Gelatin in Photography

VOLUME I

By S. E. SHEPPARD, D. Sc.

Monographs on the Theory of Photography, from the Research Laboratory of the Eastman Kodak Company.

No. 3

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Preface to the Series

The Research Laboratory of the Eastman Kodak Company was founded in 1913 to carry out research on photography and on the processes of photographic manufacture.

The scientific results obtained in the Laboratory are published in various scientific and technical journals, but the work on the theory of photography is of so general a nature and occupies so large a part of the field that it has been thought wise to prepare a series of monographs, of which this volume is the third. In the course of the series it is hoped to cover the entire field of scientific photography, and thus to make available to the general public material which at the present time is distributed throughout a wide range of journals. Each monograph is intended to be complete in itself and to cover not only the work done in the Laboratory, but also that available in the literature of the subject.

A very large portion of the material in these monographs will naturally be original work which has not been published previously, and it does not necessarily follow that all the views expressed by each author of a monograph are shared by other scientific workers in the Laboratory. The monographs are written by specialists qualified for the task, and they are given a wide discretion as to the expression of their own opinions, each monograph, however, being edited also by the Director of the Laboratory, by Mrs. Schramm, and by Miss Garvin, who is now active editor of the series.

Rochester, New York
March, 1923

Preface

Since all modern photographic processes depend upon the use of gelatin as the medium in which the silver salts are suspended, the properties and behavior of gelatin are of the utmost importance in photographic theory. It has been found necessary to divide the account of the subject into two parts, and this volume deals with the historical, manufacturing, and analytical aspects.

The technology of the manufacture and testing has been discussed with a view to the improvement of gelatin as a photographic raw material. Consequently, certain aspects have been considered more fully than a more general standpoint would require, while others have been neglected. The manufacture of gelatin and glue is only now emerging from the infantile state of a "secret art"; the principles discussed in the succeeding volume will undoubtedly have increasing bearing on the processes of manufacture. The physical and general chemistry of gelatin is developing rapidly at the present time, and it is proposed to deal with this in the second volume, which will be published as soon as possible.

Although the work is to a considerable extent a compilation from the authorities cited, practically all the essential processes discussed, both those used in the preparation of the material and in its analysis, have been tested in this laboratory. Where any divergence has been observed the fact is reported. Much original work in connection with the analysis and physical testing of gelatin which has been developed in this laboratory is also included.

Gelatin in Photography

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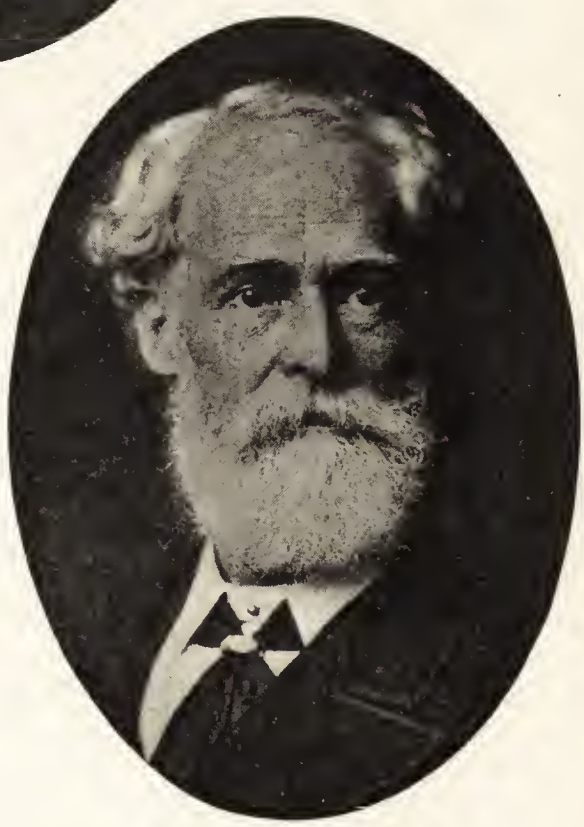

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Acknowledgment is made to J. Werge from whose book, "The Evolution of Photography", these copies were made.

Gelatin in Photography

CHAPTER I

History of the Applications of Gelatin in Photography

PART I

Gelatin with Silver Salts—Negative Processes

The earliest recorded attempt to utilize gelatin in photographic work is that of Niepce, in 1847, who experimented with it as a vehicle for holding silver iodide salts on glass plates. He abandoned the idea, however, as the gelatin layer became loose in the nitro-acetic silver bath.

Poitevin's unsuccessful efforts, in 1850, are of interest as indicating the possibilities of gelatin plates, though in his experiments he used silver iodide and gallic acid (a practically useless combination), so that the great advantage of gelatin as a medium for silver salts escaped him.

In 1853 Gaudin, in describing his "photogènes," gave a formula for what would now be termed a gelatin-iodide emulsion. Though he was unsuccessful with these "photogènes," his experiments led him to recognize the fact that the bromide rather than the iodide of silver is the important constituent of photographic emulsions.

Gelatin was "proposed as a substitute for collodion by a Tavistock gentleman in 1854, who made an iodized solution with which the plates were coated, and after being dried (in which state they would keep for a long time), they were sensitized by a brief immersion in a neutral nitrate bath, exposed in the camera while wet, and developed with iron."[1]

Norris was the first to recognize the advantage of a gelatin coating as a preservative for collodion dry plates, and the possibility of stripping collodion emulsions, so treated, from the glass in sheets. His process, which gave rise to the first successful manufacture of photographic dry plates, was patented September 1, 1856, and by 1862 the plates were on sale in all the largest cities in England.

A similar idea was advanced in 1857 by the great French professional printer, Blanquart-Evrard, who proposed to protect and strengthen prints by means of varnish composed

[1] Taylor, J. T., Brit. J. Phot. Almanac 1874: 21. 1874.

of gelatin and tannic acid. The publication of his discovery, in 1858, called forth a letter from an Englishman, W. L. Scott, who stated that he had practiced such a process for two or three years.

In the seventh (1864) edition of his *Photographic Chemistry*, Hardwich speaks of the action of light on a sheet of transparent gelatin which has imbibed a solution of silver nitrate, and also deals briefly with the use of gelatin in positive printing, in sizing paper, in mounting prints, in dry processes, and in the production of tannin plates by the Russell process.

The work of Bolton and Sayce, in 1864, though not directly connected with the use of gelatin, should be mentioned here, for by mixing the silver salts in the collodion while it was in the liquid condition and forming an emulsion they laid the foundation for the process which later revolutionized photographic manipulation.

In a short article on dry plates, which appeared in 1868, and which Eder designates as "a premature publication of mistakes", Harrison gave the results of his investigations on the emulsification of silver bromide in gelatin. Plates covered with the mixture were dried, and developed with alkaline pyrogallol. The picture came quickly and was of great intensity, but the rough and uneven surface of the emulsion greatly impaired its value. Though his attempts to improve this condition by increasing the amount of gelatin were unsuccessful, his work was significant in that he applied alkaline development to an emulsion in gelatin.

In 1871 an amateur photographer, Dr. R. L. Maddox (see frontispiece), successfully used gelatino-bromide emulsion in the preparation of dry plates by which he obtained satisfactory results. The introduction of his method, which resulted in the production of dry plates capable of a high degree of sensitiveness, marked an epoch in the development of photographic processes.

Inasmuch as Maddox stands as the discoverer of the modern silver bromide emulsion, a brief discussion of his method will not be out of place. Finely divided silver bromide was suspended in gelatin, the emulsion containing an excess of silver nitrate and a small quantity of aqua regia. Plates were coated with the emulsion, dried, printed under a negative with tolerable rapidity, and the invisible image brought out with pyrogallic acid. The necessary density was obtained by treatment with fresh pyrogallic acid to which a little silver nitrate had been added.

Maddox published his results in a more or less imperfect condition in the hope that other investigators would repeat and improve upon his work. It was nearly two years, however, before a gelatin emulsion was again attempted. Then, in July, 1873, Burgess placed on the market an emulsion made by his secret process, which was never published. Though his product was not a success commercially, he "first showed practically that excellent results were obtainable with a dry gelatin plate, and with exposures no longer than were required for wet (collodion) plates." The notable—and very important—advance made in connection with Burgess' gelatin plates was the use of alkaline pyro in development. Although alkaline development was discovered in 1862 by Russell and by Leahy (independently), it was not until it was applied to gelatin emulsions that its great advantages were realized; and conversely, the superiority of gelatin over collodion was not apparent until it was used with an alkaline developer.

In September, 1873, in an article on "Sensitive Sheet Gelatin," Sutton suggested pouring the gelatin emulsion upon a sheet of plain collodion previously set on glass, and stripping the whole from the glass in sheets which may then be cut to any desired size. This is interesting as it is the first recorded suggestion for making a film support for a gelatin emulsion.

A great step in gelatin photography was the recognition of the importance of removing the superfluous salts in the emulsion. Provision for this was made by King and by Johnston in two methods, worked out independently and published simultaneously in the *British Journal of Photography* for November 14, 1873. King, who has been credited with being the first to describe a practically workable gelatino-bromide formula, advocated dialysis followed by filtration as a means of removing the soluble salts. Johnston recommended washing the cold emulsion in distilled water. Johnston also called attention to the advantages of using an excess of the soluble bromide—a suggestion which was highly important.

In the next month—December, 1873—announcement was made of Kennett's quick and easy method of preparing gelatin emulsion plates by using the "sensitive pellicle"—i. e., the sensitive emulsion dried. This pellicle was patented and was quite successful, remaining on the market for about ten years.

About this time Foxlee recommended substituting alcohol for a large portion of the water used in the preparation of the

emulsion, to serve the double purpose of preventing decomposition and accelerating drying.

Thus it will be seen that 1873 was marked by great advances in photography, for during that year the preparation of a gelatin emulsion in a practical form was successfully accomplished. That gelatin was attracting much attention at this time is attested by the fact that the first advertisement of gelatin for photographic purposes to appear in the *British Journal Photographic Almanac* was in 1873.

The most important improvements made in 1874 were Bolton's suggestion that only a small portion of the gelatin be used in preparing the emulsion, the main part being added afterward—a procedure which proved of great value—and Stas' observation that various modifications[1] of silver bromide and chloride were possible, and that prolonged warming would induce the green condition, supposed to be the most sensitive form of the bromide.

In this year—(1874)—appeared the first advertisement of gelatino-bromide dry plates. These were manufactured by the Liverpool Dry Plate Company, by whom paper coated with the same emulsion was also prepared, as a substitute for the glass plates. This paper, the advertisement states, "may also be used for positive proofs, by contact and development printing, an exposure of a few seconds to gas or other artificial light being sufficient."

Gelatin was also being used in developers at this time, as Wortley advertised a new gelatin-containing developer which gave "wonderfully good results" with his Uranium dry (collodion) plates.

For several years little or nothing of importance relative to gelatin was published. The year 1878, however, was another one of great progress with the gelatin emulsion, and marked the advent of the rapid gelatin plate, which was first prepared by Bennett.

Following Stas' suggestion made in 1874, Charles E. Bennett demonstrated that by keeping the gelatin emulsion at a temperature of 90° F.—no higher, and little lower—for a prolonged period, extraordinary rapidity was obtained. He recommended from two to seven days' heating, the length of time of digestion depending on the degree of rapidity desired. The Liverpool Dry Plate Company manufactured plates by Bennett's formula, and Mawson and Swan soon followed with plates of "exquisite sensitiveness." The publication of

[1] Stas' "modifications" are discussed in another monograph, No. 1—The Silver Bromide Grain.

Bennett's results, accompanied by photographs convincingly demonstrating the sensitiveness of the plates made by him, was followed by a general acceptance of the gelatin process as superior in speed to wet collodion.

Later in 1878, Mansfield declared this long heating unnecessary, as he had obtained equally good results by using boiling water to heat the emulsion, thereby inducing the desired sensitiveness in minutes instead of days. Wortley brought about the same results by heating the emulsion at 150° F. for several hours.

In this year Palmer was successful in his experiments, begun a year earlier, in preparing gelatin films as a substitute for glass. They were made, dried, and coated with the emulsion on glass, after which the latter was removed.

The first advertisement of an "instantaneous" gelatino-bromide plate to appear in the *Almanac* was that published by Wratten and Wainwright in 1879, which would indicate that their attempts to prepare an extra-rapid gelatin emulsion had been successful during 1878.

In 1879 Bolton reported on results obtained by the use of his earlier suggestion—i. e., emulsifying the silver salt in a small quantity of the requisite amount of gelatin, the major portion being added after the emulsification is completed—in the preparation of emulsions by boiling. In this way the danger of destroying the setting power of gelatin is obviated. Abney says this method "first indicated the true method of preparing emulsions by boiling . . . . . and opened out a new era in gelatin emulsion".

In 1879 Van Monckhoven, following Abney in varying the method of mixing emulsions, pointed out that an emulsion obtained by the action of hydrobromic acid on silver carbonate contained no soluble salts to be removed. Later, he suggested that the increased sensitivity of the bromide emulsion brought about by prolonged digestion may be connected with a molecular change and demonstrated that ammonia greatly facilitated this "ripening" by rapidly changing the silver bromide from the ordinary state to the green condition referred to above.

Eder, experimenting with Van Monckhoven's discovery in 1880, perfected an emulsion process with ammoniacal silver oxide, and, somewhat later, recognized the advantageous influence of ammonia and ammonium carbonate on the ripening of emulsions in the cold.

The principal innovation during 1880 was Abney's introduction of iodide to replace part of the bromide in gelatin

emulsions, which made at first a slower emulsion (thus allowing more light in the dark-room), but one which produced clearer images. A certain percentage of iodide is invaluable in modern practice.

In 1880 the Paget prize for the best emulsion process was awarded to W. J. Wilson, whose formula called for potassium bichromate, alcohol, hydrochloric acid, ammonium bromide, and silver nitrate, gelatin and distilled water. A company, known as the Paget Prize Plate Company, was organized to manufacture these plates under Mr. Wilson's supervision.

There were many manufacturers of gelatin dry plates established by this time in England, and some in Belgium and in America; and during the next year (1881) there was a "gradual spread of gelatino-bromide plates . . . . . in every portion of the globe."

In 1881 Eder perfected his so-called hard gelatin, which found a ready market in Europe. Other minor contributions in 1881 include a plan for making an alcoholic gelatin emulsion to induce rapid drying of plates, devised by Herschel, and a method, outlined by Vogel, of combining gelatin and pyroxylin by means of a solvent (acetic acid) which would act on the gelatin and allow the addition of alcohol for dissolving the pyroxylin.

1882 was marked only by the commercial introduction of gelatino-chloride plates by Edwards. A "flexible glass" support to be considered later (see p. 17) was also put on the market early in this year.

In 1883 the first isochromatic plates were placed on sale by Tailfer, who was the first to apply to commercial gelatin plates Vogel's discovery of the sensitizing action of dyes, made in 1873.

By this time manufacturers of gelatin plates were known all over the world, and constant advances and improvements were being made. Among them may be mentioned Edwards' Special Instantaneous plates for drop-shutter exposures, advertised in 1884, Wratten and Wainwright's drop-shutter plates, advertised in 1885, and, in America, Eastman's introduction of the flexible film and "new and simple devices for exposing the same in single sheet and in the roll," in 1884, and in 1885, Carbutt's Keystone Special portrait and instantaneous plates, with which fully exposed negatives were obtained from exposures of one two-hundredth of a second.

SUPPORTS

A brief resumé of the various supports used for negative gelatin emulsions should perhaps be included here.

Paper was early suggested as a substitute for glass as a support for emulsions, and in 1874 the first commercial negative gelatin emulsion paper was introduced by the Liverpool Dry Plate Company (see p. 14).

Palmer's gelatin support (cf. p. 15), suggested in 1877, was not generally adopted, being supplanted by Milmson's "stripping film," introduced the same year.

Two years later (1879) Stebbing introduced gelatino-bromide emulsion on a hardened gelatin film between two thin layers of collodion, and Ferrier patented a collodio-gelatin film. Both were placed on the market.

In 1881 Pumphrey began manufacturing his "flexible glass" support of gelatin and collodion, and in 1884 a negative paper, coated with emulsion on both sides to prevent curling, was introduced by Warnerke.

In the latter year an extra-rapid bromide paper for negative work was introduced, and Eastman and others soon followed with improvements, among which may be mentioned especially Eastman's "stripping film," patented October 14, 1884. This "stripping film" consisted of a temporary paper support coated with soluble gelatin, which in turn was coated with the gelatin emulsion. After exposure and development the negative was placed face down on glass, the soluble gelatin softened by moistening the paper, and the paper "stripped" from the film negative, which was then reinforced by a gelatin skin. This type of film proved very satisfactory and was widely used until the introduction of the rollable transparent nitrocellulose film by The Eastman Dry Plate and Film Company in 1889, when paper negatives were largely supplanted. A further improvement was effected in 1903 when the Eastman Kodak Company prepared this film with a non-curling gelatin backing.

THE USE OF GELATIN IN POSITIVE PRINTING

Mention of the use of gelatin to hold sensitive salts for paper printing is made in Hardwich's Manual of Chemistry, published in 1856, where formulae are given for coating paper with citric acid and sodium carbonate in gelatin, sensitized later with nitrate or "ammonia nitrate" of silver. This is really a plain paper process, however, the quantity of gelatin recommended being very small and serving merely to keep the image at the surface of the paper.

In 1874 the gelatino-bromide emulsion was applied to paper for positive printing and commercially introduced by the Liverpool Dry Plate and Photographic Company. It was not until 1880, however, that this paper came into general use

A great advance in printing papers was made in 1881, when Abney introduced his gelatino-citro-chloride process for printing-out paper. This paper is the analogue of the salted papers, containing silver chloride, free silver nitrate, and silver citrate, and toning with gold.

The next improvement of note was in 1883, when Eder showed that a mixture of chloride and bromide for gelatin emulsion papers is more sensitive than the chloride alone.

In 1884 the first machine for coating gelatino-bromide emulsion paper was built by Walker and Eastman, and the production of these papers was begun on a large scale.

Before the end of 1886 the Ilford Company placed on the market their "Alpha" paper, a gelatino-chloride emulsion paper which, according to the advertisement, "may be dried with either glazed or matt surface, and does not deteriorate by keeping."

No noteworthy improvements were made in positive papers until 1891, when The Eastman Kodak Company introduced Solio, a gelatin printing-out paper which is still in use; and in 1893 another great advance was accomplished when the Nepera Chemical Company produced Velox from the formula of Dr. Leo Baekeland. This is a chloride emulsion developing-out paper without free silver, which, being about five hundred times slower than bromide paper, can be handled in a fairly bright light. Velox was the first of the "gaslight" papers, and it was due to its great success that the present large series was developed.

The obvious possibilities of gelatin emulsions for the preparation of opals, transparencies, and lantern slides were recognized and utilized commercially soon after the success of gelatin dry plates, so that by 1885 collodion and albumin were displaced as completely for these purposes as they had been for plates and paper.

Part II

Gelatin with Bichromates

Gelatin has also been extensively used in various photo-engraving and printing processes when sensitized with potassium or ammonium bichromate. There are two methods of using gelatin for these purposes: (1) the wash-out process,

in which the exposed film is treated with hot water to dissolve out the unexposed parts (utilized in the so-called "carbon printing" process and in the photogravure and rotary photogravure processes of today), and (2) the swelled gelatin process, in which the film is soaked in cool water to cause the protected parts to swell, making an image in relief (utilized in intaglio photo-engraving and photo-lithography). Both processes are based on Becquerel's discovery, noticed below.

The sensitiveness to light of paper coated with a solution of potassium bichromate was first recorded by Mungo Ponton in 1839. Becquerel, investigating the phenomenon, decided that the darkening in light was due to the presence of organic substances in the size of the paper, and discovered that gelatin, gum, and similar substances were rendered insoluble when exposed to light in the presence of potassium bichromate. His discovery was put to practical use by Fox Talbot, who based on it a process of photo-engraving patented in 1852.

The contributions of outstanding figures in the development of the applications of bichromated gelatin to various photo-printing processes will be briefly considered under three heads: (A) Carbon printing; (B) Photo-lithography; (C) Other photo-mechanical processes.

A. CARBON PRINTING

In 1855 Poitevin patented his method of adding powdered carbon to the bichromated gelatin used by Talbot in his "photoglyphic engraving" process (to be discussed under section C), and thereby obtaining direct carbon prints. The specifications called for gelatin, fibrin, albumin, gum, or some similar substance, bichromate, and powdered carbon. All later and practical methods are considered by many authorities to be based on Poitevin's process, whereas others agree that his directions are vague and uncertain, and that Pouncy, whose process was published January 1, 1859, should be given credit for producing the first veritable carbon prints.

Prints obtained by these processes were generally unsatisfactory, as the half-tones were seldom properly reproduced. The fact that only the surface of the gelatin is rendered insoluble under relatively dense silver deposits, the lower strata remaining soluble and being washed away in the treatment after exposure, thus carrying away the entire film, was pointed out in 1857 by Laborde as the cause of this difficulty. No remedy was suggested, however, until Blair, Schouwaloff, and Burnett announced almost simultaneously, in 1858, that the essential element of perfect gradation could be obtained

only by exposing the sensitized paper from the back. But this method proved unsatisfactory because of the long exposure necessary, and because the image was marked by the texture of the paper.

A great impetus was given carbon printing in 1860, when Fargier suggested coating the surface of the exposed gelatin with collodion, which served as a support when the original backing (Fargier used glass) with the unexposed (and therefore still soluble) gelatin was dissolved away. After washing, Fargier caught the developed picture on a piece of paper, collodion side down. Thus he made Poitevin's process definitely usable, but the manipulation was too difficult for his method to be practically or commercially utilized.

In 1864 Swan introduced a process which for the first time put forth a really practical, successful, and comparatively easy method of producing carbon prints. Two important features of his process were: (1) the production of "carbon tissue" (paper coated with a mixture of gelatin, bichromate, and powdered carbon or other pigment), which permitted exposure at one side and washing away at the other, and (2) the method of double transfer. The method as at present practiced is Sir Joseph Swan's, with a few minor modifications, among which may be mentioned the work of: Davies, in 1864, who simplified the process by demonstrating that Swan's use of caoutchouc or other cements was unnecessary, as the wet tissue would adhere to any waterproof surface; Johnson, who in 1869 published a method which consisted mainly of modifications of the processes of Blair, Fargier, Swan, and Davies; and Sawyer, who in 1874 demonstrated the practicability of a temporary flexible support during development. These various improvements have made it possible to obtain beautiful results by Swan's process.

In 1873 Marion introduced his "Mariotype" process, which is interesting because of its possibilities, utilized later by Manly. An image of so-called chromium chromate ($Cr_2O_3.CrO_3.H_2O$) was made on plain paper "salted" with potassium bichromate and used to insolubilize the gelatin of a separate pigmented gelatin layer pressed into intimate contact with it, and left for some hours. This was then developed with warm water, as in the carbon single transfer process, the applied pigment paper being removed from the copy, the insolubilized gelatin left adhering to the image.

The names of Testud de Beauregard, Heinecke, Sutton, Simpson, Gabriel de Rumine, Lea, Eder, Liebert and Liesegang should be mentioned because of their investigations of and

contributions to the process of carbon printing. Eder's monograph on the chemistry of bichromated gelatin, published in 1878, is especially noteworthy, embodying the results of his extensive researches into the nature of oxidized gelatin products.

Later processes deserving mention are the "velours charbon" process introduced in 1899 by Artigue, in which development is effected without transfer, and Manly's two processes, the "ozotype" and "ozobrome." In the ozotype process (also described in 1899), there is no lateral inversion, as no transfer of the image is produced by exposure, and no actinometer is necessary, the change on exposure being visible. This method, apparently based on the "Mariotype" process mentioned above, is an unusual one, and perhaps should be described.

The paper to be used is sized with fish glue or gelatin and sensitized with a solution containing potassium bichromate and manganese sulphate, the latter increasing the sensitivity. After exposure to light under a negative until the desired density is obtained, the print is washed, then placed, simultaneously with an unsensitized sheet coated with pigmented gelatin, in a solution containing oxidizing and reducing substances. The two sheets are pressed together face to face, squeegeed to remove excess liquid, and left for half an hour. Then they are put in hot water, the paper backing of the gelatin stripped off, and development completed as usual. The half-hour wait is to allow the acid in the solution to dissolve the chromates from the image, and the reducing substances to change these chromates to basic chromium salts, which tan the exposed parts of the gelatin. The diffusion of the reacting substances from the original image into the gelatin is controlled mainly by the degree of acidity; and, by altering the ratio of acid to reducing agent in the ozotype bath, the contrast of the pigment image may be varied within fairly wide limits.

The name ozotype was given to this process by its inventor because of the mistaken idea (refuted by Haddon) that the gelatin was made insoluble by ozone formed by the photoreduction of the bichromate. He supposed that the ozone oxidized the manganous salt to manganese dioxide or manganates, which then acted on the gelatin.

A few years later (in 1905) Manly introduced the ozobrome process, a modification of the ozotype, which surpasses the latter both in simplicity and in generality of application, and which makes the printer independent of light. By this process it is possible to change an ordinary bromide print into

a carbon print, and to make an enlarged carbon print directly from a small negative. An additional advantage of the ozobrome process is that the bromide print used may be restored by any ordinary developer and utilized for additional carbon prints.

The bromide print is pressed against a sheet of pigmented paper previously sensitized in an "ozobrome" solution containing a bichromate, a ferricyanide, and a bromide. This solution is reduced by the metallic silver of the image, and soluble diffusing products of this complex reaction enter the gelatin pigment layer, tanning the gelatin in proportion to the mass of silver (image density) locally present.

A special type of pigment emulsion for use with the ozobrome process, which gives pigments of great color intensity and transparency, has been worked out by the author.[1]

The catatype method of Ostwald and Gros, patented in 1901, is of interest because it is one of the few applications of the catalytic action of silver or platinum photographs. The method is as follows: A silver or platinum print is flooded with an ethereal solution of hydrogen peroxide, which produces an invisible image by the decomposition of the peroxide by the finely divided metal. A gelatin film (paper backing must be used, as other backings do not allow the evolved oxygen to escape) pressed against this hydrogen peroxide image is sufficiently impregnated with the peroxide to be affected by an alkaline solution of a metallic salt, with formation of an image. Images of various colors, produced by using different alkaline solutions of metallic salts or dyes, as well as carbon prints, may be obtained by this process.

B. PHOTO-LITHOGRAPHY

Bichromated gelatin, because it becomes insoluble and does not absorb water after being exposed to light, occupies an important place in photo-lithography, which was the first of the photo-mechanical reproductive processes to be commercially profitable. The possibilities of the use of bichromated gelatin for this purpose were first mentioned by Talbot in 1852, in connection with his description of his photo-engraving process, but it is to Poitevin that we owe the first method of photo-lithography. His process, which consisted in exposing a stone covered with bichromate and gum or gelatin under a negative, washing it in water, and inking the relief obtained, was patented in 1856; but there were many defects in the

[1] Sheppard, S. E., U. S. Patent 1, 290, 794. Jan. 7, 1919.

details of manipulation, and it was several years before a practical method was devised.

The honors for improvements in the process seem about equally divided between Asser of Amsterdam, James of England, and Osborne of Australia, who almost simultaneously, in 1859-60, described their successful methods, independently conceived. Asser's procedure was to obtain a laterally inverted image on the stone by first preparing a print on paper coated with bichromate and gelatin or other organic matter, and transferring this direct image in ink to the stone or zinc. He then multiplied the impressions by mechanical lithography.

Osborne and James also prepared a "transfer" in order to get a direct print, their methods differing from Asser's mainly in improved manipulation, which made the process practical and commercially successful.

C. OTHER PHOTO-MECHANICAL PROCESSES

In 1852 Talbot patented a process of photo-engraving which he called "Photoglyphy," and which was the first of many forms of permanent printing based on Becquerel's discovery. He used a mixture of gelatin (or some similar substance) and bichromate on a steel plate, and etched the image obtained after treating the exposed film with water by means of platinic or ferric chloride solution. He obtained half-tones by placing black gauze between the diapositive and the emulsion, thereby laying the foundation for later screen processes. He also mentioned the possibility of obtaining photo-zincotypes and photo-lithographs by similar methods. Talbot's etching process, modified by Klic, is the method now used in the production of photogravure plates.

Pretsch utilized Talbot's observations in a commercial venture in 1854, but his enterprise was not a commercial success and was abandoned.

The first use of bichromated gelatin in photo-mechanical printing processes is ordinarily credited to Poitevin, in 1855, but it would seem to be due to Oreloth, who stated, in his patent granted in 1854, that he inked his gelatin images before transferring them to stone or zinc. The success of this process ("collotype" process) depends largely on the proper formation of an irregular grain by reticulation of the gelatin during drying.

Half-tones by this collotype process were first obtained by Tessié du Motay and Maréchal in 1865. Though their results were good, their methods were tedious, and relatively few impressions could be obtained from a plate. Albert simplified

the process and about 1867 succeeded in making a plate from which over a thousand impressions could be made. At present, however, the number of impressions from one plate averages about five hundred. Albert's method was not published until several years later, when it was described by Ohm and Grossman.

An English patent was granted Ernest Edwards for "heliotype," a modification of the collotype process, for which a thick gelatin film without support was used. This process, however, was not generally adopted.

The Woodburytype, originated by W. B. Woodbury in 1864, deserves especial mention, for, in the words of Chapman Jones: "In these methods of Woodbury a perfect gradation of tint may be produced, a result which is practically impossible by any other photo-mechanical process". A Woodburytype may be defined as a pigmented gelatin relief in which the thickness of the relief determines the amount of color. Various improvements of the process were made by the inventor, one of the last being a modification of his Stannotype process, reported on in 1880. For this a gelatin intaglio was used instead of a relief, and the exposure was made under a transparency instead of under a negative.

Others noteworthy for their improvements of the various processes were Husnik and Obernetter, through whose labors the collotype process was brought to its present perfection; and F. E. Ives and Levy, who, by their respective contributions —the discovery of the correct optical principles of the cross-line screen, and the commercial production of these screens, patented in 1893—made the widespread use of the half-tone process possible.

CHAPTER II

Manufacturing Processes

Part I

Manufacture

As is commonly known, gelatin and its humbler relative, glue, are products of animal origin, the result of the action of hot water or steam upon certain tissues and structures of of the body. Primarily, it is obtained from the connective tissues by hydrolytic separation from the skeletal, fleshy, and fatty substances which are suspended in it in the organism. Though by manufacture somewhat of an artificial substance of varied character, it has originally a definite physiological and biochemical unity which may be diminished, but is neither destroyed nor created, in the process of preparation.

Gelatin is generally regarded as consisting of glutin and "chondrin", of which the latter has recently been found to be a mixture of glutin and chondromucoid. Glutin is the comparatively pure gelatin; chondrin is the main constituent of glue and poor grades of gelatin. A gelatin which can be considered as mainly glutin may be obtained from the corium (the sensitive and vascular layer just beneath the epidermis), while chondrin in a relatively distinct form is a product of the hydrolytic treatment of various cartilaginous tissues.

The principal sources of gelatin are the bones, the cartilage and ligaments, and the hide. Cartilaginous tissues are not very suitable for the production of gelatin stock, for (as stated above) they yield a large proportion of chondrin. Bones, from which ossein stock is obtained by dissolving out the mineral constituents with dilute acid, are also more suitable for glue and the edible grades of gelatin. The preferred material for gelatin, and especially for photographic gelatin, is the hide. Of hides, those of calves are recognized as the very best, as others—e. g., those of sheep—make very dark gelatin. In addition to the kind, the age and condition of the animal are of importance.

The actual material consists of the leavings of tanneries and slaughter-houses—i. e., trimmings, so-called skips, ears, cheek-pieces, pates, fleshings, etc. The stock may be either "dry" or "green," and is sometimes degreased and dehaired before shipping.[1]

[1] See also H. G. Bennett, Animal Proteins, p. 222.

The process of preparation is briefly as follows (Fig. 1):

A. Liming;[1]
B. Washing;
C. Neutralizing;
D. Cooking (Digesting and Extraction);
E. Clarifying or Filtering;
F. Concentrating;
G. Bleaching. (This may be combined with washing, or, as is often the case when a good grade of stock is used, may be omitted altogether);
H. Cooling (Jelling and Slicing);
I. Drying and Packing.

In practice, of course, details are varied by different makers, and we shall attempt to give only a general idea of the progress of the operations.

A. LIMING

In this operation the hides are steeped in the suspension known as milk of lime, as lime itself is but slightly soluble—one part in 700—in water.[2] This saponifies any fatty material. removes dirt, and dissolves blood and shreds of flesh, beside acting as a mild antiseptic to help preserve the stock. The period of liming depends on the pretreatment of the stock, its greenness, and the age and condition of the hide. Treatment is complete when the stock is "plumped" and relatively free from grease. Completion may be determined by examining a freshly cut surface to see that the tissue is thoroughly impregnated. The use of "sharpeners," as caustic soda, will reduce the time, which generally may vary from two months to two weeks.

Either fresh stone lime, recently slaked to calcium hydroxide, or lime which has been previously hydrated at the kiln and is furnished in a stable form, is used.[3] The value of the lime depends upon its content of free calcium hydroxide, $Ca(OH)_2$, which is gradually reduced by interaction with atmospheric carbon dioxide, CO_2, to form the carbonate—

$$Ca(OH)_2 + CO_2 = CaCO_3 + H_2O.$$

The antiseptic action of the lime has been attributed partially to a superficial coating of the stock with a film of carbonate,

[1] In the case of "green" stock, which is salted for shipping, washing precedes as well as follows liming, and, indeed, a preliminary cleansing process is generally necessary. For hide stock the so-called Roller Wash Mills are generally used. These consist of a circular tank 16 feet in diameter and about 40 inches high containing a conical roller revolving at from 14 to 21 revolutions per minute.

[2] For a discussion and tables of the solubility of lime, see Guthrie, F., Phil. Mag. V. **6**: 35. 1878.

[3] The advantages of hydrated lime for preparing the milk of lime are discussed by Roenitz, q. v. Roenitz, L. T., J. Amer. Leather Chem. Assoc. **7**: 165. 1912.

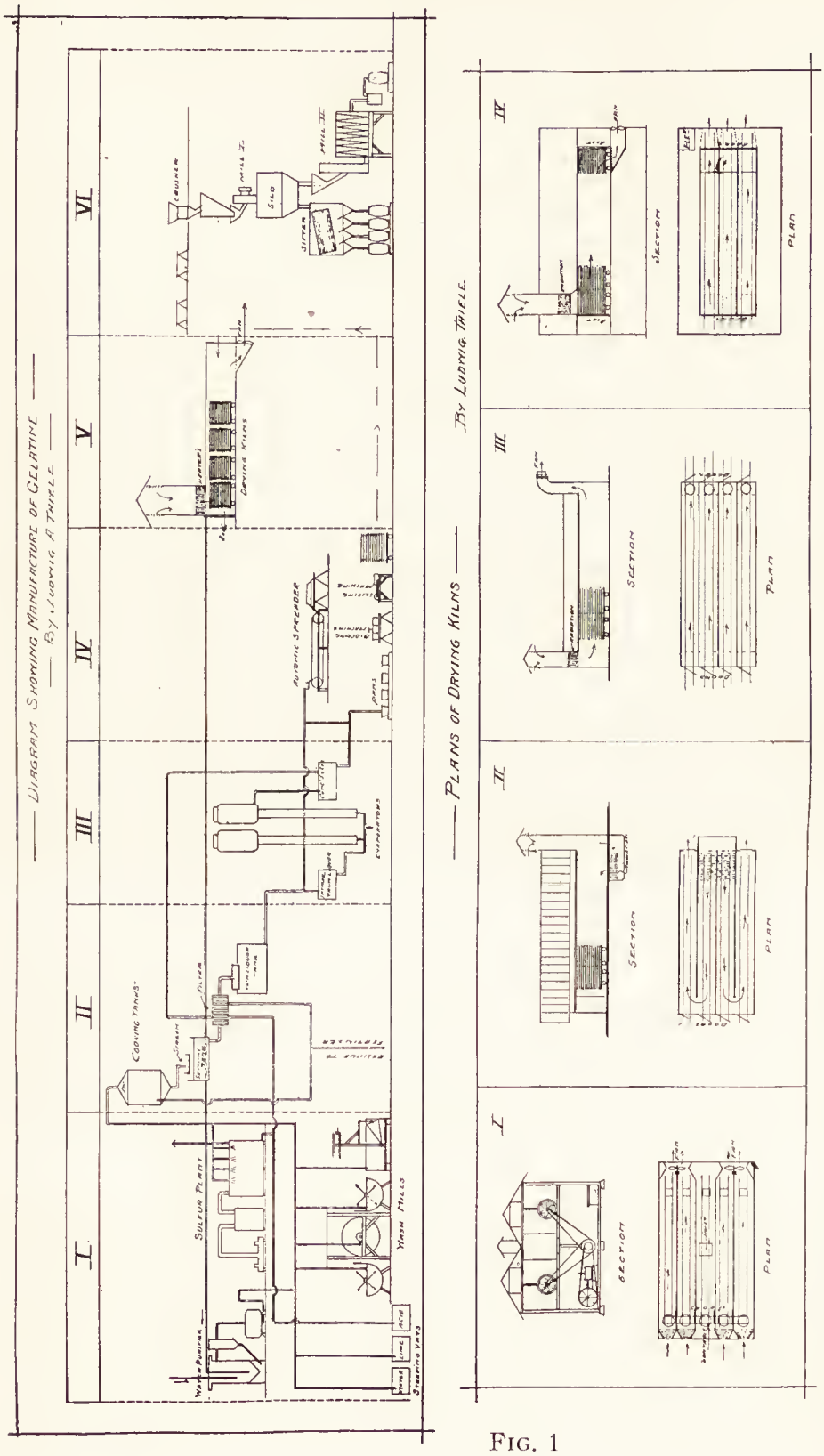

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FIG. 1

but is due chiefly to the fact that few bacteria can flourish in the relatively high hydroxyl-ion concentration present. However, bacteria are present, especially in old lime baths, and potentially play an important part in the process. (See p. 63.)

The temperature of the liming bath should be kept approximately constant, in summer at that of the surrounding atmosphere or even slightly cooled, in winter at 15-20° C.

B. WASHING

After liming, the stock is thoroughly washed with fresh water agitated by paddles. It may be remarked here that the importance of the character and supply of the water used, for boilers as well as for cleansing and washing the material, cannot be overestimated. The purity of the water must also be carefully investigated, as invasion of molds and bacteria will ruin everything.

A good water for manufacturing purposes should be reasonably soft, containing not more than ten to fifteen grains per gallon (14 to 20 parts per 100,000) of lime and magnesia salts; it should be free from iron and hydrogen sulphide, and should not contain excessive quantities of mineral salts, especially carbonate of soda, nor even small amounts of ammonia. Sodium chloride in any quantity is objectionable, as it has a tendency to soften and liquefy the gelatin. A definitely hard water may, of course, be improved by artificial softening, and suspended matter may be removed by sand or other filtration.[1]

The washing after liming is continued until the wash liquor is colorless and nearly neutral (showing but faint color with phenolphthalein), and yields only the natural mineral residue on evaporating a definite amount on the water-bath. The operation must not be unduly prolonged, and is effected best by successive treatments with relatively small quantities, thoroughly drained off and frequently renewed.

Antiseptics are sometimes added during the washing process. Here, choice is limited, as those used must be cheap, on account of the large volume of liquid necessary, and must neither harden nor color the stock. Metallic compounds, such as mercuric chloride, chlorine, and hypochlorite are excluded; boric acid is of low antiseptic power; phenol is hardening, and imparts its odor to the glue; formaldehyde, sufficiently diluted (1 in 10 to 100,000) has been recommended,

[1] Purification of water here is partly a chemical, partly a bacteriological question, as for drinking water. A softening process leaving any quantity of alkali metal salts present is undesirable.

as in this small quantity it does not harden the stock and is volatilized in the subsequent cooking. The minimum antiseptic strengths are:

Phenol	1 in 1,000;
Borax	1 in 200;
Formaldehyde[1]	1 in 20,000.

C. NEUTRALIZATION

To remove the impregnated lime, neutralization may follow washing, or be combined with it. Sulphuric or hydrochloric acid up to about two per cent strengths may be used, but care must be exercised in washing out the acid, as acids greatly accelerate the hydrolysis of glue and gelatin, and may injuriously affect the subsequent clarifying. Rideal discountenances the acid treatment as letting the fatty acids from the lime soaps go to waste, and as giving rise to loss of gelatin by too free use of acid; but it is generally questionable if the lime could be sufficiently removed by water alone on a commercial scale. The stock, at this stage, will contain three to ten times its weight of water.

D. COOKING

This consists in the digestion of the purified stock with hot water. It is usually carried out in large wooden vats provided with water feed-pipes and false perforated bottoms, below which are steam coils for injecting steam and raising the temperature as desired. For the higher grades of gelatin temperatures from 130-170° F. are used, while for glue the stock is heated to boiling. In France, batteries of closed metal kettles are very generally used, working together in a manner similar to a sugar diffusion battery. It is claimed that, by this method, there is less manipulation, lower steam consumption, higher strength of liquor, and more exhaustive extraction.

In any case, fractionated cooking is usually practiced, a relatively small quantity of water being admitted and the cooking continued till an extract of the desired strength is obtained. This is run off, and the process repeated until the liquid will not gelatinize. Three to four runnings are generally made, the first and second being the best, the third and fourth yielding material fit only for size. The residues go for fertilizer.

[1] Great caution is necessary in using formaldehyde for photographic gelatins, as it may cause "fog" in the emulsion. Sodium silicofluoride has been recommended recently.

E. CLARIFYING

After extraction, the liquors are clarified, either in clarifying vessels or tanks, or by a preliminary settling followed by press-filtration.

In the first method, the liquor is run into large shallow vats, in which it is kept warm so that grease will rise to the surface to be skimmed off, and insoluble matters settle out. Settling is assisted by a clarifying agent which is usually a potash-alum, sometimes followed by a little milk of lime. The action of the clarifying agent depends upon the coagulation of suspension colloids by electrolytes. This coagulation is attributed to the neutralization of the electric charge of the colloidal particles by an ion of the opposite sign, and the efficiency of the electrolyte depends upon the valency of the ion. A very small quantity of a polyvalent ion will be as effective as a much larger quantity of a monovalent ion. Suppose the coagulating power of potassium chloride to be one, that of magnesium chloride would be 182, that of aluminium chloride 2518—that is, the coagulating powers of the cations for a negatively charged hydrosol are as

$$R' : R'' : R''' = K^1 : K^2 : K^3$$

where $R' : R'' : R'''$ are concentrations, K a constant.[1] Hence alum is a very good clarifying agent. Conversely, the coagulating power of anions for positively charged particles increases in like measure with the valency.

Clarification can also be effected by electro-osmosis, the colloidal particles being flocculated at the electrode. The possibilities of this method in the preparation of gelatin have been little investigated, nor have experiments upon the possible use of ultra-violet light to clarify and bleach gelatin sols simultaneously been carried out on any considerable scale.

Another method of clarification consists in the addition of albumin to the glue or gelatin. This method is more expensive than that with alum, and is less frequently employed on a large scale. The albumin (e. g., blood albumin) is added below the temperature of coagulation—at about 50° C.—and the temperature then raised to about 90° C., when the coagulated albumin drags down other suspended matter.

Clarification by settling may be replaced, partly or entirely, by press filtration, a method which is of advantage in that it requires a shorter time to produce a clear liquid, and a firm cake is formed of the solid matter. This cake is much more

[1] This so-called Whetham's rule does not hold absolutely.

easily handled than the stratified sludge of the sedimentation process, and the clear liquid is usually of homogeneous density and character. Paper pulp forms the best filler medium, at not too high pressures. Centrifugal separation is also used.

F. CONCENTRATION

Concentration of the clear liquor cannot be accomplished by simple evaporation in bulk, as this would cause a tough skin to form on the surface, and the interior portions would lose their jelling power. Therefore, the method of surface evaporation is employed, by means of either the open (aerobic) system—this consisting in rotating cylinders half-in, half-out of the liquor (which is kept at 70-80° C.), thereby continually exposing fresh surfaces to evaporation—or the closed or vacuum pan evaporation at reduced temperature and pressure. The Yaryan evaporator, for double or triple effect, is very generally employed, but for experimental work on the relation of the concentration conditions to the quality of the product a modified form of the Kestner evaporator is very suitable. It is simple in construction and works upon a cycle very similar to that of the Yaryan, the main difference being that the former system is vertical, that of the Yaryan horizontal.[1] The high velocity possible in the Kestner type makes it very suitable for photographic gelatins. The Yaryan and Kestner types are illustrated in Fig. 2.

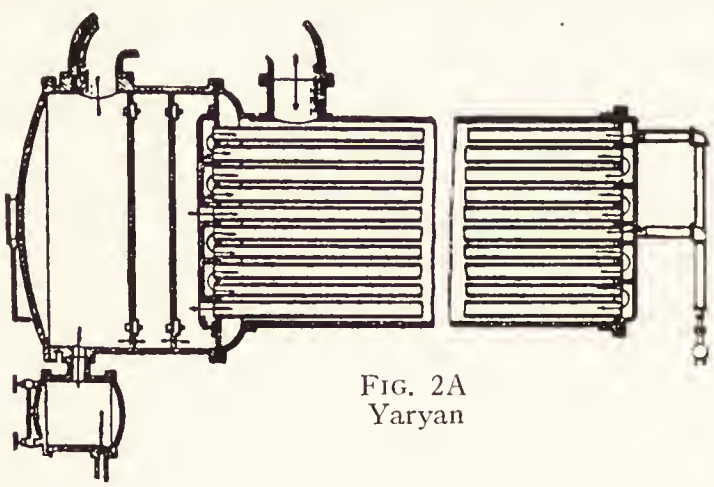


FIG. 2A
Yaryan

As will be noted later in more detail, prolonged heating of gelatin solutions at 70° C. or above destroys the setting power of the gelatin, so that the temperature must be kept down—

[1] A good account of recent advance in evaporator design is given by D. Dunglinson, (Chem. Met. Eng., **25**: 110. 1921), and J. A. Reavell (J. Soc. Chem. Ind., **37**: 172T. 1918).

that is, the proper vacuum secured. The degree to which concentration is carried varies with different makers. It is, however, highly important, since, other things being equal, the water-absorbing capacity of a gelatin depends upon the concentration at which it was set prior to drying, the dried gelatin tending to swell to a limit near its previous concentration as a gel. It may be emphasized here that gelatin is not a chemical substance of definite composition and constitution, of properties independent of origin and preparation, but a material embodying a history, which, from first to last, affects its behavior.

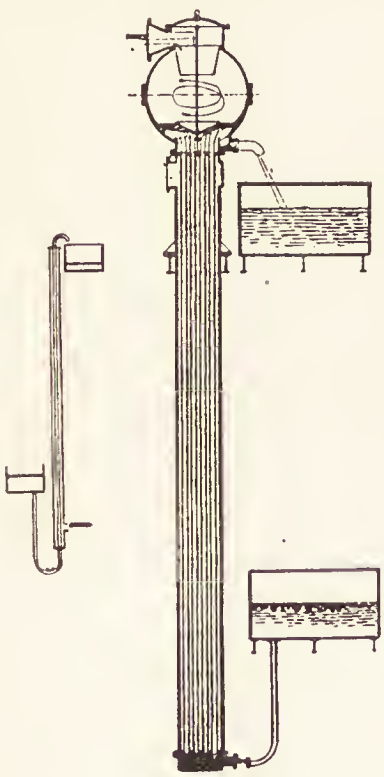


FIG. 2B
Kestner

The concentration limit will depend both upon the purpose for which the gelatin is intended (being relatively lower for photographic gelatins), and upon the form in which the dry material is desired. Thus, if it is proposed to produce gelatin in thin sheets (a usual form for photographic gelatins), it is advisable not to concentrate the thin solutions too far, but to stop at five or six per cent and chill these dilute solutions. The difficulty of spreading such relatively thin solutions by hand has led to the invention and introduction of various automatic spreading and chilling devices. (See under H.) The concentration is readily controlled with the hydrometer.

G. BLEACHING

The next stage consists usually in lightening the color, which is intensified by concentration. This is done only in those cases where low grade stock (which produces a darker gelatin) is used. Bleaching is generally effected with sulphurous acid, introduced either as gas or as solution in water. It has also been proposed to utilize crude sulphite cellulose liquors from paper pulp factories. Ozone is claimed by some to be a good bleach, but by others it is declared to destroy the setting power, and to be the effective agent in the spoiling of gelatin in electrical weather. Hydrogen peroxide is used for bleaching food gelatin.

This operation may be combined with clarification, and it would seem worth while to experiment upon the bleaching and clarifying of gelatin by ultra-violet light under conditions similar to those employed on a large scale in the sterilization of water. The method would allow working under continuously sterile conditions.

H. COOLING AND SLICING[1]

The liquor is now ready for chilling and setting. This may be accomplished either by discontinuous setting in shallow metal trays placed in a suitably cooled chamber, or by a more or less continuous process, the liquor being fed over a series of slowly moving chilling rolls which are cooled internally by brine fed through the stuffing boxes, and from which the gelatin layers can be continuously and automatically stripped and cut up. Or, the process may be only partly mechanical, the material being hand-stripped, and cut into sheets of the desired thickness with a wire cutter. This process is due to Peter Cooper Hewitt. In more recent methods continuous belt-chilling is used.[2]

I. DRYING

This is in some respects the most critical period in the process, loss here being the more weighty because of the time and labor already expended. The cut sheets are suspended upon netting of hemp or heavily galvanized iron or other metallic wire. (Cotton netting is unsatisfactory as it rots easily and, being absorbent, becomes a breeding place for bacteria.)

The rate of drying must be regulated according to the concentration and thickness of the sheets. The temperature must be little over 20° C. or the jellies will soften or melt, and become more liable to infection. In winter it may be necessary to warm the air, in summer to cool it. The best method is to have a properly regulated air current, the temperature, saturation, and velocity of which can be controlled.

Processes for preparing gelatin in powder form by precipitating it with benzene or alcohol have been worked out, but it is more usual to obtain a powder by grinding and for many purposes, the sheet, flake, or shredded products are preferred. The enormous increase of surface which is effected by powdering, lays a product of the character of glue or gelatin

[1] On the applications of refrigeration in the gelatin industry, see Cavalier, P., J. Soc. Chem. Ind. **30**: 143. 1911.

[2] Schill and Seilacher, Germ. P. 119,447 (1899). Kind, M. (Kind and Landesmann) U. S. P. 1,046,307 (1912).

open to contamination or infection, so that the preference of many users for the traditional forms of the material is possibly justified, although no definite trouble has been found in this connection.[1]

The gelatin is dried down to a water content of from ten to fifteen per cent. A higher water content gives a somewhat flexible and easily attacked product, a lower makes it objectionably brittle, and probably involves in its attainment actual alteration in the character of the gelatin. Generally, however, a "summer gelatin" will have a higher water content, about fifteen or sixteen per cent, in equilibrium with the higher humidity, and be flexible, whereas a "winter gelatin" will have a lower percentage—eight to ten per cent—and be brittle.

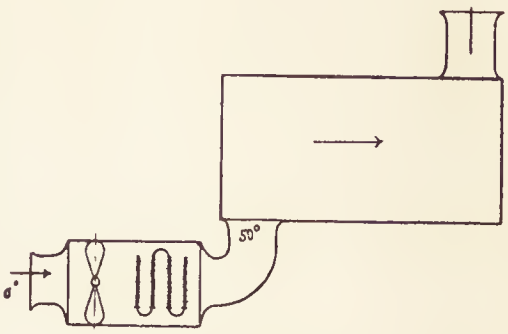


FIG. 3
Drying chamber

Since drying is such an important part of the manufacture of gelatin, a description of various methods of drying and a brief discussion of the theory of drying is included.

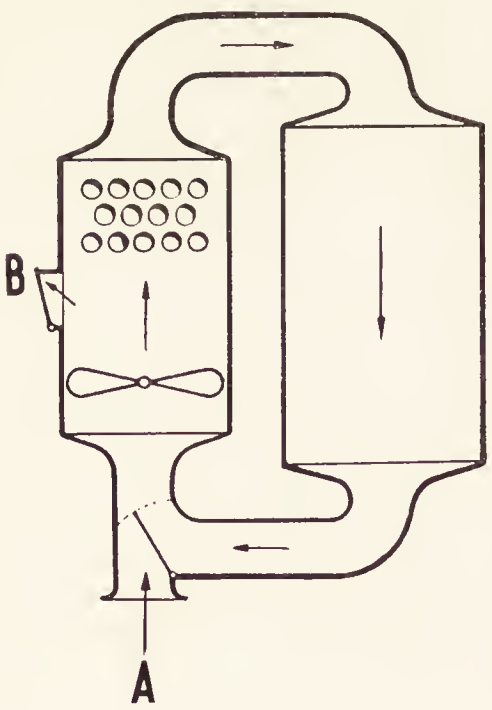


FIG. 4
Drying chamber

A drying chamber in which pre-heating is accomplished by means of a blower in front of the heating coils is shown diagrammatically in Fig. 3, and a partially regenerating system —i. e., one making partial use of the increased temperature of the outgoing moisture-laden air —is shown in Fig. 4. In the latter system, sufficient fresh dry air is admitted at *A*, sufficient moist warm air is passed out through *B*, to eliminate the evaporated water.

[1] The danger of minute *iron* particles, or other metal, being carried off by the gelatin powder must be considered for photographic gelatins.

Systems providing for complete regeneration and internal circulation, in which the moisture-laden air is dried by refrigeration and then reheated, have been devised.

The drying is carried out in channelled galleries or kilns provided with rails for the trucks. The alleys are from 70 to 100 feet long, and six and one-half feet square in section. They have heating coils at one end, and, at the other, either blowers or exhaust fans, so that a continuous current of air is drawn through the gallery. The trucks conveying the nets are passed through the gallery, usually in the direction opposite that of the current.

The temperature during drying usually ranges from 85 to 105° F. Higher temperatures liquefy the gelatin, causing it to run through the nets. Insufficient circulation of air favors molds and bacterial growth (since the slower the current, the more easily dust and spores settle), and is apt to cause adhesion to the nets; too strong a current tends to dry the gelatin too rapidly, hardening and warping the sheets.

THEORY OF DRYING

Drying consists in the transfer of water from the material to air or vacuum. Hence it depends severally and totally upon:

(i) The moisture-holding capacity of air (properly of space at certain temperatures);

(ii) The physico-chemical properties of the material;

(iii) The mechanical conditions of transference.

We may consider (i) and (ii), representing (iii) only by a constant velocity. The removal of moisture by evaporation into air depends upon the capacity of air to hold moisture as vapor in increasing quantities as its temperature is raised. The moisture in any substance to be dried may be regarded as of two kinds—free moisture and hygroscopic moisture—the latter being designated variously as "water of imbibition" or "adsorbed water," water of condition, etc. Since it is not desired to carry the drying of gelatin (in manufacture) to absolute dryness, it is dried only to a point at which the hygroscopic moisture is in equilibrium with air of a certain normal temperature and humidity. For most material it is convenient to term the difference between this "equilibrium moisture" and the total moisture content of the material its free moisture.[1] Whether this is theoretically valid for gelatin

[1] Lewis, W. K., J. Ind. Eng. Chem. **13**: 427. 1921.

is uncertain, but practically it is permissible. The equilibrium moisture curves of a material are conveniently plotted as equilibrium moisture against relative humidity of air, giving a series of isotherms. (See Fig. 5.)

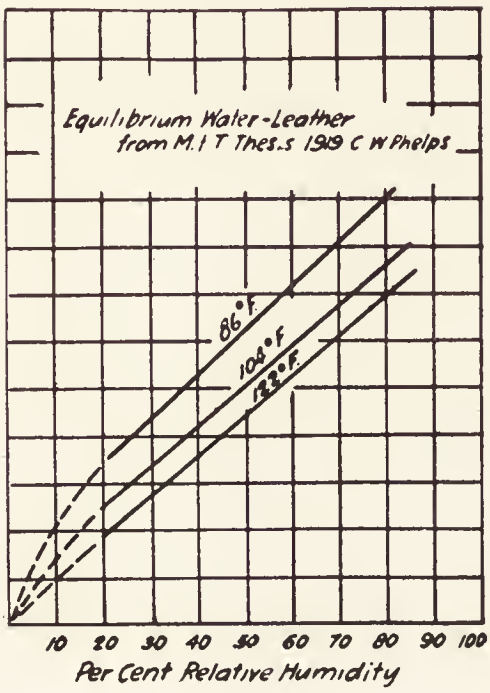


FIG. 5
Equilibrium-moisture curves[1]

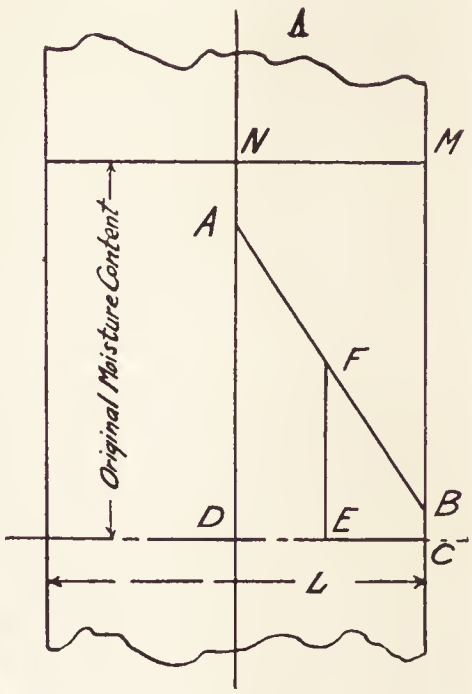


FIG. 6
Cross section of sheet of material being dried

THE RATE OF DRYING. The most satisfactory method of considering the rate of drying is due to Lewis, who regards this rate as a function both of the rate of evaporation of free moisture from the surface, and of the diffusion of the interior water to the evaporating surface. As his treatment is illuminating in respect of gelatin and photographic materials, it is freely reproduced here. In Fig. 6 suppose a cross-section of a sheet of material be represented, the surface by CM, and the middle line by DN. From DC as base, let concentrations of moisture as parts by weight per unit volume be plotted as ordinates. Let CM equal the initial concentration, so that the area under NM represents the initial moisture content of the sheet or film. When surface evaporation starts the moisture of the surface drops to a point B. Diffusion starts simultaneously, and at a given time the moisture content will have decreased to the amount represented by the line AB. In general this line will not be straight, as shown in the

[1] Data on gelatin, now being obtained, will be given in Vol. II.

figure, its equation being determined by the definite integral of the Fick diffusion equation

$$\frac{\partial^2 y}{L} = \frac{-\partial y}{\partial t}$$

However, Lewis makes the simplifying assumption that the line is straight, to secure conditions for approximate integration. Let $EF = y$ be the average moisture in the sheet, y_s the surface concentration of moisture, and w the total weight of moisture in the sheet for each unit of surface. Then $w = \frac{L}{2}y$. The rate of diffusion of moisture from interior to surface will be proportional to the concentration difference $(y - y_s)$, and inversely as the distance $\frac{L}{4}$, the proportionality constant being H. The rate of surface evaporation will be equal to some coefficient R times the surface concentration. These two must equal each other and be equal to the rate of loss of moisture by the sheet; hence

$$\frac{d\,w}{d\,x} = R\,y_s = \frac{4\,H\,(y - y_s)}{L},$$

whence

$$\frac{d\,w}{d\,t} = \frac{8\,H\,R\,w}{L\,(4\,H + R\,L)}.$$

Since water content appears as $\frac{dw}{w}$ the expression is independent of the units in which the water is measured, and we can write $\frac{dW}{W}$ for $\frac{dw}{w}$, w being the free water content of any desired amount of the material.

If the equilibrium moisture E is not negligible, the equation takes the form

$$-\frac{d\,(W - E)}{d\,t} \cdot \frac{1}{(W - E)} = \frac{8\,H\,R}{L\,(4\,H + R\,L)}.$$

This equation may be regarded, for a first approximation, as the basic differential equation governing drying of materials in sheet form. Integration will depend, *inter alia*, upon whether the material shrinks much in drying. With gelatin this is the case.

Special Cases. The simplest case is where shrinkage is negligible, when

$$\frac{8\,H\,R}{L\,(4H + R\,L)} = \text{constant } K,$$

which may be called the drying coefficient, and on integration

$$\log\ (W - E) = -R\,T + \text{constant}$$

or

$$\frac{l}{t}\log\frac{W_0 - E}{W - E} = \text{K}.$$

Again, K in general varies with the rate of diffusion and of surface evaporation, and with thickness. If diffusion is rapid compared with surface evaporation, RL may be neglected compared with $4H$, and $K = 2\,R/L$. If diffusion is very slow compared with surface evaporation, $4H$ is negligible compared with RL, and $K = 8H/L^2$.

For sheet material with rapid diffusion ($K = 2\,R/L$), shrinkage is usually great. If the thickness varies linearly with the moisture content—a case realized for heel board and also for gelatin drying down on rigid supports—then $L = L_0\,(1 + a\,W)$, where L_0 = thickness of dry sheet, and a = constant; hence $K = 2\,\text{R}/(1 + a\,W)\,L_0$, and integration of the drying equation gives

$$a\,W + 2.3\log\,(W - E) = K\,t + \text{constant}.$$

Skin Effect. In drying thick layers of material, where internal diffusion is slow—which applies to jellies of high concentration—the diffusion gradient does not extend rapidly to the center of the mass. Hence, the basic differential equation must be modified. In Fig. 7 let GD be a cross-section of the layer. Let water concentrations be plotted up from CD, the initial concentration $GE = y_0$, and the surface concentration $y_s = B$. Then y_s will be substantially in equilibrium with the drying air. Let AB be the diffusion gradient, the point A regressing as drying proceeds. Let the thickness actually effective in diffusion, $AE = \text{I}$. This is the variable in the equation

$$-\frac{dw}{dt} = \frac{H\,(y_0 - y_s)}{\text{I}}.$$

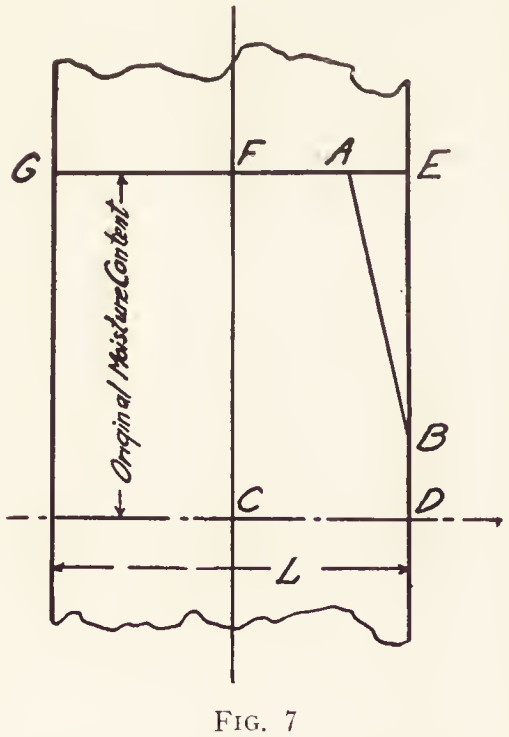


Fig. 7
Cross section of sheet of material being dried to illustrate skin effect

This layer AE determines the "skin effect" of the drying operation. By comparing areas in the diagram (Fig. 7) it will be seen that

$$I = \frac{L\,(y_o - y)}{(y_o - y_s)}$$

Also, $$w_o - w = \frac{L}{2}\,(y_o - y), \quad \text{and}$$

$$w_o - E = \frac{L}{2}\,(y_o - y_s),$$

so that $$-(w_o - w)\,dw = \frac{2H}{L^2}\,(w_o - E)\,dt\ ;$$

or, $$\frac{(W_o - W)^2}{(W_o - E)^2} = \frac{4Ht}{L^2}.$$

It is pointed out that the conclusion from this—that the drying time is proportional to the square of the moisture loss from the beginning of drying (over initial free water), and to the square of the sheet thickness—is obtained by simplifying assumptions. Hence, it is probable that more generally a power function, the power approaching 2, represents the conditions. Further, when the point A has receded to the center of the sheet, F, the character of the drying curve, will change and pass into the case originally considered.

The Rate of Drying in Relation to the Concentration of Gelatin. In drying gelatin an apparent paradox exists in that the more concentrated a solution, the longer it takes to dry; and conversely, the weaker a solution, the quicker the process.[1] The explanation of this is as follows: a slice of four per cent gelatin weighing 75 grams will weigh, when dried, about three grams—that is, 72 grams of water must be removed in drying. The first fifty grams of water are readily taken up by the air current; but after this the surface of the gelatin sheet hardens to an extent which makes it much more difficult to remove the remaining twenty-two parts.

Since the more concentrated the solution, the sooner surface hardening or impermeability will occur, the final stages of evaporation in the concentrated sol are much slower. Practically, the dilution at which drying can be effected is limited by the difficulty of spreading and netting the weaker jellies, and by the available space.

[1] Consider in relation to Lewis' theory of "skin effect." The existence of actual impermeability is open to question.

Temperature and Humidity of the Air.—The constant R, or coefficient of surface evaporation of free water, is determined by the rate of transfer of water vapor through the stationary film of air surrounding the surface. The case is analogous to the rate of solution of a solid in a liquid, the solid being bounded by a stationary layer of saturated solution. The rate of diffusion of dissolved solid out of this, is proportional to the differences in concentration in this layer and in the outer solution. The diffusion of water vapor through the stationary layer is proportional to the difference between the partial pressure of water at the surface of the sheet, and that in the drying air. This difference, which may be termed $p - p' = \triangle p$, will be proportional to the *wet bulb depression*, and can be read from psychrometric tables.[1] At a constant velocity of air transfer, $R = \frac{K L}{2} = \frac{b \triangle p}{2}$, where b is a proportionality constant (depending on the velocity), and K is the slope of the logarithmic functions already obtained. As long as the free moisture content of a material is reasonably high in the absence of direct exposure to a heating element, the material remains at a definite minimum temperature of the drying air. This, which Carrier terms the minimum temperature of evaporation, corresponds to the vapor pressure p' and is known as the *wet bulb temperature* in psychrometry. If the free moisture becomes very low, the temperature of the material tends to approach the dry bulb temperature of the air; but under usual conditions the material remains at the wet bulb temperature.

The proportionality constant b depends upon the velocity of the drying air current. We may write

$$\frac{dw}{dt} = (a + b\,v)\ (p' - p),$$

where a = rate of evaporation in still air,
b = proportionality to velocity, and
v = velocity (cu. ft. per minute).

Carrier finds that the effect of velocity depends upon whether the air current is parallel to the surface or perpendicular to it. For parallel flow, the heat transfer is given by

$$H = 97\ (1 + \frac{v}{230})\ (p' - p),\ (\text{in } b.\,t.\,u.)$$

and the water evaporated by

$$W = 0.073\ (1 + \frac{v}{230})\ (p' - p)\ (\text{lbs. per sq. ft. per hour}).$$

[1] The relation of the effective vapor pressure difference to wet bulb depression is discussed quite fully by W. H. Carrier. (J. Ind. Eng. Chem. **13**: 433. 1921.) Carrier shows that the rate of evaporation is substantially proportional to the wet bulb depression $()-()'$, as well as to $p'-p$, the difference of vapor pressure.

With perpendicular incidence of air current, the rate is nearly twice as great for the same velocity; but with the same frictional losses, the rate is practically the same for both types of current. The effect of velocity may be regarded as reducing the thickness of the stationary layer of saturated air at the surface.[1]

The capacity of air for the absorption of water is directly proportional to its wet bulb depression. Fig. 8 (from Carrier's article), shows the capacity of the air in grams moisture per pound of air.

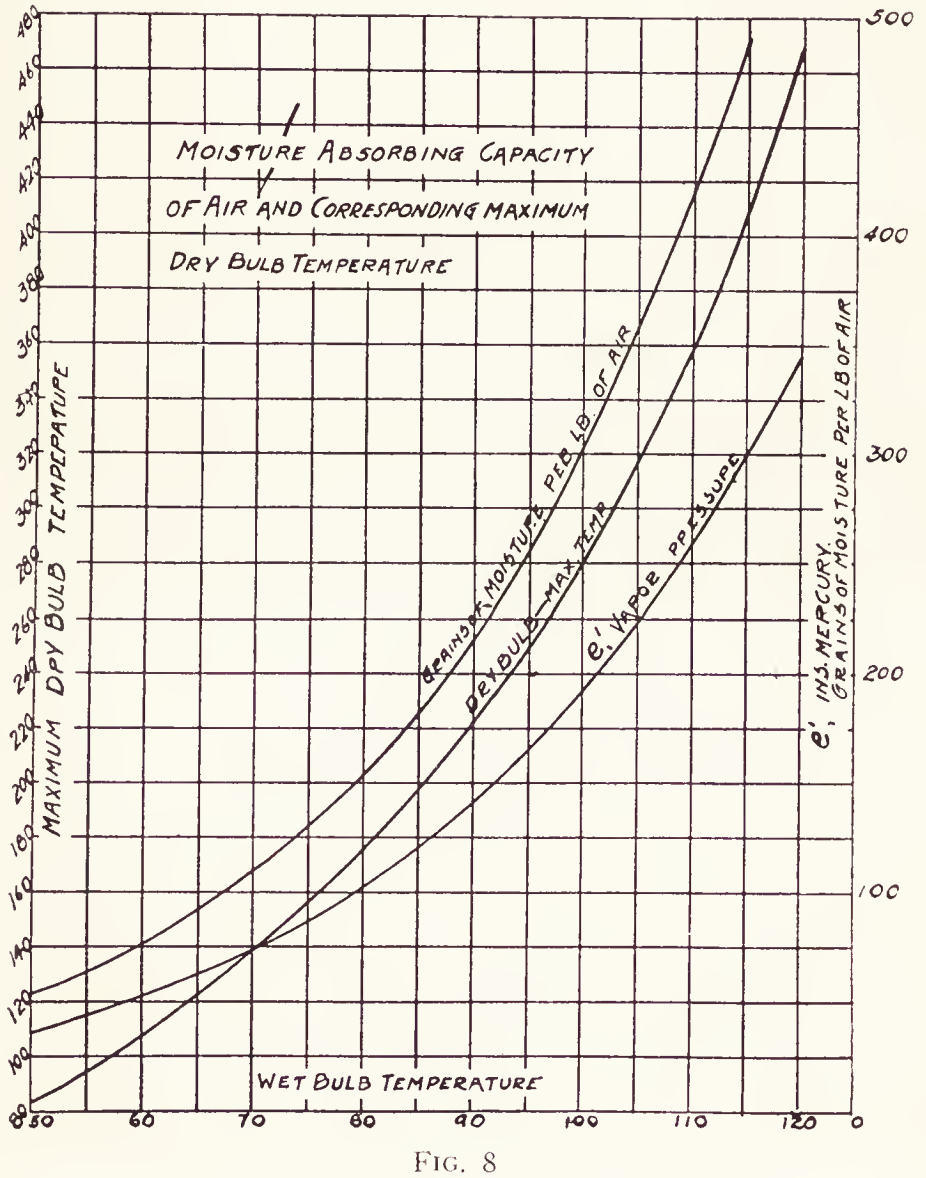


FIG. 8
Curves showing moisture absorbing capacity of air

[1] In tunnel driers the velocity of the air decreases from the hot to the cold, and as the volume of air is reduced by cooling.

PSYCHROMETRIC CHARTS

The psychrometric data necessary for calculation of evaporative and drying conditions will be found discussed in full in various publications cited.[1] The results have been conveniently collected in a chart by Carrier.[2]

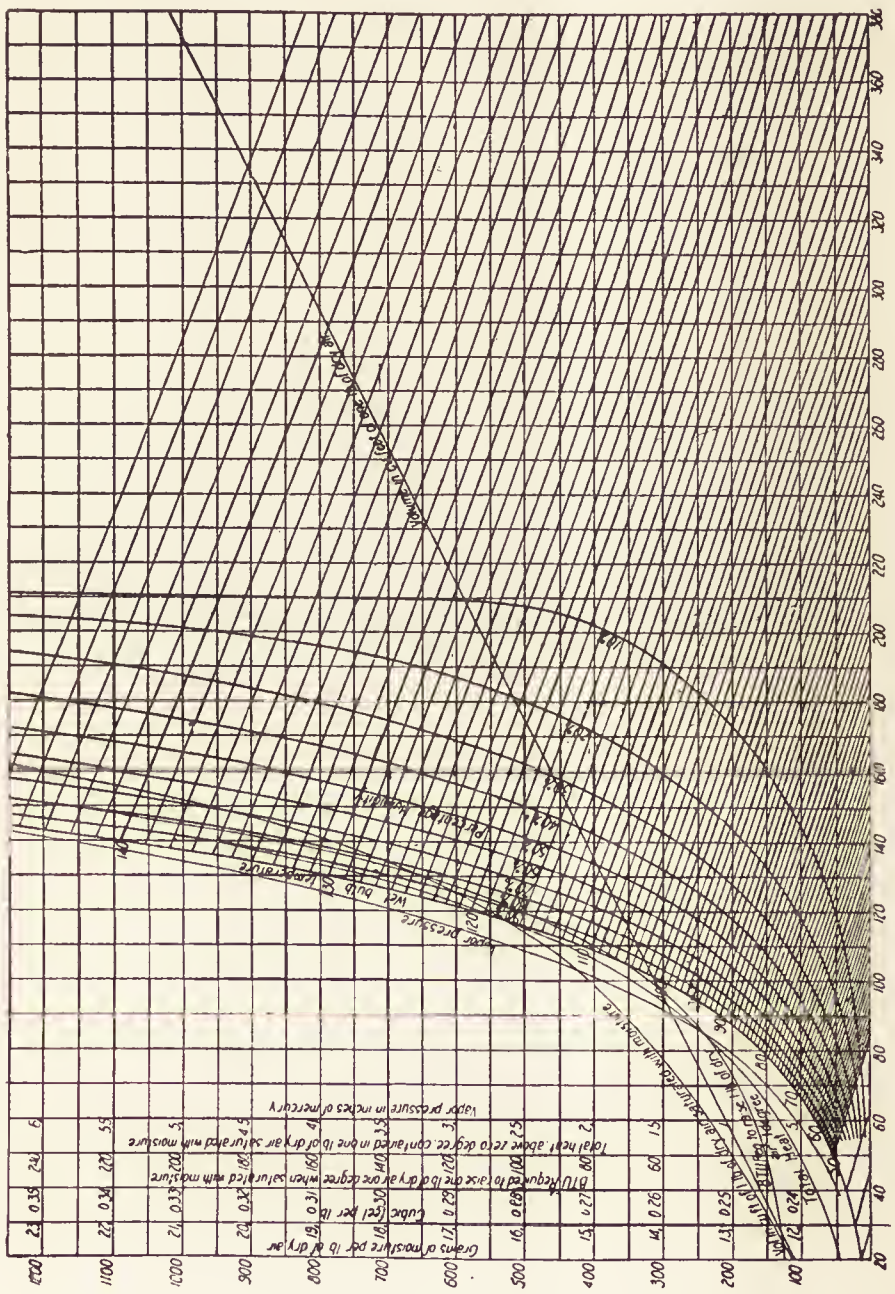


FIG. 9—Psychrometric Chart

[1] Cf. Grosvenor, W. H., Amer. Inst. Chem. Eng. **1:** 184. 1908; Marvin, C. F. Government Print, W. B. No. 235, 1900; Marr, O., Das Trocknen und die Trockner, p. 462.

[2] Carrier, W. H., l. c.

The principal curves are (Fig. 9):

(a) Saturation curve, lbs. weight of water per lb. dry air at saturation. (barometer B = 29.92 in.)

(b) Corresponding vapor pressure curve.. The slanting lines represent definite wet bulb temperatures with corresponding dry bulb temperatures, and weight of water vapor per pound of air. In using the chart, it should be remembered that the temperature drop and the corresponding increase in weight of water vapor always occur along a constant wet bulb line; and that, in heating air, the weight of water vapor remains constant.

Zone Driers. The greatest efficiency in drying is secured when the process is divided into a series of consecutive steps, the air being taken alternately through heaters and drying chambers. In this way it is possible to dry the material gradually and to regulate the temperature so as to remove the gelatin at practically the same temperature at which it entered the drying chambers. Systems of this type are termed zone driers. These, although more costly to install, offer several advantages in operation. It has been calculated that such a zone system for the drying of gelatin, in addition to giving a considerably superior type of product, requires 25 to 30 per cent less heat expenditure per unit weight of water evaporated than the direct type.[1] Where (for sensitive materials) the least possible effect of temperature change is required, vacuum driers, made continuous for quantity production, are essential.

Concurrent versus Countercurrent Drying. The counter current method of drying—i. e., one in which the driest material meets the hottest driest air—is not by any means the most effective, as the material is very liable to be partially dessicated and spoiled. On the other hand, the small amount of moisture which necessarily remains after concurrent drying can be removed, if undesirable, by a separate operation after cooling the material to normal temperature.

Since in the drying gallery as usually constructed for countercurrent drying the temperature of the entering air must not be higher than that permissible for the material, and on exit will be much lower, the system suffers a corresponding disadvantage compared with the concurrent system, for which the temperature permissible may be maintained throughout the operation. The countercurrent system has no advantage in respect of quality of product nor of economy of heat, but it is relatively easily constructed.

[1] Marr, O., l. c.

In a recent German process (system Otto Rupp, Munich), claim is made for a new continuous drying process, delivering the dried material either as flakes or powder. The solution, either dilute or concentrated, is beaten to a foam with air in an emulsor from which it is fed, as a stable foam, into a drying drum, heated with low pressure steam (0.1 to 0.6 atmosphere). At three revolutions per minute the material is dried in 20 seconds. The "crêpe" thus formed is stripped by a doctor and carried over to a disintegrator on a continuous belt.[1]

It has already been noted that the concentration at setting prior to drying affects the subsequent water absorption. This is probably due, as pointed out recently by the writer and F. A. Elliott[2], to "case-hardening" which takes place on drying gelatin, whereby the original surface extension is conserved. This factor is naturally most evident in the case of sheet drying.

PREPARING FOR PACKING[3]

Leaf gelatin is usually put up in one-pound packages, consisting of some 100-110 sheets of about 8½ by 3½ inches. When the gelatin is to be packed in flake or powder form, the dried sheets are thrown into a crusher running at some 250 revolutions per minute and requiring about two horsepower. The crushed gelatin, so-called "flakes," may then be passed into storage bins or towers, and fed thence to mills of the disintegrating type, to be screened and packed as required. If still finer gelatin is desired, it is ground in another pulverizing mill, where the "granulated" gelatin is reduced to powder of fifty mesh and upwards, as needed.

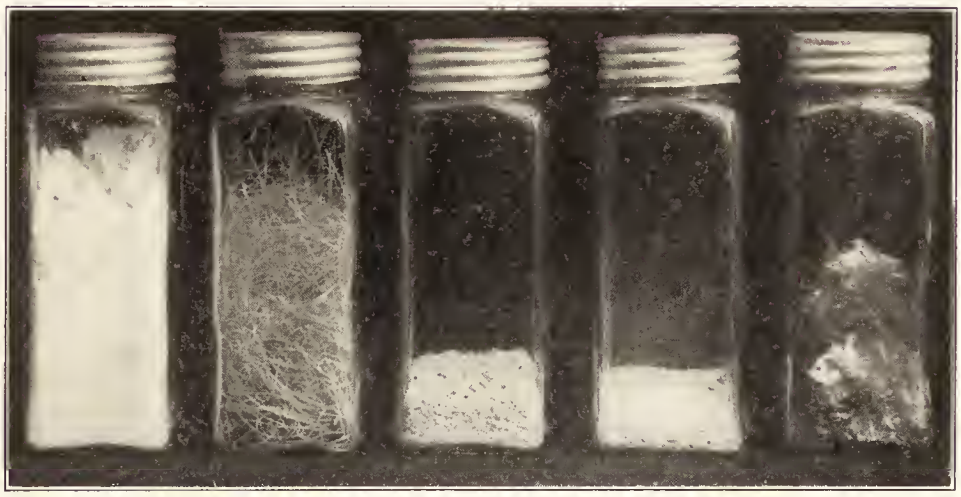
Another method of granulating gelatin has been patented.[4] In this method a warm solution of the gelatin is sprayed into liquids which precipitate it, the solidified particles being removed and washed with a non-aqueous solvent. Spray drying in air is also used, producing rapidly soluble gelatins. (See Fig. 10). The mechanical disintegration of the product obviously facilitates blending, an important manufacturing point.

[1] See Rupp, O., Chem. Ztg. **45**: 771. 1921.

[2] Sheppard, S. E., and Elliott, F. A., J. Amer. Chem. Soc. **44**: 373. 1922.

[3] Cf. Thiele, L. A., J. Ind. Eng. Chem. **4**: 446. 1912.

[4] British Patent No. 15,365, 1913, from Aktien für ehemisehen Produkte.



A B C D E

FIG. 10

Relative bulks for equal weight of gelatins variously dried and prepared.

A—Spray dried
B—Sheet dried and shredded
C—Drum dried
D—Sheet dried, disintegrated, and powdered
E—Sheet dried, disintegrated to flakes

OTHER METHODS USED IN MANUFACTURING GELATIN

Caustic alkalies are used instead of lime in the process employed by Nelson[1] in England. The parings, etc., of skin are scored on the surface and digested in dilute soda lye for ten days. They are then digested at 70° F. in an air-tight cemented vat, washed with cold water in revolving cylinders, and bleached with sulphurous acid gas. After the surplus liquid is removed by pressure, the stock is extracted with water in earthenware vessels enclosed in steam chests and the resultant solution is strained off at 100-120° F. It is then solidified in thin layers on marble slabs, redissolved at the lowest possible temperature, and dried on nets.

In Cox's process (Edinburgh), the pieces of hide (shoulders and cheeks preferred) are washed, cut up, and pulped by special machinery. The pulp is pressed between rollers, mixed with water, and extracted at 150-212° F. Cow's blood in small amount is added to the gelatin solution at a temperature not exceeding 170° F., the solution heated further, and the scum formed by coagulation skimmed off. The purified solution is run off and concentrated, preferably in vacuo.

[1] From Thorpe, E., Dictionary of Applied Chemistry. Article on Gelatin.

THE MANUFACTURE OF GELATIN FROM BONE

The processes differ from the production of hide gelatin because of the high mineral content, and the large proportion of fatty substances.[1]

There are two principal methods:

A. Preparation of bone glue and gelatin by boiling crushed bones. (Boiling process);

B. Preparation of *ossein* stock by demineralizing bones with acid. (Ossein process).

The boiling process is said to be more suitable for the preparation of bone glue than for gelatin preparation. In the *ossein* process, the crushed bone is first degreased, generally by extraction with fat solvents, such as carbon bisulphide, benzole, naphtha, etc. Of these, a solvent naphtha, from petroleum, boiling near 100° C. is the most satisfactory. After degreasing, the bone is leached with acid, usually hydrochloric, to remove the mineral substances—chiefly calcium phosphate and carbonate. Hydrochloric acid is generally used, in a countercurrent system, at about 6 per cent concentration (3 to 4° Bé.) as stronger acid hydrolyzes the gelatin. The acid liquor, saturated with the calcium salts is discharged, for precipitation and recovery of the phosphate, while the demineralized bone or *ossein* is ready for washing and extraction. The acid leaching treatment may last from five to ten days, depending on the condition, age, and size of the bones. In a recent investigation by Manning and Schryver[2] only the middle portions of the femurs of young oxen were used. After cleaning and degreasing they were crushed to various sizes, the largest being between $\frac{1}{4}$ and $\frac{3}{16}$ inch mesh, the smallest passing $\frac{1}{32}$ inch. For demineralization 3 per cent hydrochloric acid was used. The process was followed by titration and required from three to seven days, depending upon the size of bone fragments.

The crude ossein stock after the acid extraction, is washed in several changes of water, with the object of removing salts and acid. The removal of acid is, however, usually facilitated by neutralization, either with soda, or with lime, so that liming treatment may be superposed.

[1] The proximate composition of bone is:

Protein (ossein, etc.)	21.5 per cent
Fat	12.5 per cent
Calcium phosphate	48.0 per cent
Calcium carbonate	3.0 per cent
Silica, salts, etc	2.5 per cent
Water	12.5 per cent

[2] Manning, A. B., and Schryver, S. B., Biochem, J., **15**: 523. 1921.

Prior to extraction, a bleaching treatment with sulphurous acid may be used in place of hydrochloric to effect demineralization. The "ossein" stock, washed and neutral, is then extracted with hot water to give gelatin, the "ossein" being equivalent to the "collagen" of hides. Manning and Schryver in the paper referred to above, followed the rate of extraction of gelatin from decalcified bones of various graded sizes at both 100° C. and 90° C. The curves in Fig. 5 show their results. They consider with Hofmeister, that ossein (or collagen) is an anhydride of gelatin (see p. 57). They were able to confirm his statement that gelatin heated to 130° C. loses solubility in water, this being restored only after boiling several hours. They find that the regeneration of the anhydrized gelatin is similar in course to the formation of gelatin from ossein, which consists essentially in the hydrolysis of the anhydride and the solution of the gelatin. The speed of the process depends, however, on other factors also. A preliminary calculation from the mesh size of the bone particles, gave the relative surface as proportional to $\sqrt[3]{n}$, where n = the number of particles per gram, and as a general formula for the rate of extraction:

$$\frac{dx}{dt} = k \left\{ S^2 + f(x) \right\} (a - x)$$

where x = quantity of gelatin extracted,

a = total quantity

k = constant

kS_o^2 = initial rate (S_o being initial surface), while $f(x)$ represents the change in the surface effect as the reaction proceeds. The simplest assumption for this is that

$f(x) = x$, which gives:

$$\frac{dx}{dt} = k \, (S_o^2 + x) \, (a - x).$$

On integration:

$$\frac{1}{a + S_o^2} \log \frac{a(S_o^2 + x)}{S_o^2 \, (a - x)} = RT.$$

This does, in fact, give a group of curves of the type found experimentally; when the initial surface is small, the curve has a point of inflection, which, however, disappears beyond a critical value, when $S_o^2 = a$, and for larger values of S, i. e., very small sizes of particles, the equation approaches the monomolecular reaction formula. The average for k for different sized particles was:

Size	S_o	$k_{100°C.}$	$R_{90°C.}$
1/4 inch	3.75	.00478	.00238
3/16 inch	5.71	.00436	.00206
1/8 inch	9.65	.00463	.00189
		.00444	.00211

Temperature coefficient for 90°-100° = 2.1.

On testing the actual initial surface by iodine and dye adsorption, it was found to be independent of the size of the ossein pieces. Hence, owing to high porosity of the bone, the available surface is independent of subdivision. The time to reach adsorption equilibrium depends on the size, however, consequent on the difference in diffusion path. In the extraction of gelatin from ossein (demineralized bone) the variation of rate with size of pieces is not a true surface effect, but the rate of extraction is controlled by the rate at which gelatin diffuses out and water diffuses in.

REFERENCES AND PATENTS ON THE MANUFACTURE OF GELATIN

The list given below is not intended to be comprehensive, but merely to illustrate certain aspects of the process.

A. *Liming.*
See Chapter II, part 2. Schaeffer, J. E., U. S. Patent 1,377,367 and with W. Crow, U. S. Patent 1,377,401.

B. *Washing Machines.*
Cf. Thiele, L., Die Fabrikation von Leim und Gelatine, p. 86. Second edition, M. Jänecke, Leipzig, 1922.

C. *Deliming.*
See Chapter II, part 2.

D. *Cooking.*
Cormack, U.S. Patent 728,205 (1903).
Thiele, L., U.S. Patent 989,826 (1911).
Lehmann, J., U. S. Patent 964,980 (1910); French Patent 420,726 (1909).
Upton, G., U. S. Patent 1,063,229 (1913).
Löw, A., and Fisher, E., U.S. Patent 1,086,149 (1913).

E. *Clarifying.*
Cf. Chapter II, part 2.
Hank, E., German Patent 234,859 (1910).
Kiefer, K., British Patent 1821.

F. *Concentration.*
Moore, H. K., Chem. Met. Eng. **18**: 128,186. 1913.
Reavell, J. A., J. Soc. Chem. Ind. **37**: 172T. 1918.
Dunglinson, D., Chem. Met. Eng. **25**: 110. 1921.

G. *Bleaching.*
Hulbert, H., British Patent 18,042 (1902).
Devos, H. D., French Patent 446,549 (1912).
Mumford, U. S. Patent 1,289,053 (1919).

H. *Chilling.*
See Thiele, L., l. c.
Dietz, J., U.S. Patent 103,852 (1870).
Upton, K., U. S. Patent 338,374 (1886)
Cooper Hewitt P., U. S. Patent 521,371 (1894).
Schill and Seilacher, German Patent 119,477 (1899.)
Kind, M., and Landesmann, U. S. Patent 1,046,307 (1912).

I. *Drying and Packing.*
See Thiele, L. l. c.
Campbell, C. H., U.S. Patent 1,147,-165 (1912).
Scheidenmandel, H., British Patent 13,365 (1915) and 100,392 (1916).
Fleming, R. S., J. Ind. Eng. Chem. **13**: 447. 1921.
Merrill, I. S. and O. E., U. S. Patent 1,102,601 (1914)
Grosvenor, W. M., U. S. Patent 1,138,751 (1915).

Part II

The Technochemistry of Gelatin Manufacture

The importance, from the viewpoint of photographic chemistry and technology, of a somewhat complete and detailed account of both the technochemistry of gelatin manufacture and the chemistry of gelatin and its congeners, the proteins, is reflected in the following statement of Mr. B. V. Storr, of the Ilford Company, England, in his second annual report of the progress of photographic manufacture.[1] Speaking of methods of determining physical properties, he says: "Apart, however, from the fact that certain minima of strength, setting-power, viscosity, etc., are necessary, and that a stronger gelatin is easier and more economical to work, it is the writer's experience that the physical properties are a very insufficient guide to the suitability of gelatin for making photographic emulsions. There are certain chemical differences between different types of gelatin and even between different batches of the same type, which are more effective than are the physical properties in determining speed, freedom from fog, and such qualities in different types of emulsion. As to the exact nature of these chemical differences there are few available data, but it is very possible, if not probable, that they are due to the presence or absence of very small quantities of specific substances, rather than to variations in the proportions of the main constituents of the gelatin. A process such as washing the gelatin, for instance, will improve it for some purposes and spoil it for others." This statement is in substantial agreement with facts familiar to emulsion-makers, and affords justification of what might otherwise be regarded as too detailed a description of the chemistry of gelatin and of the materials from which it is derived or to which it is related.

There is probably much room for improvement in respect of both research and chemical control work in glue and gelatin manufacture. As pointed out by Powell,[2] only in the last decade has the recent awakening to the possibilities of better manufacture and control of all food products made a more exacting chemical control of food gelatin a commercial necessity. Once established, this control becomes a real aid to the manufacturer, but it is still in a large measure lacking in the manufacture of technical gelatins and glues. Such control, as stated by Powell, falls naturally into three divisions:

[1] Storr, B. V. Annual Report of the Society of Chemical Industry on the Progress of Applied Chemistry **37**: 465. 1918.

[2] Powell, J. R. J. Ind. Eng. Chem. **8**: 932. 1916. See also Sauer, E. Koll. Zeits. **17**: 130. 1915; Chem. Abst. **10**: 1445. 1916.

1. Investigation and inspection of raw materials and chemicals;

2. Control of the manufacturing process;

3. Inspection of the finished product.

In the following we shall not attempt to give particulars of a definite scheme of control, which would in any case vary with the magnitude of production and the special ends in view. It appears more useful to group together material bearing both on research into and control of the phases of production as already outlined.

RAW MATERIAL[1]

Under "raw material" three topics come up for consideration, viz.: (i) Classes and condition of stock; (ii) The anatomical and histological structure of the hide; and (iii) The condition and chemical composition of skin.

(i) CLASSES AND CONDITION OF STOCK. Information on the glue-yielding value of various sources of skin glue and gelatin is given by Rideal.[2] The differences between stock in various conditions are noted and given in condensed form, as follows:

Material	Yield of Glue	Quality of Glue	Quality of Gelatin
Calf skin	—	Superior	Good
Sheep skin	—	Superior	—
Horse hide	—	Dark and poor	—
Tannery waste (foot, head and buttock)	44–46%	Good	Depends upon animal and condition
Scarf skin and fleshings from bullocks' hides and tendons	30–35%	Good	—
Bullocks' feet	62%	Good	—
Parchment shavings	—	—	—
Dehaired rabbits' skins	—	Fair	Poor
Ear laps of sheep and cows	38–43%	Fair	—
Sheeps' feet and tendons	—	—	—
Skin waste	—	Poor	—
Hogs' skins	—	—	—

Rideal states that good stock should yield fifty per cent glue and that poor may run as low as twenty per cent.

Stock before drying is termed green and is stored in winter in wooden or cement-lined vats filled with half-saturated lime water, covered from the air.

[1] See particularly Yocum, J. H. J. Amer. Leather Chem. Assoc. **7**: 135. 1912.
[2] Rideal, S., Glue and glue testing.

The following table indicates the general condition and methods of treatment of raw material:

Material	Condition	Treatment	Remarks
Raw hide South American hides	Dry, uncured, or salted	Soaked in cold water 12 hours	Should gain 50% in weight, remain firm and water sweet. Moisture, dirt and salt not over 10%.
Hide pieces, calves' heads and pates	Green salted		There should be no excess of salt, no foulness or discoloration. Should be firm, hair loose. Moisture and salt not over 40%.
	Dry limed	Soaked in water 12 hours	Should be firm and fibrous, not slimy. Water not dark. Lime and dirt not over 5%.
	Green limed		Should be smooth and soft, hair, if any, easily rubbed off. Liquor sweet and clean.

As has been stated (Part I, this chapter), the age of the animals giving the stock is of importance, as the product from younger animals is generally lighter in color and more abundant; but it contains more chondrin, and therefore the product from older animals usually has greater jelly value.

Kopecky[1] points out that the quantity of ammonia salts in well-preserved hides depends chiefly upon the sex of the animal, being greater for those taken from male animals. Also, the feeding of the animals reacts on the skin. Andreis[2] points out that the skins of milk-fed and of grass-fed calves act very differently in liming, the former requiring much less time than the latter.

(ii) Anatomical and Histological Structure of the Hide. The animal skin is made up of several different structures which were formerly divided into three principal layers:[3]

(a) *Scarf skin* or epidermis, O, a thin horny layer consisting chiefly of *keratin*. It is removed by scraping before tanning, and is not considered of importance for glue and gelatin production.

(b) The actual *leather hide*, L, composed chiefly of connective tissue fiber bundles, the primary material for both the tanner

[1] Kopecky, F. K. J. Amer. Leather Chem. Assoc. **2**: 307. 1907.

[2] Andreis, E. J. Amer. Leather Chem. Assoc. **7**: 609. 1912.

[3] Cf. Davidowsky, F., Die Leim-und Gelatine-Fabrikation, p. 35.

and the glue manufacturer. According to Rideal, this true skin or corium consists of some 95 per cent of interlacing white fibres embedded in a structureless matrix of coriin. In liming, the coriin is dissolved and the fibres loosened. It is important to see that liming is not too prolonged, as loss results.

(c) The *under-skin*, U, consisting of cellular tissue frequently contaminated with fat and flesh particles, objectionable in glue making. These elements are (somewhat diagrammatically) illustrated in Fig. 11.

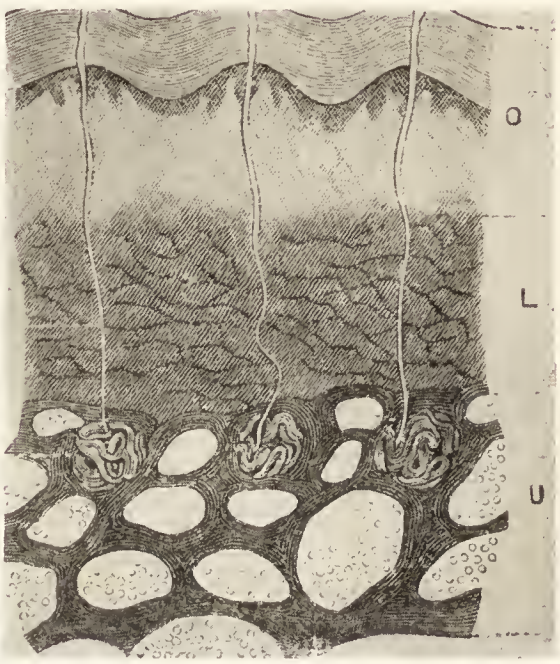


FIG. 11
Section of epidermis

Among other valuable papers which should be consulted for complete and minute descriptions of the anatomy and histology of hides are those by Seymour-Jones,[1] Proctor,[2] and Rosenthal.[3] The latter paper includes a detailed and illustrated description of the degradation taking place in the process of liming, which should, perhaps, be outlined here.[4]

The samples examined were fresh calf skin, taken from a five-weeks-old calf one hour after death. The skin was divided into shoulder, belly and butt, in order to compare

[1] Seymour-Jones, A. J. Amer. Leather Chem. Assoc. **11**: 41. 1916.

[2] Procter, H. R., The principles of leather manufacture.

[3] Rosenthal, G. J. J. Amer. Leather Chem. Assoc. **11**: 463. 1916.

[4] Terrasse, G. L. J. Amer. Leather Chem. Assoc. **15**: 608. 1920, and Seymour-Jones, F. L. J. Ind. Eng. Chem. **14**: 130. 1922.

the composition from different parts of the animal. The pieces were soaked in water for forty-two hours, then placed in lime water, using five per cent slaked lime, calculated on the weight of the skins, with two and one-half per cent more lime added after twenty-four hours. The pieces were unhaired on the fifth day, washed and bated with oropon.

The samples were taken from:

(1) Fresh skin (Fig. 12);
(2) Skin after 42 hours' soaking (Fig. 13);
(3) Skin after 24 hours' liming (Fig. 14);
(4) Skin after 66 hours' liming (Fig. 15);
(5) Skin after 120 hours' liming, unhairing and washing (Fig. 16);
(6) Skin after bating (Fig. 17).

In view of their particular interest, the figures (see at end of chapter, pp. 97-102) giving the microscopic appearance of the sections are reproduced, by courtesy of the Journal of the American Leather Chemists' Association.

The changes taking place may be summarized as follows:

(1) In soaking, the corneous layer begins to scale off and the lower layers of the epithelium and the connective tissue are swollen;

(2) In liming, the corneous layer disappears; the lower layers of the epithelium show indistinct cell boundaries and transverse shrinkage spaces. They finally dissociate from the papillary layer. The connective tissue is less fibrillated and presents large shrinkage spaces;

(3) After bating the connective tissue alone is left, the shrinkage spaces are not so large as those seen after liming, and the tissue shows a faint fibrillar structure, but stains poorly.

(iii) Condition and Chemical Composition of Skin. In regard to the condition of hides in the preliminary stages of treatment, reference may be made to a paper by Parker[1] which deals particularly with bacteriological control.[2] In addition to this Parker points out the possibility of estimating the loss of material (hide substance) in soaking by a method which is an application of the salting out of proteins such as gelatin, and which will be discussed later. The procedure is to shake up 100 cc. of the liquor with ten to twenty grams of salt, then acidify with acetic acid. This salt-acid combination will precipitate dissolved albuminoids.

[1] Parker, J. G. J. Amer. Leather Chem. Assoc. **2**: 361. 1907.
[2] Compare also Wood, J. T. J. Soc. Chem. Ind. **25**: 109. 1906.

The effect of salt (used as a preservative) on the solubility of hides in soak is discussed by Balderston.[1]

As to chemical composition,[2] the skin is composed primarily of albuminoids. The epidermis consists chiefly of keratin, the true skin or corium largely of collagen, the glue yielding tissue.

According to Uuna,[3] three different substances are found in horny materials—A-, B-, and C-keratin. A-keratin, forming the envelope of the horn, hair-cells and hair, is not dissolved by fuming nitric acid and does not give the xantho-proteic test.[4] Both B-keratin, which occurs in nail cells, and C-keratin give the xantho-proteic test, but only B-keratin is soluble in fuming nitric acid.

Rosenthal points out that it is difficult to isolate keratin without some decomposition. This, together with the probable existence of several different keratins, helps to explain the wide variations in the recorded values of the elementary composition of keratin. Analyses of certain keratins and tissues rich in keratins give:

Material	C	H	M	S	O	Observer
Human hair.....	43.72	6.34	15.06	4.95	29.93	Rutherford[5]
Nail.....	51.00	6.94	17.51	2.80	21.75	Mulder[6]
Neurokeratin.....	56.61	7.45	14.17	2.27	.....	Argiris[7]
Horn.....	54.89	6.56	16.77	2.22	19.56	Mulder[6]

The quantity of sulphur in various keratinous substances has been determined by Mohr.[8] This sulphur is partly in loose chemical combination, and is readily removed as sulphide by alkalies, or even partly by boiling water. The sulphides produced in this way play an important part in the liming process, which will be discussed shortly.

Keratin is insoluble in water, alcohol, ether and dilute acids. Digestive enzymes (pepsin, pancreatin, trypsin) do not dissolve it directly, but render it soluble after considerable

[1] Balderston, L. J. Amer. Leather Chem. Assoc. **12**: 193. 1917.

[2] Cf. Seymour-Jones, A., l. c., and Rosenthal, G. J., l. c.

[3] Uuna, in Monatsheft f. prak. Dermatologie. Cited by Rosenthal.

[4] For protein reactions see Chapter III.
Rutherford, T. A., and Hawk, P. B., J. Biol. Chem: **3**: 459. 1907.

[6] Cited by Rosenthal, l. c., p. 464.

[7] Argiris, A., Zeits. physiol. Chem. **54**: 86. 1907-08.

[8] Mohr, P., Zeits. physiol. Chem. **20**: 403. 1895.

time, and in bringing it into solution decompose it. The question of the solubility of keratin is an extremely important one in the gelatin, glue and leather industries.

Keratin is removed during the liming process and its chief interest in gelatin and glue-making consists in a possible residuum, either of unchanged keratin or of products of its decomposition.

Of more direct importance are the proteins of the corium or connective tissue. There are several kinds of connective tissue fibres, of which the yellow and the white forms are readily distinguishable. The yellow fibres, which are tough and elastic, consist largely of the protein elastin, and rival keratin in stability and insolubility.

Elastin, which is a distinct albuminoid, is not affected by a cold five per cent solution of caustic potash, and but little by a hot one per cent caustic solution. The digestive enzymes, trypsin and pepsin-hydrochloric acid, slowly decompose it, forming albumoses.

Elastin may be prepared relatively pure from the ligmentum nucheae.[1] The tissue is extracted under thymol for three or four days with five per cent sodium chloride solution to remove albumins and globulins, then repeatedly treated with boiling water to gelatinize and remove the collagen. The residue is thoroughly washed with water, extracted with alcohol and ether, and dried. Alternate acid and alkali extraction has also been used to remove associated substances.

Insoluble in water, alcohol, and ether, elastin is dissolved slowly by boiling alkali and by cold concentrated sulphuric acid, but easily by warm concentrated nitric acid. Its behavior toward hydrochloric acid depends upon the source from which it is derived.

Elastin gives the xantho-proteic and the Millon reactions, but not the Hopkins-Cole test.[2]

Richards and Gies[3] have shown that sulphur is present in small amounts, their analyses being:

C	N	H	S
54.14	16.87	7.33	.14

The presence of sulphur apparently depends to a large extent upon whether or not the elastin is obtained by alkaline treatment. That is, the sulphur is loosely combined. Richards

[1] Rosenthal, l. c., p. 465. (Tendon at back of the (ox-) head.)

[2] See Chapter III, p. 104.

[3] Richards, A. N., and Gies, W. J. Amer. J. Physiol. **7**: 93, 1903.

and Gies used half-saturated lime water in their determination. Chittenden and Hart[1] used no alkali nor caustic soda, and obtained values as follows:

C	N	H	S
54.08	16.85	7.2	0.3

Emil Fischer's pupils obtained the following amino-acids by hydrolysis of elastin:

	Per cent
Glycocoll	25.75
Alanine	6.6
Amino-valerianic acid	1.0
Proline	1.7
Leucine	21.4
Phenylalanine	3.9
Glutaminic acid	0.8
Asparaginic acid	trace
Tyrosine	0.34
Arginine	0.3

Mann notes that elastin gives no indol derivatives upon digestion, and that, of all albuminous substances, it is the poorest in tyrosine and arginine.

Two products, termed by Horbaczewski[2] hemi-elastin and elastin-peptone, by Chittenden and Hart proto-elastose and deutero-elastose, are obtained by heating elastin with water in sealed tubes, by boiling it with dilute acids, or by the hydrolytic action of enzymes.

Further details of the chemistry of elastin may be found in the articles cited; or, for collected data, see Abderhalden's Biochemisches Handlexikon, Volume IV, p. 185.

Beside the *collagen* of the white fibres (which, as the chief glue- and gelatin-yielding substance, is noted more specifically later), the corium contains quantities of soluble albumins in the blood serum or lymph. The principal albumin present is *serum-albumin*, which is still suspected of being a mixture of closely related proteins.[3]

On hydrolysis it gives a number of amino-acids, of which leucine is the principal constituent. It is usually closely associated with a carbohydrate.[4]

Serum-globulin, which is distinguished by solubility in dilute salt solutions, and which has been fractionated, is also present in the serum.[5] *Mucins* and *mucoids*, between which

[1] Chittenden, R. H., and Hart, H. S. Studies from the Laboratory of Physiological Chemistry, Yale University. **3**: 19. 1887-88.

[2] Horbaczewski, J. Zeits. physiol. Chem. **6**: 330. 1882.

[3] Cf. Abderhalden's Biochemisches Handlexikon. Vol. IV, p. 62, and Vol. IX, p. 15.

[4] For details of the chemistry of this typical albumin, reference may be made to Abderhalden, l. c., and Mann, l. c.

[5] See Abderhalden, l. c., and Hardy, W. B., and Gardiner, S. J. Physiol. **40**: 68. 1910.

the relation is uncertain (see Chapter III, pp. 139, 140), also occur in the skin and connective tissues. They are glucoproteins, yielding reducing carbohydrates on hydrolysis with acids. Their elementary composition is given by different authorities as follows:

	C	H	N	S	O
Tendon-mucoid	48.76	6.53	11.75	2.33	30.63
Tendon-mucoid	47.47	6.68	12.58	2.20	31.07
Osseo-mucoid	47.07	6.69	11.98	2.41	31.85

The mucins, like the nucleins, are acid substances, soluble in dilute alkali and precipitated by dilute acids, being redissolved by stronger acids. They may be prepared by extracting the tissues with dilute alkali, then acidifying with acetic acid.

The remaining substances of interest in the chemistry of skin are the fats or lipoids, secreted in the loose fibres of the corium. Seymour-Jones[1] found that the amounts of these substances present depend directly upon the food of the animal, increasing greatly with oil-cake feeding. He also noticed the interesting fact that the fatty substance has considerable solvent power on the cementing substance of the connective tissue fibres, if not on the fibres themselves.[2] These fats are chiefly composed of the alcohols cholesterol and iso-cholesterol. They may be removed from the skin either by a suitable solvent, such as benzene or ligroin, or by hydraulic pressure.

Although collagen and its immediate derivative gelatin are necessarily treated more fully in a later chapter, it is desirable to summarize the facts known as to the former, the chief constituent of the fibrils of white connective tissue. There are possibly several varieties of collagen, since the composition varies somewhat. Hofmeister[3] found the elementary composition to be:

C	H	N	S	O
50.75	6.47	17.86	—	24.92

By hydrolysis with boiling water, especially if assisted by acid in small quantity, it is converted into gelatin. On heating gelatin to 130° C., Hofmeister obtained as an anhydride of

[1] Seymour-Jones, l. c., p. 60.

[2] This interfibrillar cementing substance is apparently a mucin, or mixture, with some nucleo-protein.

[3] Hofmeister, F. Zeits. physiol. Chem. 2: 299. 1878.

gelatin a product insoluble in water, which he considered to be collagen, according to the equation:

$$\text{Collagen} + H_2O \rightleftharpoons \text{Gelatin}.$$[1]

CHEMICAL ASSAY OF SKIN

The following method for the chemical assay of skin is due to Rosenthal,[2] who employed it to determine whether there are material differences in the composition of skin from different parts of the animal, and also to follow the changes occurring in soaking, liming, and deliming hides.

The pieces of skin, immediately after removal from the animal, are placed in a vacuum oven and dried at 55-60° C. under a vacuum of twenty-nine inches. After eight hours the skin is sufficiently dry to be cut up and ground in a mincing machine. Samples of the ground material (weighing three and one-half or four grams each) are then placed in stoppered bottles containing 150 cc. of ten per cent sodium chloride solution under toluol. This is then digested at 37° C. until no coagulable protein is obtained, the solutions being renewed daily. Normally, two changes (450 cc. of solvent in all) are sufficient. The combined solvents are then heated with a few drops of acetic acid, and the combined coagulum of albumin and globulins washed with warm water, alcohol, and ether, and weighed.

The residual skin is washed with warm water and extracted under toluol with 150 cc. of half-saturated lime water. Three changes (a total volume of 600 cc.), are usually sufficient. The combined extracts are made neutral to phenolphthalein by adding ten per cent hydrochloric acid, and then acidified with 0.2 per cent hydrochloric acid. The prepared mucoid is washed with 0.2 per cent hydrochloric acid, water, alcohol and ether, dried and weighed. All filtrates should be tested with additional acid for unprecipitated mucoid.

The washed skin residue is then digested at 37° C. with alkaline trypsin under toluol. After twenty-four hours the solution is decanted and the digestion continued, with fresh pancreatin, for ninety-six hours.

The amount of elastin present is computed by multiplying the total nitrogen in the combined filtrates, determined by the Kjeldahl method, by a factor of six. (A correction should be made for nitrogen of the enzyme.)

[1] Cf. also Manning and Schryver, l. c.

[2] Rosenthal, G. C., l. c., p. 469.

Finally, the washed skin residue is digested in a pepsin—0.2 per cent hydrochloric acid solution, as in the method given for elastin, and the amount of nitrogen here is obtained by multiplying by the factor 5.58. From the analysis of collagen, N = 17.90 per cent.

Using this method, the composition of calf skin from the shoulder, belly, and butts was followed through various stages, as indicated in the tables given below. The percentages of coagulable protein, elastin, mucoid, collagen, and keratin are given on a dry basis.

Per cent of	Belly	Butt	Shoulder
No. 1. Fresh skin—			
Coagulable protein	4.30	4.14	5.16
Elastin	19.43	12.31	16.74
Mucoid	1.24	4.81	2.29
Collagen	51.46	58.83	39.66
Keratin	25.73	19.91	36.15
No. 2. After soaking 42 hours in water—			
Coagulable protein	4.67	7.18	5.37
Elastin	19.72	11.75	15.04
Mucoid	1.07	4.56	2.11
Collagen	50.75	53.00	46.88
Keratin	23.79	17.31	30.60
No. 3. After 24 hours in lime water—			
Coagulable protein	3.81	4.64	11.87
Elastin	19.08	13.06	14.65
Mucoid	1.07	3.65	1.76
Collagen	56.47	62.88	43.61
Keratin	19.57	15.77	28.11
No. 4. After 66 hours in lime water—			
Coagulable protein	7.45	5.96	9.25
Elastin	10.34	9.15	14.60
Mucoid	1.10	2.29	1.58
Collagen	63.01	66.78	54.43
Keratin	18.10	15.82	20.14
No. 5. Unhaired after 120 hours in lime water—			
Coagulable protein	9.88	8.55	10.00
Elastin	8.92	10.36	5.55
Mucoid	0.93	2.42	0.85
Collagen	78.90	76.93	82.08
Keratin	1.37	1.74	1.54
No. 6. After bating with "oropon" (trypsin)—			
Coagulable protein	14.81	10.02	5.66
Elastin	0.85	0.31	0.00
Mucoid	0.94	0.82	0.34
Collagen	82.85	87.71	93.81
Keratin	0.75	1.14	0.19

These results show that skin from different parts of the body varies in composition, that during soaking and liming there is loss of cellular material (shown by the increase of coagulable and meta-protein), and that elastin is removed during bating.[1]

From additional studies of the ash content and of the water absorption, Rosenthal concludes that water is absorbed in soaking and in the early stages of liming, but that the period of maximum swelling is followed by a gradual loss of water; that the ash content decreases during soaking and increases during liming, the maximum of total ash being about seven per cent.

METHODS FOR DETERMINING NITROGEN AND ARSENIC IN HIDES

NITROGEN. If putrefaction or hydrolytic decomposition has taken place in the skins to be examined, the degraded nitrogenous bodies should be washed out with cold water before the total nitrogen content is determined. The nitrogen content of calf skin after a treatment which leaves only the corium is given as 17.8 per cent.[2]

The percentage of peptonizing nitrogen may be determined approximately by extracting the finely pulped skin with cold distilled water and saturating the solution with magnesium sulphate. The precipitate of gelatin and albumoses is filtered off and the filtrate treated with bromine to precipitate the peptones, which are filtered off and their nitrogen content determined; or the filtrate from the magnesium sulphate precipitation may be concentrated and its nitrogen content determined directly.

ARSENIC.[3] If it is necessary to test for arsenic, a weighed portion of the skin is shredded, covered with pure magnesia, dried, and ignited in a muffle furnace. The ash is taken up with dilute sulphuric acid, digested for some time, and filtered. About one gram of potassium metabisulphite is added to the filtrate and the mixture boiled till all the sulphur dioxide is expelled. This reduces arsenates, which are not so readily acted on by nascent hydrogen, to arsenites. The liquid is made up to a given volume, an aliquot part placed in the inner cell of an electrolytic Marsh's apparatus, and the mirror produced compared with a standard.[4] The modified Gutzeit method may also be used.

[1] Also shown microscopically by J. A. Wilson: see J. T. Wood, Scientific aspects of tanning.

[2] Von Schroeder and Paessler, cited by Trotman, S. R., Leather Trades Chemistry, p. 222.

[3] Trotman, S. R., Leather Trades Chemistry, l. c.

[4] Trotman, S. R. J. Soc. Chem. Ind. **23**: 177. 1904.

ANALYSIS OF WATER USED IN GELATIN MANUFACTURE

The water used in gelatin manufacture should be free from organic matter, which indicates and favors the presence of putrefactive organisms; iron, which is undesirable, particularly for photographic gelatin; and chlorides, which in considerable quantity are also detrimental.

The analysis of water should include determination of:

1. Total dissolved solids;
2. Suspended matter;
3. Chlorides;
4. Nitrates and nitrites;
5. Iron and alumina;
6. Temporary and permanent hardness;
7. Sulphates;
8. Organic matter and organic ammonia.

There are other values, such as other mineral constituents, which may become important, but those listed are the most essential. Where the water is obtained from some natural source, subject to seasonal or other variation, it is very desirable that the routine analyses should be filed graphically as functions of time. Graphic records of this type become increasingly valuable. Descriptions of the methods used in the above determinations may be found in various text books,[1] as are also methods of purification—e. g., softening, iron removal, filtration of suspended matter and filtration (by sand filters) for removal or at least reduction of bacteria.

LIMING AND THE LIME BATH

In the outline of the chemistry of skin the extraction of various substances from the hide has been mentioned. It is evident that control of this extraction, much of which occurs in the lime bath, is a matter of importance not only in leather manufacture but also in the glue and gelatin industries.

The lime liquors are generally prepared in old lime pits, and a fresh lot of skins are usually started in the oldest liquor (which contains bacteria and enzymes), and worked back to fresh lime solutions.

The chemistry of the action of the lime-bath has been much debated, particularly by leather chemists. In the process of leather manufacture the whole epidermis from the surface to the hyaline layer, being practically of no value, is removed. This is accomplished usually by soaking the skins in milk of lime, occasionally by the use of caustic alkalies or

[1] E.g., Trotman, S. R., Leather Trades Chemistry, pp. 18 et seq.

alkaline sulphides. Only the dissolved lime has any action on the skin, and saturated lime-water at ordinary temperatures is less than N/20. Stiasny[1] divides the various unhairing solutions into three classes:

(a) Straight alkaline solutions: potassium hydroxide, sodium hydroxide, calcium hydroxide, barium hydroxide, ammonium hydroxide;

(b) Strengthened solutions, such as lime fortified by additional alkali; or lime with sulphides, such as sodium sulphide, calcium sulphide, arsenous sulphide;

(c) Old lime solutions.

He considers that, with the straight alkaline solutions, the greater the hydroxyl-ion concentration, the greater the swelling action, the more thorough the unhairing, and the greater the loss of hide substance. In the series potassium hydroxide, sodium hydroxide, barium hydroxide, calcium hydroxide, ammonium hydroxide, decreasing plumping and increasing loosening of hair is shown progressing from left to right, and there is a selective adsorption of the hydroxyl-ion over the metal cation. Generally, with different solutions, absorption by the skin is greatest for calcium hydroxide and barium hydroxide, least for ammonium hydroxide. The loss of hide substance depends not only on the concentration but on the kind of solution. Thus the loss is less with lime and baryta than with lyes and ammonia; and there is greater loss of hide substance with old lime solutions containing ammonia. It has been found, however, that ammonia dissolves but little gelatin (collagen).

Generally, salts increase the action of unhairing solutions, though ammonium salts, in particular, have a greater effect in fortifying lime solutions because of the specific effect of the ammonia. However, such solutions do not behave like free ammonia solutions, so that they offer an effective method of controlling the action.[2] Stiasny also points out that the amount of ammonia in old solutions of lime does not affect the hair-loosening properties.

A considerable difference of opinion has existed as to the solubility of hair and epidermis in lime liquors. Among industrial chemists, solubility is generally accepted.[3] On the other hand, biochemists[4] find that keratin is not soluble in less than twenty per cent caustic alkali in the cold. It has

[1] Stiasny, E. J. Amer. Leather Chem. Assoc. **2**: 55. 1907.

[2] Cf. Andreis, E., The process of liming, l. c.

[3] Cf. Procter, H. R., Principles of leather chemistry, l. c.

[4] Cf. Mann, G., Chemistry of the proteids, p. 567.

been suggested by Seymour-Jones[1] that reconciliation of these opposing results lies in the existence of bacteria and digestive ferments in the unhairing baths. His analysis of the action is briefly as follows: While digestive ferments have great difficulty in rendering adult (dying) keratin soluble, young keratin is readily dissolved by pepsin. The Malpighian layer is built up of young keratin cells and it was observed on many occasions that the lime solutions appeared to attack the lower layer (rete malpighi) first, thus loosening the upper layer in large sheets.

On the other hand, Griffiths contends that unhairing is a purely chemical process, not involving bacterial agency. This view, however, is opposed to the balance of the evidence.

Wood and Law[2] conclude that unhairing in lime water is effected partly by ammonia from bacterial action in old limes, partly by chemical action of the lime, partly by the proteolytic enzymes of bacteria, and partly by sulphur compounds formed by the action of lime on easily decomposed sulphur-containing bodies of the hair and skin. In this connection Seymour-Jones points out that the action of alkaline sulphides on keratin "appears to have been completely overlooked by pure scientists whereas from the experience of applied scientists it may be definitely stated. Alkaline sulphides seem to attack the harder tissues with at least the same facility as they do the soft ones, the hair being often completely disintegrated, while the epidermis is still almost intact; hence their applicability to unhairing by destruction of hair." The action is supposed to be due to the sulphohydrate, according to the reaction

$$Na_2S + H_2O = NaOH + NaSH.$$

The corrosive action is said to be exerted to some extent on not only the keratin, but on the collagen as well. It appears probable, then, that the action of the liming-bath is to a certain extent auto-catalytically influenced by the young keratin dissolving first by enzyme attack and thus supplying sulphohydrates which attack the older keratin. At the same time the swelling of the tissues, due to the influence of the hydroxyl-ions, plays a large part; and there is also a specific effect of ammonia. A detailed investigation, both histological and chemical, using old limes, fresh limes, and lime-baths inoculated with enzymes, carried out along the lines of Rosenthal's research, would contribute much to a solution of this problem.

[1] Seymour-Jones, A., l. c., p. 48.

[2] Wood, J. T., and Law, D. J. J. Amer. Leather Chem. Assoc. **7**: 346. 1912; ibid. **9**: 318. 1914. See also Schlichte, A. A., J. Amer. Leather Chem. Assoc. **10**: 526 and 585. 1915; cf. editorial comment, ibid. **10**: 612. 1915.

The direct use of enzymes in unhairing hides and skins in place of liming has recently been suggested[1]; it is claimed to be quicker and cleaner.

CHEMICAL CONTROL OF THE LIME BATH AND THE LIMING PROCESS

For discussions of this question the reader is referred to articles by Kopecky,[2] Wood and Law,[3] and Bennett.[4] For a critical review and bibliography a paper by Burton[5] should be consulted. Very recently Atkin and Palmer[6] have published an important paper, in which certain errors in the methods previously proposed are corrected, and the interrelationship between the varying determinations is emphasized. It should be noted here that so far as liming hides for gelatin is concerned, strengthening or sharpening with sodium sulphide need not be considered. Hence the main alkalinity is due to lime, ammonia, and amines. Some sulphide, due to the action of alkali on the proteins of hair and adhering blood and parts of the skin, will be present, as well as nitrogenous bodies from the same source.

Atkin and Palmer propose to restrict the analysis for works control to:

1. Total nitrogen;
2. Ammonia and amines;
3. Sulphides;
4. Total alkalinity;
5. Caustic alkalinity.

To determine these some six estimations are necessary, and from these there may be calculated:

6. Amino-acids;
7. Acid break-down derivatives of proteins combined with calcium or sodium hydroxides, referred to as calcium or sodium salts of hydrolysates.

The liquor should be filtered or centrifuged before the estimations (Wood and Law give details showing the importance of this), and care should be taken in accomplishing this. Absorbent cotton removes lime particles insufficiently, ordinary centrifuging only particles larger than 1μ in diameter.[7] Filtration through analytical paper removes some colloidal nitrogenous matter, and is recommended by Atkin and Palmer, who used Green's folded No. 605.

[1] Hollander, C. S., J. Amer. Leather Chem. Assoc. **15**: 477. 1920.
[2] Kopecky, F. K., Chemical control of soaking hides and skins, l. c.
[3] Wood, J. T. and Law, D. C., l. c.
[4] Bennett, H. G. J. Amer. Leather Chem. Assoc. **11**: 98. 1916.
[5] Burton, D. J. Amer. Leather Chem. Assoc. **15**: 308. 1920.
[6] Atkin, W. R., and Palmer, W. E. J. Amer. Leather Chem. Assoc. **15**: 620. 1920.
[7] High speed centrifuges of the Sharples type might be more satisfactory.

1. Total Nitrogen may be determined by a slight modification of the Kjeldahl method, as described by Procter. 25 cc. of filtered liquor are heated in the digestion flask with 15 cc. concentrated sulphuric acid, and some potsherd or pumice to reduce bumping. The flask is carefully heated to evaporate water, and 5 grams of potassium sulphate are added after carbonization. Then the mixture is heated vigorously. Heating should be continued for about one hour after the liquid becomes colorless or light yellow. On cooling, the liquid is diluted, 60 cc. of 40 per cent caustic soda added, and the ammonia distilled off into excess of standard acid. The excess of acid is titrated back, using carminic acid as indicator.

2. Estimation of Ammonia and Amines. Volatiles are determined by distillation, according to Thompson and Suzuki,[1] except that instead of neutralizing the lime with acid and adding excess of magnesia, a ten per cent solution of magnesium sulphate is added without neutralization.[2] Some foam inhibitor, such as turpentine or diphenyl ether is added, and distillation carried out at 40° C. for 35 minutes. The absorbing sulphuric acid is rinsed into a large evaporating basin and boiled a few minutes to drive off any hydrogen sulphide. Carminic acid may then be used as indicator for the back titration of the acid.

3. Sulphides. (See note, p. 68). This estimation is carried out by titrating 25 cc. of the lime liquor with N/10 ammoniacal zinc sulphate solution made up according to Bennett's directions, using sodium nitroprusside as indicator. Since 1 cc. of the zinc sulphate solution (16.14 grams $ZnSO_4$ per liter H_2O) corresponds to .0028 grams of NaSH, and 1 cc. N/10 hydrochloric acid corresponds to .0056 grams NaSH, the number of cc. of N/10 zinc sulphite solution used must be divided by two to obtain equivalence to cc. N/10.

4. Alkalinity. The following substances may cause alkalinity in lime liquors:

(a) Calcium hydroxide;
(b) Sodium hydroxide;
(c) Calcium and sodium hydrosulphides;
(d) Ammonia and amines;
(e) Calcium and sodium salts of weak acids (protein hydrolysates).

(a) and (b) will be termed caustic alkalinity. It is not practicable to determine this by a single titration, owing to

[1] Thompson, F. C., and Suzuki, K. J. Soc. Leather Trades Chem. 1: 140. 1917.
[2] Procter, H. R., Leather Industries Laboratory Book, p. 63.

the presence of the weak bases. It may be found by difference, by estimating:

(i) *Total alkalinity*, by boiling 25 cc. of the filtered lime liquor with excess of N/10 sulphuric acid, and titrating back with N/10 caustic soda, using methyl red. This gives $a+b+c+d+e$;

(ii) $a+b+c$—*amino-acids:* 10 cc. of 40 per cent formaldehyde solution are diluted with 10 cc. distilled water, and neutralized with N/10 caustic soda to a faint pink with phenolphthalein. 25 cc. of clear lime liquor are added, and the solution titrated back with N/10 hydrochloric acid until the pink color disappears.

The reactions occurring are, according to Bennett:

(α) Calcium and sodium hydroxide are neutralized by acid and are not affected by the formaldehyde;

(β) Ammonia reacts with formaldehyde, forming neutral hexamethylene tetramine—

$$4\ NH_3 + 6\ H.CHO = (CH_2)_6N_4 + 6H_2O;$$

(γ) Formaldehyde reacts with hydrosulphides, releasing equivalent hydroxide—

$$NaSH + H.CHO + H_2O = H.CH\ (OH)\ (SH) + NaOH;$$

(δ) Formaldehyde reacts with amino-acids giving methylene amino-acids, which react acid with phenophthalein—

$$NH_2\ .R.COOH + H.CHO = CH_2{:}N.R.COOH + H_2O.$$

(iii) d + *amino-acids*, by another formaldehyde titration.[1] 25 cc. of filtered lime liquor are made neutral to phenolphthalein with glacial acetic acid until the pink color is discharged, and then N/10 iodine solution is added to definite excess. N/10 caustic soda is then added until the pink color reappears, when 10 cc. of neutral formaldehyde are added. The solution is then titrated with N/10 caustic soda until the pink color again appears.

Atkins and Palmer find that hydrochloric acid gives a sharper end-point than acetic.

This second formaldehyde titration measures the amino-acids plus ammonia and amines.[2] Therefore, the sum of the two formaldehyde titrations gives $a + b + c + d$, and by subtracting $c + d$ (that is, cc. N/10 acid required for ammonia, amines, and hydrosulphides), $a + b$, the caustic alkalinity of the lime liquor, is obtained.

More recently Atkin and Atkin[3] have described a method of direct estimation of the caustic alkalinity, involving two titrations:

(a) Titration with standard acid to a definite PH (=10.0), using a color comparator and thymolphthalein as indicator. This titration is a measure of the caustic alkalinity plus 24 per cent of the ammonia, but causes no decomposition of the sulphites;

(b) Determination of the ammonia by distillation. Then $a - 0.24b =$ caustic alkalinity.

[1] Earp, R. A., J. Amer. Leather Chem. Assoc. **3**: 63. 1908.

[2] Cf. Atkins and Palmer, l. c.

[3] Atkin, W. R., and Atkin J. J. Amer. Leather Chem. Assoc. **16**: 85. 1921.

Wood and Law's earlier procedure for total alkalinity is given in abbreviated form in the footnote.[1]

Finally, it may be noted that the amino-acids *e* (salts of the hydrolysates) are calculated by subtracting the sum of the formaldehyde titrations $(a+b+c+d)$ from the total alkalinity $(a+b+c+d+e)$, and that the degree of hydrolysis of the lime liquor can be calculated by a formula due to Stiasny

$$\alpha_m = \frac{\text{mgs.N by Kjeldahl}}{\text{cc. N/5 NaOH in second formaldehyde titration}}.$$

For unhydrolysed gelatin, the factor is 178, for ammonia and mono-amino acids 2.5, for arginine 11.2, for lysine 5.6; for completely hydrolysed gelatin (according to Van Slyke) 4.0. The percentage hydrolysis is therefore

$$100\,\frac{(178-\alpha_m)}{178-4} = 0.575\,(178-\alpha_m).$$

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[1] TOTAL ALKALINITY OF BATH ACCORDING TO WOOD AND LAW

Saturated lime water at 15° C. contains 1.211 grams of calcium oxide per liter, equivalent to 433 cc. N/10 acid. Limes in use contain also neutral salts of lime, lime combined with skin substances, sulphides from the hair, and ammonia, and are usually more alkaline than lime water.

Before titration it must be decided whether filtration or merely decantation is to be used. This has considerable influence on the alkalinity observed, as is shown in the following:

10 cc. weak old lime unfiltered = 4.5 cc. N/10 HCl;

10 cc. weak old lime filtered cotton wool = 4.2 cc. N/10 HCl;

10 cc. weak old lime filtered S. and S. 605 = 3.2 cc. N/10 HCl.

When filtered through S. and S. 605 very few of even the oldest limes gave more than 4.7 cc. N/10 hydrochloric acid for 10 cc. of the liquor. (A cap, devised by Trotman,[1] may be used to avoid loss of ammonia in filtering.) Stiasny proposed centrifuging, which, when tested, gave a result intermediate between that obtained by filtration through cotton wool and through filter paper. Cotton wool removes but few of the lime particles, and ordinary centrifuging for five minutes only particles larger than 1 μ in diameter. After centrifuging, the liquor still contained great numbers of particles measuring 0.5 μ and less. Particles of calcium carbonate, chiefly calcspar, are present, and it is mainly these which increase the alkalinity of the unfiltered liquor. Centrifuging is better than filtration unless an optically clear liquid is required, because filtration removes some colloidal skin substance. Methyl red is preferable to phenolphthalein as an indicator.

Hydroxyl-ion, or rather hydrogen-ion concentration (or effective alkalinity) (see volume II) may be obtained electrometrically. This was found to be much the same for old and new limes at $P_H = 12.5 - 12.3$, from which it appears that the substances formed in the action act as buffers. The addition of sodium sulphide had no effect on the hydrogen-ion concentration of lime liquor, even when present in sufficient quantity to double the titration figure.

The alkalinity due to caustic lime is obtained by subtraction of that due to ammonia and to sulphide, determined separately.

Bennett recommends alizarin as indicator in the determination of total alkalinity as it is sensitive to weak acids and to ammonia. Titration is made roughly to phenolphthalein, the alizarin being used last, after a preliminary value for phenolphthalein has been determined. Half the sodium as sulphide is determined at the same time.

Determination of Ammonia. Calcined magnesia is recommended for decomposing ammonium salts.[2] No appreciable hydrolysis of protein material takes place, so that unfiltered solutions can be used. 100 cc. of the liquor is placed in a distilling flask with a little methyl orange, and dilute hydrochloric acid added to acid reaction. Considerable excess of magnesia is then added, and the ammonia distilled off into a measured quantity of standard acid to be titrated back. (Bennett[3] adds magnesium sulphate to prevent

More recently Enna[1] has described a method of determining available sulphides in lime liquors. It depends upon the fact that carbonic acid, CO_2, passed through a solution quantitatively liberated hydrogen sulphide, H_2S, from its alkaline salts. The hydrogen sulphide is collected in copper acetate solution, the cuprous sulphide filtered off, and the residual copper determined iodimetrically. A blank titration of the copper acetate solution is likewise made, the difference being due to hydrogen sulphide only.

For the separate determination of soda, of total lime, and of caustic lime, the papers by Bennett and by Blockley and Mehd should be consulted.

DISSOLVED SKIN SUBSTANCE IN LIME LIQUORS

The way in which hide substance is lost in liming by solution in the lime is shown by the curve by Blockley.[2] (Fig. 18, page 72.)

The graph shows the amount of hide substance dissolved in liming, as obtained by the Kjeldahl method for nitrogen determination. He also shows the amount of hide substance dissolved in a lime liquor used in the "three pit" system—i. e., used (a) for four days as fresh lime for hides having passed through two others; (b) for three days as a medium lime for hides having been limed once; (c) for three days as a stale lime for a fresh lot of hides. This is an intermittent counter-

[1] Enna, F. G. A., J. Soc. Leather Trades' Chem. **5**: 131. 1921.

[2] Blockley, J. R., J. Amer. Leather Chem. Assoc. **8**: 383. 1913.

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decomposition, but in view of Kober's work[4] on ammonia distillation this can not be considered as desirable. Where greater accuracy in this respect is required it would be better to distill in vacuo at 40° C.) Frothing is minimized by adding a few drops of turpentine.[5]

Sulphides, small amounts of which will be found in all old lime liquors, even when none are added (due to the decomposition of sulphur-containing proteins), are conveniently estimated by titration with standard zinc sulphate solution.[6] 25 cc. of the filtered or centrifuged liquor is titrated with a solution containing 14.35 gms. of crystallized zinc sulphate per liter. Before making the solution up to one liter, ammonia is added drop by drop till the precipitate just redissolves. To this ammonium chloride should be added in the proportion of 50 gms. per liter. Drops of the solution are removed as the titration proceeds and placed in contact with equal-sized drops of lead acetate solution on filter paper. The zinc sulphate solution is run in until no further black or brown color is produced, corresponding to precipitation of all the sulphide.[7] As the zinc solution is N/10, each cc. corresponds to 1.6 milligrams sulphur, or 12 milligrams crystallized sodium sulphide.

[1] Trotman, S. R., Leather trades chemistry, p. 63.

[2] See Chapter III, p. 131; also Procter, H. R., l. c., p. 63.

[3] Bennett, H. G., J. Soc. Chem. Ind. **28**: 291. 1909.

[4] See Chapter III, p. 169.

[5] Caprylic alcohol may also be used alone or with phenyl ether.

[6] Procter, H. R., Leather industries laboratory book of analytical and experimental methods, p. 55.

[7] For discussion of the ionic mechanism of the reaction, see Blockley, J. R., and Mehd, P. V. J. Amer. Leather Chem. Assoc. **7**: 358. 1912; J. Soc. Chem. Ind. **31**: 369. 1912.

current leaching process, in so far as concerns extraction of soluble material.

From this it appears that, as the age of lime liquor increases, its power of dissolving hide substance increases; and the rate at which hide substance is lost is greater in an old lime than in a fresh one. By finding the lowest curve of loss at which unhairing and plumping (swelling) will take place, all other curves can be compared therewith, and thus the limes may be kept at the lowest limit of freshness possible.[1]

To determine the hide substance in the bath, Wood and Law estimate the total nitrogen in all the products of hydrolysis of the skin substance by the Kjeldahl method. The total nitrogen found in this way is evidently not necessarily derived from material useful either for leather or for glue and gelatin, but largely from epidermal (keratin) material and interfibrillar matter (mucins and mucoids).[2]

It may be emphasized here that little is definitely known as to how far the "stripping" in lime should be allowed to proceed to secure respectively: culinary gelatins, photographic gelatins, technical gelatins, and glues. Up to the present adjustment of this has been largely a matter of rule of thumb. Valuable or invaluable as this may be, it appears very probable that it should be supplemented by chemical research and control.

Other processes proposed for the control of dissolved hide are:

(a) Salting out with saturated salt solution made just acid, a method which has been noticed (p. 58) in connection with control of soaking. As pointed out by Wood and Law, this method is not very accurate, and should be used only as a comparative test. The amount and kind of acid added has a great effect on the quantity of substance precipitated. (See volume II, on the salting out of gelatin.)

(b) Bennett[3] has proposed titration using phenolphthalein as indicator followed by one with methyl orange, the difference between the two being a measure of the dissolved skin substance. The validity of this method has not been confirmed by other workers.[4]

[1] It is feasible to fortify with ammonium salts and use only fresh or else sterile limes.

[2] It is claimed that the precipitates obtained by neutralizing lime liquors with acids and by the so-called tanners' test have been shown to be due entirely to dissolved epidermal substances, and not at all to true hide substances. These precipitates are not given by solutions obtained by treating pure gelatin or hide powder with solutions of lime or of lime and sodium sulphide. Cf. F. C. Thompson and W. R. Atkin, J. Soc. Leather Trades Chem. 4: 15. 1919.

[3] Bennett, H. G., l. c.

[4] Cf. Wood. J. T., and Law, D. J., l. c.

(c) Stiasny's method, which utilizes the neutralization of amino-groups by formaldehyde, is, after removing any sulphides in the liquor, as described on p. 62.

DIFFERENTIATION OF DISSOLVED PROTEINS IN THE LIME LIQUOR

Trotman and Hackford acidified the hot filtered lime liquor and saturated it with zinc sulphate to precipitate the "albumoses,"[1] then precipitated the remaining "peptones" in the filtrate with tannin. (This will include any gelatin not thrown down by zinc sulphate.) After filtering these off, the nitrogen was determined in the dry precipitate by the Kjeldahl method, using the factor 5.42 to convert the percentage nitrogen to peptone.

Wood and Trotman precipitate unchanged gelatin by saturating the solution with zinc sulphate, and estimate the nitrogen in the precipitate.[2] Peptones are then precipitated in the filtrate by bromine water in excess, and the nitrogen estimated in this precipitate. Finally, the nitrogen in the filtrate from the bromine precipitate represents amino-acids, amines, and ammonia.

Procter[3] precipitates with tannin from faintly acid solution and determines nitrogen in the precipitate as well as in the original filtered liquor. The percentage ratio may be regarded as indicating what proportion of the dissolved nitrogenous matter could have been converted to gelatin or glue. This tannin-precipitable fraction is of the same order as the matter precipitable by alcohol from filtered and neutralized liquor, and such alcohol precipitation may be used to determine the proportion of glue-yielding material which is being dissolved out.

Stiasny's method consists in combining the aforementioned formaldehyde titration process with a determination of the total nitrogen by the Kjeldahl method. The factor expressing the degree of hydrolysis of dissolved protein—the amino index—is taken as N/C, where N = milligrams nitrogen per unit volume of liquor, and C = number of cc. of N/5 soda required to neutralize the same volume after addition of formaldehyde. This method is at present probably the most satisfactory for following the progress of hydrolysis.[4] To

[1] See Chapter III, p. 131. In this method albumins and globulins should be coagulated and filtered off first.

[2] Separation of "gelatin" from other proteins is not complete here.

[3] Proctor, H. R., Leather industries laboratory book, p. 89.

[4] This will be discussed more fully in Chapter III. See p. 171. For details, see Jessen-Hansen, H., Die Formoltitration, in Abderhalden's Handbuch der biochemischen Arbeitsmethoden, vol. VI, p. 263.

represent this as a percentage, Wood and Law propose to take the factor found by Stiasny for gelatin (178) as 0; i. e., to assume that there is no hydrolysis beyond that required to bring collagen (hide substance) into solution. If the factor for the final stage (ammonium salts 2.8) be taken as 100, any intermediate index between these can be recalculated as per cent hydrolysis. Thus, suppose the index $\frac{\text{mgs. N}}{\text{cc. N/5 soda}}$ for a given lime liquor be 12, the percentage hydrolysis in the liquor equals

$$100 - \left[(12-2.8) \times \frac{100}{178}\right] = 94.8\%.$$

Stiasny[1] has pointed out in connection with this method that it is important not only to remove sulphides, but also to convert free ammonia into sulphate.

For other approximate methods of determining the amount of dissolved hide substance in soak and lime liquors, reference should be made to the literature.[2]

A valuable report on the analysis of materials for the treatment of hides is given by Oberfell,[3] who considers that the following items demand attention for a complete scheme of analysis of lime liquors: Filtration, total alkalinity, total and caustic lime, ammonia, caustic alkali, sulphides, and dissolved hide substance. Most of the recommendations have been discussed, but a definite procedure is outlined which should be of use in framing a control method. Helfrich[4] has also published a valuable paper, in which typical schemes for arranging control data are given.

As desiderata in the liming process, Griffiths gives the following:

(1) The actual lime content (as calcium oxide) of the lime used should be not less than fifty per cent;
(2) The amount of lime, used as milk of lime and reckoned as calcium oxide, should be not less than five per cent, calculated on the weight of the hides;
(3) It is desirable in renewing the lime bath to use a mixture of old lime liquor with new milk of lime, or else to control the ammonium chloride;
(4) Eight to nine days' action is generally sufficient;
(5) Adsorption of lime takes place to the extent of from 0.5 to 1 per cent of the weight of the skin. This lime should be washed out until maximum swelling is effected;

[1] Stiasny, E., Collegium **3**: 398. 1908.
[2] Kopecky, F. K., l. c., Earp, R. A., l. c., also Blockley, J. R., and Mehd, P. V., l. c.
[3] Oberfell, C. R. J. Amer. Leather Chem. Assoc. **10**: 252. 1915.
[4] Helfrich, J. J. Amer. Leather Chem. Assoc. **10**: 396. 1915.

(6) Just as sufficient lime is necessary to secure proper unhairing, etc., so the volume and quality of water for swelling and washing out lime is important, since it determines the removal of hide substance in solution. The wash waters, both after soaking and after liming, may contain soluble hide substance and ammonium salts of organic and mineral acids. Well washed hide does not show an appreciable amount of water-soluble organic matter unless there has been bacterial action. Hence the amount of hide substance (or nitrogen) in wash waters is a useful control.

CHANGES IN PHYSICAL CONTENTS OF HIDES DURING LIMING, ETC.

The changes in the area, volume, weight and density of hides in the processes of soaking and liming, as well as in the subsequent tanning operations, have been followed quantitatively by Schlichte.[1] Generally, the volume increases rapidly at first on soaking and liming, then less rapidly, falling again in the deliming, bating, pickling and tanning stages, the density undergoing a reciprocal alteration.

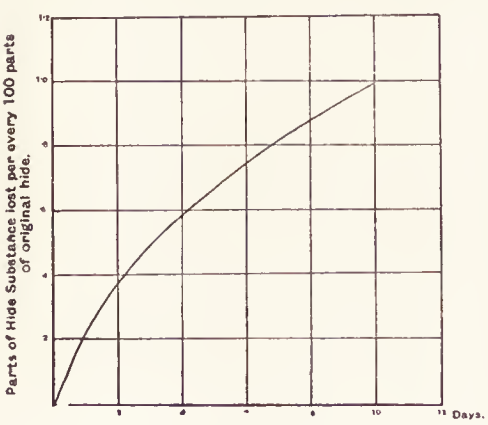


FIG. 18
Curve showing hide substance lost in liming

The curves given below illustrate this; but it must be remembered that in practice, where the nature of the raw stock, etc., varies, there are great variations in the absolute values. (See Figs. 19A and 19B.) The curves showing change in weight are generally similar to those giving change in volume, though showing less rapid increase. The limes in every case contained bacteria, but no added sulphides.

The area increases twenty per cent in the first swelling by soaking, but there is no further change during liming, etc. Hence, the increase in volume is due mainly to increase in thickness. It will be seen (Volume II) that sheet gelatin, on swelling, behaves similarly.

V. DELIMING HIDES[2]

The next step is the removal of the retained lime. The removal is insufficiently effected by washing alone, so that

[1] Schlichte, A. A., l. c.

[2] In the leather industry, part of the process of bating or puering. The fermentation processes involved here play no essential part in glue and gelatin manufacture.

various dilute acid solutions are employed to neutralize the remaining lime. Both in the leather industry and in glue and gelatin manufacture it is undesirable that the acid used be too strong; in the first case because this will produce increased hydrolysis and loss of hide substance, seriously affecting the quality of leather derived from the hides; in the second, because not only will there be loss of material in the neutralizing bath, but hydrolysis on cooking the stock for gelatin will proceed too far, reducing the quality of the product.

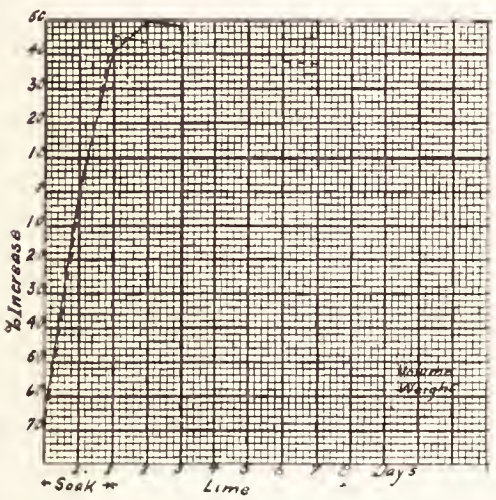


FIG. 19A

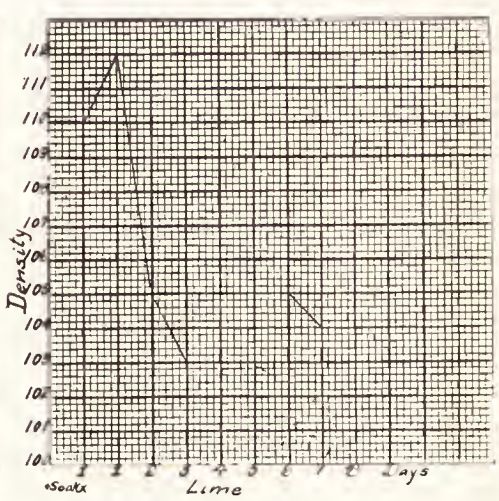


FIG. 19B

Curves showing increase in volume and weight

The chemistry of the factors involved, particularly in relation to the strength of acid permissible and the relation of acids to the hide substances, has been elucidated by Stiasny and by Procter. It is pointed out by the former[1] that thorough deliming requires much care, as an excess of acid must be avoided lest an excessive acid plumping or swelling be produced. The danger is greater the stronger the acid. The strength of an acid is given by its hydrogen-ion concentration, depending upon its dissociation constant.[2] The magnitude of the dissociation constant must first be determined for any acid which in N/20 solution has a hydrogen-ion concentration of about 10^{-5}, this being about the concentration of hydrogen-ion for which the limit of the swelling power lies, as found by Procter.[3] Hence, an acid which, when diluted for use, gives a lower hydrion concentration than 5×10^{-5}, and which is

[1] Stiasny, E., J. Amer. Leather Chem. Assoc. 7: 301. 1912.

[2] See any text book of physical chemistry.

[3] See Volume II. The minimum swelling of hide powder lies at about $P_H = 4.8$ to 5.0. The actual swelling of the stock allowed before extraction varies with different processes and manufacturers.

present in sufficient quantity for complete deliming, will not do any harm if used in excess, and will produce an absolutely lime-free hide.

Assuming that the deliming is done with an acid solution weighing four or five times as much as the pelt, and that the amount of lime in the hide is about 0.6 per cent, N/20 acid should be used, since $600/28 \times 400 = .054$. To determine the dissociation constant desirable for this, we have from the mass law,

$$K.C_{HX} = C_H \times C_X = C_H^2,$$

in which K is the dissociation constant, C_{HX} the concentration of undissociated acid, and C_H the concentration of hydrion. For $C_H = 10^{-5}$ and C_{HX} for a very weak N/20 acid about .05, the dissociation constant for the acid required is given by the formula—

$$K = \frac{C_H^2}{C_{HX}} = \frac{10^{-10}}{0.05} = 2.10^{-9}.$$

Any acid which shows a somewhat low dissociation constant can be used for liming provided it fulfills the following conditions: It should form a soluble lime salt, be non-poisonous, odorless, and low in cost. The only acid fulfilling these conditions and at the same time having a dissociation constant of 10^{-10} to 10^{-8} is boric acid. Actually, however, the choice of acids may be widened by utilizing "buffer" action—that is, by mixing the solution of a suitable acid whose dissociation constant lies between 10^{-5} and 10^{-6} with an equivalent amount of one of its soluble salts, so that the hydrion concentration is reduced below that producing an excess swelling action.

From the mass law, $K.C_{HX} = C_H \times C_X$, and hence $C_H = \frac{K.C_{HX}}{C_X}$, where C_X = concentration of the acid radicle ion (anion). As C_{HX} for a weak N/20 acid is about 0.05 and C_X of an N/20 salt solution equals about 0.05 also, $C_H = K$. That is, the hydrion concentration of an equivalent mixture of a weak acid and its salt is equal to the dissociation constant of the acid. Consequently, any harmless cheap acid which forms soluble lime salts and of which the dissociation constant is not much above 10^{-5} can be used for deliming.

Such an acid, mixed with an equivalent amount of one of its salts (or half the acid neutralized), gives a deliming solution fulfilling all conditions. This solution should be neutral to

methyl orange and give, when titrated with phenolthalein, the total acidity available for deliming. For example, for 100 lbs. weight of hide, we should need 20 ozs. acetic acid and 32 ozs. sodium acetate to 40 gallons of water, assuming 0.6 lb. of lime in the hide.

Procter,[1] from the result of experiments to be described later (in volume II), concludes that acids combine with the hide substance (collagen) to form, in true chemical combination, salt-like compounds which are very easily hydrolyzed. In a slightly swollen hide there is a good deal of uncombined hide substance. With more concentrated acid liquors, as the free acid increases in the hide, the uncombined hide substance diminishes. Every acid-swollen hide is a system of hide-salt, free acid, uncombined hide, and water, each bearing a definite relation to the strength of the solution in which the hide is swollen. Removal of lime by acid is apparently very simple, as to secure adequate removal it should be necessary only to use sufficient acid to form a soluble lime salt. And the fact is that even such dangerous acids as hydrochloric and sulphuric may be used quite successfully by skillful workers. If abundant clean water is used and slightly less acid than is necessary to combine with the lime present is added gradually, with gentle agitation of the hides (sufficient time being allowed for effects to equalize throughout the hide), perfect deliming will result, no matter which acid is used. (Even calcium sulphate is soluble in a sufficiently large volume of water.)[2] It should be added that while hides for tanning go subsequently to a bate or drench, where a slight alkaline reaction is immaterial or even desirable, it is better that hides for glue and gelatin be extracted or cooked in a slightly acid solution.[3] If carbonation be suspected or if the water be hard, it is best to use all the acid at once, as calcium (and, even more so, magnesium carbonate) is not very rapidly attacked by very weak acids.

It is when acids are used in excess, whether to save time or to dispense with accurate chemical control, that the process becomes more complicated because of the combination of hide substance with acids. If hide is placed in a very dilute acid it does not (as a strong base would) combine with the whole of the acid necessary to saturate it. The water, acting as a very weak base, competes with the hide for the acid, so that a

[1] Procter, H. R., J. Amer. Leather Chem. Assoc. 8: 244. 1913.

[2] Procter, H. R., J. Amer. Leather Chem. Assoc. 8: 350. 1913.

[3] The isoelectric point of collagen is in the neighborhood of PH = 5, and photographic gelatins are generally extracted at PH 5 to 6. The PH determination may be made colorimetrically on the delimed stock, as well as on the liquor.

state of hydrolytic equilibrium results, the proportion of hide-salt to free acid being less as the acid is weaker or the quantity of water larger. Hence with weak acids or with sufficiently dilute acid, excess is much less harmful, and many tanners prefer to pay the much higher cost of organic acid rather than risk the heavy penalties of insufficient scientific control.

There is, as has been noted above, a method of still further weakening a naturally weak acid by adding a suitable quantity of its neutral salt. In practice, since neutralization with an organic acid forms neutral salt of lime, similar to the sodium salt in its buffer action, it is necessary only to retain sufficient of the old liquor to restrain the action of newly added water and acid. This method is equally applicable to formic, acetic, lactic, and butyric acids, though the largest amounts of old liquor will be required for formic, the least for butyric. Butyric acid is valuable because of the solubility of its calcium salt. Boric acid requires no restrainer, and must usually be frequently renewed, as otherwise insoluble calcium borates accumulate.

The indirect use of strong mineral acids to liberate the weaker acids from their lime salts and enable them to be used a second time has much to recommend it, as the stronger acids are much cheaper. In this case, however, the effect of the neutral salts of the strong acids which remain in the liquor must not be overlooked. Procter has shown that if hide treated with formic acid alone be placed in a strong solution of common salt, the formic acid is practically entirely replaced by hydrochloric, of which as much as three and a half per cent may be fixed as dry hydrochloric acid. Procter says: "Whatever neutral salt of a strong acid is present in the deliming liquid will replace a certain amount of the weaker acid in a similar way and we do not know how far it is indifferent in tanning whether the hide salt is a chloride, a sulphate, an acetate or unaltered hide—and on this point properly conducted scientific research is urgently needed." *Mutatis mutandis*, the same may be said in regard to the preparation of gelatin and glue.

The accumulation of soluble salts of strong acids in the deliming liquor, and the consequent fixation of acids in the hide is probably equally objectionable for tanning and for gelatin making. Several methods of avoiding it have been suggested, the most obvious of which is to employ a strong acid forming a lime salt which is but slightly soluble. For this reason, and on account of its cheapness, sulphuric acid

is the most suitable. A method involving its use, but effected in stages and requiring chemical control, is described by Procter.[1] The general practice in glue and gelatin manufacture is to use a strong mineral acid, usually sulphuric, to complete deliming.

This discussion of the deliming process has been included because it shows the possibility of effecting an insurance against trouble and loss at this stage. References to the use of various acids will be found in the literature cited.

The relative amounts of hide substance (collagen) dissolved out by different acids were found by Salomon[2] to be as follows:

Reagent		Percentage of Dissolved Substance
Water		0.45
0.8% butyric acid		0.20
Hydrochloric	At concentrations equivalent to this concentration of butyric acid.	0.18
Formic		0.36
Sulphuric		0.60

Procter's ionization theory of swelling is attacked by McLaughlin[3] on the basis of work with M. Fischer, as will be noted more fully later. However, we may note the main points here. Primarily, McLaughlin finds no definite relationship between the concentration of the hydrogen or hydroxyl ions yielded by the dissociation of the reagents in water and the amount of swelling of hide produced thereby. He considers that the results shown in the table below contradict the ionization hypothesis:

Reagent	Concentration	Plumping Effect = Weight of water absorbed / Mols $\frac{+}{H}$ or $\frac{-}{OH}$
Hydrochloric acid	N/16	100
Nitric acid	N/16	60
Sulphuric acid	N/16	92
Lactic acid	N/16	1111
Formic acid	N/16	226
Potassium hydroxide	N/16	39
Sodium hydroxide	N/16	25
Ammonium hydroxide	N/16	1477
Lactic acid	2/N	329
Acetic acid	3/N	172
Acetic acid	4/N	219
Formic acid	2/N	115
Formic acid	3/N	129
Formic acid	4/N	94

McLaughlin considers that the swelling depends on the previous history of the hide and on the beam-house treatment.

[1] Procter, H. R., Leather chemists' handbook, p. 354.

[2] Salomon, T. J. Soc. Chem. Ind. **31**: 737. 1912.

[3] McLaughlin, G. D., J. Amer. Leather Chem. Assoc. **15**: 228. 1920.

Assuming proper salting by the packer, the possible changes during the beam-house treatment would be of two types—first, those due to post-mortem acid formation (chiefly lactic), secondly, those due to salt. It is considered that when the hide reaches the soaks there is, instead of the original protein mass, more or less of a mixture containing protein lactate and sodium chloride proteinate. In this condition the hide is soaked and limed, and thus largely converted into calcium proteinate. It is contended that each of these various protein compounds has a definite and characteristic hydration capacity. The analogy with soaps is pointed out in connection with Fischer's work in this direction,[1] and objection is raised to the "ionic" or "electrical" theories of swelling (hydration and dehydration) on the score of the inadequacy of such theories to deal with swelling in non-aqueous solvents.

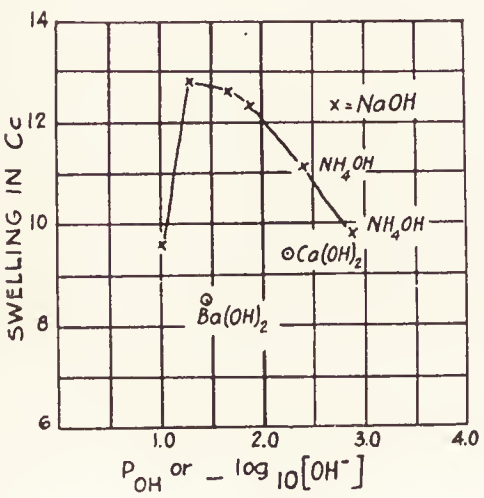


FIG. 20

The theoretical conclusions of McLaughlin are traversed by W. R. Atkin in a recent paper.[2] This author finds that the Proctor-Wilson-Loeb theory of acid swelling dealt with more fully later, can be expanded to alkaline swelling of proteins. Hide powder was swollen for three hours in various solutions. The volume of powder at equilibrium was measured, the equilibrium alkaline solution filtered off and titrated. From the titration, and tables of dissociation, the P_H values were calculated. The results are shown in the following table and the curve (Fig. 20).

Normality of Equilibrium Solution		P_H value (calc.)	Swelling in cc.
$Ba(OH)_2$	0.0405	12.55	8.5
$Ba(OH)_2$	0.016	12.11	7.9
$Ba(OH)_2$	0.007	11.81	9.7
$Ba(OH)_2$	0.0025	11.38	9.7
$Ca(OH)_2$	0.006	11.75	9.4

[1] Fischer, M. H., and Hooker, M. O. Science **48**: 143. 1918; and Chem. Eng. **27**: 155, 184, 223, 253, 276. 1919.

[2] Atkin, W. R., J. Ind. Eng. Chem. **14**: 412. 1922.

Normality of Equilibrium Solution		P_H value (calc.)	Swelling in cc.
NaOH	0.113	12.99	9.6
NaOH	0.055	12.70	12.8
NaOH	0.022	12.31	12.6
NaOH	0.014	12.12	12.3
NH_4OH	0.865	11.60	11.1
NH_4OH	0.0935	11.11	9.8
Distilled water		6.4	5.85

The addition of salt produces a repression of swelling exactly as in the case of pickling an acid swollen hide, a part in full agreement with the Proctor-Wilson theory. Although neutral salt has a tendency to repress swelling, the mixture of sodium chloride and barium hydroxide produces more swelling than barium hydroxide alone, while barium chloride added to collagen swollen chloride produces a larger depression than an equivalent amount of sodium chloride, added to collagen swollen with caustic soda. These results agree with Loeb's on combination of cations with gelatin. We have a reversible reaction:

$$Ba^{++}(Coll)_2^{-} + 2Na^{+}Cl^{-} = 2Na^{+}Coll^{-} + Ba^{++}Cl_2^{-}$$

the relative properties being governed by the mass law (and the Donnan membrane equilibrium). The collogenates of the divalent cations Ba and Ca have lower swelling powers than those of the monovalent ones.

Atkin shows that these results are in agreement with certain aspects of "sharpeners" used in lime baths; salt already present acts in this sense. McLaughlin's results are considered to be inconclusive, because no allowance was made for the lime in the pelt and the consequent buffer action of the calcium salts dissolved out by the acids, while the acidities determined were not equilibrium values; it is the equilibrium hydrogen-ion concentrations which are decisive.

VI. EXTRACTION (COOKING)

There is little to be added to what has already been said on this subject. Both under and overcooking can be controlled by analysis, the first by determination of the yield and proportion of glue-yielding residual substance (collagen), the second by determination of the degree of hydrolysis of the stock secured.[1]

From the foregoing discussion of neutralizing and deliming, the probability and importance of retained acid or alkali and

[1] See preceding discussion, pp. 29, 169 and Chapter III, p. 156. The importance of the size of the material, and of agitation will be evident from the discussion on pp. 46-48, which remains true, *ceteris paribus*, when applied to hide.

the possible detrimental action thereof is evident. As pointed out in this connection by Powell,[1] the reaction[2] of the material throughout the process must be carefully watched. "The well-known influence of acidity on the swelling of gelatin and like colloids points to the importance of such control. The points of maximum and minimum swelling of the stock must be definitely related to the extraction of the gelatin and the properties of the resulting product. The fact that salts again modify this action of acidity adds other complicating factors."

Powell also calls attention to the various possibilities of "formation of undesirable precipitates, since we may have almost any combination of acid or alkali albuminates, mucins, and related substances, together with inorganic compounds, especially the uncertain phosphates. There are conditions where a very slight change in acidity may bring down any one of these, or, more likely, a combination of several, and great care must be taken to avoid the formation of colloidal precipitates, a condition favored by the function of gelatin as a protective colloid." This point will be more fully dealt with in Volume II.

VII. CLARIFICATION[3]

The principles involved here are:

(1) The addition of a coagulating electrolyte, such as alum, which precipitates both colloidally suspended inorganic matter, and certain organic colloids. The optimum activity here also depends not only on the nature of the suspended matter and the coagulating salt, but on the hydrion concentration and the rate of mixing;

(2) The production of a colloidal oxide, hydroxide, or insoluble precipitate, in the solution. Similar considerations obtain here;

(3) The addition of a coagulable protein, such as blood albumin, and coagulation by heat. Here again the degree of acidity (hydrion concentration), is decisive.

In any research on these problems, there are two essentials which may very probably be profitably extended to routine control. These are: first, a means of determining actual acidity (hydrion concentration), a subject to be dealt with in Volume II, and secondly, a turbidimeter for quantitative expression of the results on the liquor or stock. The writer has designed a convenient instrument for this purpose, the

[1] Powell, J. R., l. c.
[2] More precisely, perhaps, hydrion concentration.
[3] See Part I, this Chapter, p. 30.

readings of which are expressed as percentage clarity, based, for example, on pure water as 100.[1]

As to procedure, Lambert[2] states that although many clarifying ingredients have been proposed, as for instance oxalic acid (giving calcium oxalate), phosphate of soda (giving calcium phosphate), basic acetate of lead, blood, etc., generally nothing fulfills the purpose better than potash alum, used in the proportion of one-half of one per cent of the weight of dry glue present in the liquor.

Lambert's method is to heat the liquor to be tested to 80° C., and stir in the necessary quantity of alum, increasing the temperature to 100° C. meanwhile. After boiling for ten minutes, the liquor is allowed to cool, when the heavy mineral and organic impurities settle to the bottom and the lighter coagulate as a scum on the surface. These impurities are then removed by filtration, for which paper pulp has been found very satisfactory. Recently the use of insoluble inorganic gels such as alumina in colloid gel form has been proposed by W. Gordon Bennett.[3] These have both decolorizing and clarifying properties, and have powerful adsorptive action upon such objectionable impurities as arsenic, copper, zinc, and lead. The clarifying power of ordinary alum is probably connected with this, since alumina tends to precipitate in solutions with $P_H > 3$, and most gelatin solutions will have at the stage in question a $P_H = 5$ to 6.

Before leaving the subject of clarification, reference should be made to proposals to utilize the phenomenon of electrophoresis in the purification, *inter alia*, of glue and gelatin. In recent years Botho-Schwerin, through a German firm, the Elektro-osmose A.-G., has been granted a large number of patents dealing with the purification of colloidal and non-colloidal mixtures and the resolving of such mixtures into their constituents.[4] The physical chemistry of this method will be discussed in Volume II. The principle of electrical osmosis is based on the fact that in a two-phase system a difference of potential sending a current through the system may produce a relative displacement of the phases. The two aspects of this are:

[1] Sheppard, S. E. J. Ind. Eng. Chem. **12**: 167. 1920.

[2] Lambert, T., l. c., p. 24.

[3] Cf. Bennett, H. G., Animal proteins, l. c., p. 240.

[4] See Briggs, T. R. Second Report on Colloid Chemistry, its general and industrial applications. Dept. of Scientific and Industrial Research, London, 1918. Schwerin, B., Electro-osmotic purification of gelatinous substances. U. S. Patent No. 1,235,064. July 31, 1917.

(a) If the solid is fixed in the form of a porous diaphragm, the liquid may be forced through the diaphragm. This is *electrical endosmosis;*

(b) If the solid is in the form of a suspension and is free to move, it may migrate through the liquid. This is *cataphoresis.* A very complete discussion, both of the scientific principles involved and of the industrial applications tried or proposed is given by Briggs in the paper cited.

The purification of gelatin from electrolytes by electrolysis was described by Dhéré and Gorgolewski[1] who first prepared ash-free gelatin in this way in 1910.

The substances causing turbidity may be both mineral and organic. The writer and H. Hudson have found, beside calcium phosphate, that traces of calcium soaps (from fats on liming) cause obstinate turbidity.

VIII. CONCENTRATION, BLEACHING, AND DRYING

There is little to add to what has been said regarding concentration, bleaching, and drying. Regulation of the temperature and pressure (vacuum) during concentration secures restraint of hydrolysis.

Decolorizing glues and gelatins by absorbent charcoals is not easy, probably on account of high adsorption. The use of bleaches, such as sulphurous acid, chlorine, peroxides, etc., requires care and control to prevent harm being done by hydrolytic decomposition. The action of these substances on gelatin will be noted in greater detail in the next chapter. (See British Patent No. 134,011.) Sulphurous acid is generally regarded as the most useful, and may be doubly utilized, both in deliming and bleaching. Its reducing action is sometimes fortified by addition of sodium hyposulphite (hydrosulphite) $NaHSO_2$ or Na_2SO_2. This may be added either directly or as a mixture of zinc and bisulphite of soda. Rideal strongly recommends calcium peroxide as a combined bleach and coagulant for use with acid bone glues.[2]

As regards drying, the relation of water absorption to, first, the hydrion concentration and salts, and second, to the initial concentration secured, will be discussed in Volume II.

[1] Dhéré, C., and Gorgolewski, A., Comp. rend. **150**; 934. 1910. *Addendum.* The method has been used recently by the writer and his collaborators as part of a process for preparing standard gelatin. (Cf. J. Amer. Chem. Soc. **44**; 1858. 1922).

[2] Cf. Bennett, H. G., l. c., p. 246.

BACTERIOLOGICAL CONTROL

The positive and useful part played by bacteria in the early stages of liming hides has already been mentioned. The harmful and deteriorating effects of bacteria on stock are of even greater importance. From the concentration and clarification stages onward the importance of bacteriological control increases, since the temperature and the dilute solution are ideal for incubation of bacteria.

The disinfection problem commences with the receiving and storing of the raw material, and the considerable possibility of the occurrence of anthrax spores increases the need and importance of care. The method of sterilization developed by Seymour-Jones[1] is of great interest in this connection, but since it depends upon the absorption of mercuric chloride by the hide from acid solution, it is not particularly desirable in the manufacture of photographic gelatin. As will be noted later, mercury salts are strong desensitizing agents for photographic emulsions, and gelatin containing even very small traces would be unsuitable for many photographic purposes. Therefore, the possibility of raw material having already been treated in this manner must be considered.

The partial sterilization effected by formic acid alone is interesting here. Seymour-Jones prefers this acid as the acid component of the bactericide because it has a high ionization constant and low molecular weight, both factors contributing toward making it the most efficient and, in the long run, the cheapest organic acid to use. It may be removed completely by washing, and is a disinfectant for all classes of fungoid growths.

Kohnstein's anthrax antiseptic method,[2] which avoids the use of mercury, and which was developed in cooperation with the Hygienic Institute of Vienna, calls for a bath of two per cent hydrochloric acid and ten per cent sodium chloride. The method is referred to (as Schattenfroh's pickling process) by Schnurer and Sevcik,[3] who state that, in four tests out of eleven, thick cattle hide still showed infection after 72 hours' action. Schattenfroh's favorable results were probably due to the thinness of the skins (lamb, sheep and goat) employed.

The Seymour-Jones method is much more positive but requires higher concentrations of mercury unless the skins are first degreased. A detailed study of both these methods, as

[1] Seymour-Jones, A., The Seymour-Jones anthrax sterilization method. J. Amer. Leather Chem. Assoc. **6**: 85. 1911.

[2] Kohnstein, B. J. Amer. Leather Chem. Assoc. **6**: 529. 1911.

[3] Schnurer, J., and Sevcik, F. J. Amer. Leather Chem. Assoc. **8**: 174. 1913.

well as of others recommended for the same purpose, was made by F. W. Tilley of the U. S. Bureau of Animal Industry, whose report[1] probably summarizes the state of knowledge on the subject at present. His conclusions regarding the various methods are:

(1) The Seymour-Jones Method: The strength of the disinfectant originally recommended by Seymour-Jones (mercuric chloride, 1 to 5000, plus one per cent of formic acid) was not found to be efficient, even without neutralization of the disinfectant.[2] A lower dilution, 1 to 2500, plus one per cent formic acid, was found to be efficient where no neutralization was attempted. But the latter strength was not sufficient to prevent infection of guinea pigs by disinfected material when the disinfectant was neutralized by a one per cent sodium sulphide solution three or four days after the completion of the process. No infection was caused by inoculation with material kept a week or more after disinfection. It seems, therefore, that the Seymour-Jones method might be employed (with dilutions of mercuric chloride 1 to 2500, plus one per cent of formic acid), provided the treated hides are not to be subjected to the action of any substance which will neutralize the disinfectant within a week or two, as would be the case, for instance, if the hides were disinfected at foreign ports before shipment to this country. This method apparently does not injure hides or leather.

(2) The Schattenfroh Method: Immersion for forty-eight hours in a bath of hydrochloric acid and sodium chloride, in the proportions of two per cent of the acid to ten per cent of the salt, proved efficient in every case. Consequently from the bacteriological standpoint the Schattenfroh method seems to be entirely satisfactory. This conclusion is supported not only by this investigation, but also by the exhaustive researches of Gegenbauer and Reichel[3] and of Hilgermann and Marmann. As stated above, the recently published work of Sevcik[4] is not so favorable to this method.

A memorandum by F. P. Veitch, chemist in charge of the Leather and Paper Laboratory of the Bureau of Chemistry, states that from samples submitted it appeared that this treatment had not injured the hides.

[1] Tilley, F. W. J. Agr. Research, U. S. Dept. of Agriculture, Washington, D. C. Vol. IV. No. 1, April 15, 1915; and J. Amer. Leather Chem. Assoc. **11**: 131. 1916.

[2] Such neutralization will be effected in the liming process, the acid being neutralized by lime, and the mercuric chloride by sulphides, converting the mercury to insoluble mercuric sulphide. Although less dangerous to photographic material in this form, small quantities getting through in colloidal solution would be liable both to cause "fog" and to produce some desensitzing effect.

[3] A complete bibliography is appended to Tilley's paper.

[4] Tilley, l. c., p. 59.

No statement as to the possible or actual effect upon the production of glue or gelatin is made in this report. The possible objection to the Seymour-Jones method has been noticed. But, as pointed out recently by F. L. Seymour-Jones[1] under the conditions specified for the process, the P_H is such that no mercury can combine with hide substance. Consequently, *if the hides are washed prior to liming* (or other alkaline treatment) all the mercury is removed. If the mercury ion is not removed, it will be fixed in the liming and either appear as black stains in pelts for tanning, or possibly be carried down colloidally in gelatin or glue extraction. As to the Schattenfroh method, the possible loss of hide substance in salt solutions should be considered—(see p. 76)—as well as the probability of increased chlorides in the gelatin or glue. For several purposes, photographically and technically, soluble chlorides are objectionable.

Other disinfectants tried were formaldehyde and carbolic acid (phenol). From a limited number of tests, it was concluded that two and one-half per cent formaldehyde is efficient bacteriologically against both anthrax spores and other organisms, and that five per cent phenol is fairly efficient against non-spore-bearing organisms, but is practically useless against anthrax spores. Pieces of hide disinfected by formalin (two and one-half per cent solution), were so seriously affected that it was impossible to tan them. Pieces treated by carbolic acid were uninjured.

Since the glue and gelatin manufacturer receives his material as a by-product from the tannery industry, his material will probably have been already treated by one or another of the approved methods.

A copy of the official Disinfection Regulations, U. S. A. Treasury Department and Department of Agriculture Joint Order No. 2, of October 15, 1917, is appended. Attention is called especially to the instructions for the disinfection of glue stock.

Although this subject represents a remote stage in the history of the product, the technological chemist has frequently (and nowhere more than in the photographic industry), to review the history of a material in an effort to discover the source of some factor, desirable or undesirable; hence the inclusion of this somewhat full account of this phase of the subject.

[1] Seymour-Jones, F. L., J. Ind. Eng. Chem. **14**: 130. 1922.

On this subject the reader is also referred to the Report of the International Commission,[1] and especially a report by Yocum[2] to the National Association of Tanners of America, in which the methods of disinfection of hides intended for shipment to the United States as permitted by the Government Regulations (T. D. 30,583, Circular 23 of May 2, 1910), are criticized as not meeting the requirements of proper disinfection, while quite recently the subject has been fully reviewed by A. Seymour-Jones[3] and his mercury-formic acid method defended.

For recent studies of disinfection processes from a physicochemical standpoint, reference should be made to a paper by Lee and Gilbert;[4] and on the application of physical chemistry to this type of biochemical problem, to Arrhenius' book.[5]

The reader's attention is also called to three papers on the purification of tannery wastes and effluents, a problem which concerns the glue and gelatin producer,[6] and to a paper of interest from the viewpoint of the efficiency of utilization.[7]

BACTERIA AND THEIR ACTION IN THE LIMING PROCESS

Wood and Law,[8] investigating this question, added a single loop of an old lime plus a drop or two of ammonia to ordinary nutrient gelatin. Fifty colonies appeared, which would mean that the estimated number of bacteria per cc. of lime liquor is about 50,000. There were undoubtedly many more organisms present, although they failed to come to development in the nutrient medium used. In the soaks there are approximately 1000 times as many bacteria.

The principal colonies developed were small roundish ones which appeared granular under low magnification, and which consisted of small bacteria in pairs (diplococci), not motile except for Brownian movement. There were also colonies of a pinkish color, the bacteria resembling *B. prodigiosum*. Some of the yellow colonies, which are probably the cause of the yellowish color of old limes, were surrounded by a ring of

[1] J. Amer. Leather Chem. Assoc. **7**: 487. 1912.

[2] Yocum, J. H., J. Amer. Leather Chem. Assoc. **8**: 526. 1913, see also committee Report, ibid. **11**: 333. 1916.

[3] Seymour-Jones, A., J. Amer. Leather Chem. Assoc. **17**: 55. 1922.

[4] Lee, R. E., and Gilbert, C. A. J. Physic. Chem. **22**: 348. 1918.

[5] Arrhenius, S., Immuno-Chemistry.

[6] Morrison, J. A. S. J. Amer. Leather Chem. Assoc. **6**: 326. 1911. Veitch, F. P. J. Amer. Leather Chem. Assoc. **8**: 10. 1913. Jackson, D. D., and Buswell, A. M. J. Amer. Leather Chem. Assoc. **12**: 229, 1917.

[7] Peck, C. Lee. J. Amer. Leather Chem. Assoc. **13**: 417. 1918.

[8] Wood, J. T., and Law, D. J., l. c.

liquefied gelatin, corresponding to *Micrococcus flavus liquefaciens*, and the non-liquefying colonies were probably identical with *Micrococcus aurantia*. Fluorescent grayish colonies were also observed, forming apparently swarming islets like *Proteus vulgaris*, but these were cocci, and not identical with Hauser's bacillus.

The relative antiseptic power of lime was first enunciated clearly by Humphrey Davy, in the following conclusions based on his experiments:

"After animal substances are subjected to the action of lime they cease to become putrescible; they resist putrefaction better if protected from air or immersed and kept in water. Lime does not exercise a destructive effect on animal substances, nor promote their decomposition, but on the contrary it has a strong preservative and a decidedly antiseptic power, which arrests putrefaction even when previously well advanced."

That bacteria play a part in the liming bath is now very generally accepted.[1] Wood and Law consider that the action is somewhat as follows: Fresh lime dissolves the interfibrillar substance of the skin, in which bacteria from the air develop, decomposing the dissolved skin substance by enzymes into gelatones (gelatin-peptones). These gelatones are then broken down to amino-acids and simpler bodies, such as ammonia, caproic acid, etc.

As against the view that bacterial action is essential must be set the record of experiments directed to this end by Schlichte,[2] who concludes that "it is possible to depilate a skin or hide under strictly sterile conditions with lime alone or with addition of sulphides. The same sterile line solution can be used to depilate successive pieces of hide. Calf skin kept for six months in sterile milk of lime shows a firm though rather glassy corium. A skin kept a similar length of time in sterile milk of lime containing arsenic or sodium sulphide is completely dissolved. The hair from the skins in the latter solution is also dissolved, while that of the skin in lime alone appears almost unchanged." For details of the technique of sterilization, etc., Schlichte's paper should be consulted.

The position appears to be that, while it is probably possible, and possibly desirable, to unhair, etc., in sterile lime solutions, it has not been established that, under usual conditions, bacteria do not play a definite part. It should be noted that it

[1] Cf. Bennett, H. G. J. Amer. Leather Chem. Assoc. **10**: 569. 1915.

[2] Schlichte, A. A., l. c.

has also been suggested that the removal of fatty matter is due less to lime than to the action of fat-splitting bacteria. In any case, from this stage on, so far as glue and gelatin manufacture is concerned, bacterial influence is inimical and the control required a preventive one. That implies:

(a) relative sterility of water used;
(b) relative sterility of containers, transporting vessels, etc.;
(c) relative sterility of air, in drying, etc.

Methods of dealing with these problems will be found in works on hygiene and sanitation, especially as applied to the conservation of foodstuffs.[1]

Recent data of value on antiseptics will be found in the literature cited.

The bacteria chiefly dangerous are anaerobic and gelatin liquefying, as for instance *B. putrificus* and *B. putilus*, active in putrefying processes in hides.[2] It was shown by Koch[3] that the liquefying character is an important one in differentiating species which resemble each other in form and in other respects.

There is no relation between liquefying and pathogenic power (relative to higher organisms), as some liquefying organisms are harmless and some are deadly disease germs; and, on the other hand, non-liquefying bacteria may be very pathogenic or quite innocent. Liquefaction, however, is perhaps the most dangerous effect of bacteria in gelatin manufacture.[4] It is produced by soluble peptonizing ferments or enzymes (so-called tryptic enzymes), formed during the growth of the cells.[5] It is important to notice that the tryptic digestive action of most bacteria is prevented by sulphuric acid,[6] but not interfered with by acetic acid, and probably depends also upon the simultaneous presence of true albumins (hemi-albumoses or hemi-proteins, as distinguished by Cohnheim[7] from anti-proteins).

For methods of investigation of air and water for bacteria and microorganisms, reference should be made to the literature cited.[8]

[1] E. g., Don. J., and Chisholm, J., Modern methods of water purification. Rideal, S., Disinfection and disinfectants.

[2] Cf. Eitner, W. J. Amer. Leather Chem. Assoc. **8**: 482. 1913.

[3] Cf. Sternberg, G. M., Textbook of bacteriology. 2nd Edition, p. 135.

[4] The activity of non-liquefying bacteria in producing fogging of photographic emulsions is possible, but has not been studied.

[5] See Chapter III, p. 00.

[6] Cf. Sternberg, l. c., p. 136.

[7] Cf. Mann, G., Chemistry of the proteids, p. 148.

[8] The use of hypochlorite of soda for sterilization is claimed by Chem. Ges. Rhenania, D. R. P. 313141, Chem. Ztg. **45**: 629. 1921.

In addition to the negative, preventive side of bacteriological control, there exists a field of positive research, especially for photographic gelatins—e. g., in the production of sensitizing dyes by chromogenic bacteria. Another field is the influence of regulated hydrion concentration on bacterial infection and immunity. The writer has observed that infection occurs much less easily in solutions having concentrations of acid of the order of $\frac{N}{1000}$ and upwards when exposed to aerial infection. This agrees with much recent work,[1] and suggests that properly buffered solutions would be partially self-protecting.

Generally, in any putrefying material, such as hide, albumin, or gelatin, "a large number of different species of bacteria may be observed, as well as monads and infusoria, and in some cases molds, all of which take part in the process. The first stage is a process of oxidation in the presence of air, in which aerobic bacteria use up the oxygen present, and only simple inorganic compounds are formed—carbon dioxide, nitrates, sulphates. This stage is generally odorless. The second stage, of true putrefaction, occurs in the absence of oxygen by anaerobic bacteria and is a process of reduction."[2]

Several molds take part in putrefaction, notably *Penicillium glaucum* and *Mucor mucedo*.

[1] Wolf, C. G. L., and Harris, J. E. C. Biochem, J. **11**: 213. 1917, also Davis, C. E and Oakes, E. T., J. Amer. Chem. Soc. **44**: 464. 1922.

[2] Wood, J. T. J. Soc. Chem. Ind. **25**: 109. 1906. The following list of putrefactive, bacteria which have been studied in pure cultures is given by Wood:

Proteus vulgaris, commonest and most active of putrefying organisms. Among products, choline, ethylene-diamine, trimethylamine.
Proteus mirabilis
P. Zenckeri
B. oldamatis maligni
B. chauvaei = B. sarcophyemator bovis
B. liquefaciens magnus. One of the chief liquefying organisms.
B. spinosus
B. putrificus
B. pseudooedamatiens
B. enteridis sporogencs
B. tetani
Clostridium foetidium
B. cadaveris sporogenes
Spirillum desulfuricans
B. coli communis
B. lactis aerogenaes
B. fermentationis cellulosae
Micrococcus flavus liquefaciens
Diplococcus grisus non liquefaciens
Streptococcus pyogenes
Staphylococcus pyogenes albus
B. filiformus aerobius
Diplococcus magnus anaerobius
B. gracilis putidus
B. perfringens

For more complete accounts of the organisms causing putrefaction, see the following:

*Jungano, M., and Diastaso, A., Les Anaerobies. Masons et Cie., Paris, 1910.

*Abt. G., Le rôle des microbes dans la putrefaction des peaux en poils et en tripe et dans les confits. Bulletin mensuel du Syndicat General de cuir et peaux de France. 416. 1908.

This includes a table summarizing the action of putrefaction bacteria.

Wood, J. T., The bacteriology of the leather industry. J. Soc. Chem. Ind. **29**: 666. 1910

Skins in their fresh state contain all the bacteria which develop later, but putrefaction begins only under favorable conditions. Drying hinders development and salting probably acts similarly, though some species can grow in the presence of salt, inducing so-called salt stains in hides.

Bacteria in the soaks (first wash liquors) are numerous, and mostly putrefactive.[1]

Part III

General Properties

As taken from the drying nets—if dried in that way—gelatin is obtained in the form of tough to brittle transparent leaves. These may be broken up into flakes, or even ground or powdered; or sometimes the leaves are shredded before drying.

The specific gravity of gelatin has been given as 1.368 for air dry α-gelatin, by Quincke (1903), and as 1.412 and 1.346 for anhydrous gelatin, by Ludeking (1883) and Frank (1912) respectively.

Purified gelatin is usually colorless, the color, when present (in unbleached material), being yellow to brown.

Optically, gelatin is a transparent material, the refractive index being 1.5. It is optically active in solution, the laevorotation varying with the nature of the gelatin, the temperature, etc. (See under Muta-rotation.) The values

$[\alpha]_D = -130°$ at 30° C., and

$[\alpha]_D = -167.5°$ are given by Hoppe-Seyler and Nasse respectively. Solid gelatin shows typical polarization effects due to strain induced by drying, and similar transient effects can be obtained by compressing or shearing concentrated gelatin jellies.

[1] Andreasch, Fr., Gährungserscheinungen in Gebrühen, (Der Gerber. 1895-7), gives a list of species found. See also Wood, J. T., l. c.

Air-dry gelatin contains from eight to fifteen per cent moisture, depending upon the conditions under which it was dried. Over-dry gelatin is brittle and possibly somewhat denatured. On the other hand, gelatin insufficiently dried is readily attacked both by molds and by putrefactive bacteria, e. g., *Bacillus liquefaciens magnus.*

Placed in water below 20° C., gelatin does not dissolve,[1] but swells up by absorption or imbibition. The maximum amount of water absorbed varies with different products, from five to ten times its own weight being a general average. The jellies thus formed melt at higher temperatures (i. e., above 20° C.) and set again on cooling—a reversibility which is an important property in practice.

Gelatin is dissolved in the cold by certain concentrated salt solutions, as, for instance, potassium iodide and barium nitrate. Concentrated acids and alkalies liquefy it, considerable hydrolysis taking place. Gelatin is insoluble in 95 per cent ethyl alcohol, in methyl alcohol, acetone and other organic solvents, but aqueous alcohol, acetone, etc., will dissolve considerable amounts. (This will be discussed more fully in Volume II.)

The inorganic electrolytes present in gelatin are of great importance. Their total amount is indicated by the ash figure, which in good commercial gelatins varies from one to four per cent.

Chemically, gelatin is a relatively neutral or inert substance. It is amphoteric—that is, capable of acting as a weak base in the presence of acids, and as a weak acid in the presence of alkalies and bases. Many specific phenomena depending upon this property will be discussed later, as also will certain characteristic reactions of gelatin and those of impurities likely to be present in it.

[1] A certain amount of material, which is largely hydrolyzed gelatin, may be extracted. See Chapter III.

Appendix to Chapter II

Disinfection Regulations

Special Order Prescribing Methods for the Disinfection of Hides, Skins, Fleshings, Hide Cuttings, Parings and Glue Stock, and Other Animal By-products, Hay, Straw, Forage, or Similar Material Offered for Entry into the United States, and the Containers of Glue Stock, Bones, Hoofs, and Horns so Offered for Entry.

UNITED STATES DEPARTMENT OF AGRICULTURE
Bureau of Animal Industry

District of Columbia, December 14, 1917.

In accordance with the provisions of the United States Treasury Department and Department of Agriculture Joint Order No. 2 of October 15, 1917, "Regulations governing the sanitary handling and control of hides, fleshings, hide cuttings, parings, and glue stock, sheepskins and goatskins and parts thereof, hair, wool, and other animal by-products, hay, straw, forage, or similar material offered for entry into the United States," the following methods for the disinfection of the above-named materials and articles are hereby prescribed, effective January 1, 1918.

I. DISINFECTION OF HIDES AND SKINS PRIOR TO SHIPMENT

Hides and skins disinfected prior to shipment as provided by regulation 1, Section 1, of said Joint Order No. 2, must be disinfected by one of the following methods:

(a) By immersion for not less than 24 hours in a 1 to 1,000 bichloride of mercury solution.

(b) By immersion for not less than 20 hours in a solution containing 2 per cent absolute hydrochloric acid (hydrogen chloride) and 10 per cent sodium chloride.

(c) By immersion for not less than 40 hours in a solution containing 1 per cent absolute hydrochloric acid (hydrogen chloride) and 10 per cent sodium chloride.

(d) By immersion for not less than 24 hours in a solution containing 1 per cent formic acid, and mercuric chloride in the proportion of 1 part to 2,500 parts of the solution. Hides or skins treated by this process shall be held for 2 weeks following the treatment before neutralization.

(e) By dehairing and pickling in a solution of salt containing a definite percentage of mineral acid, and packing in barrels or casks while still wet with such solution, provided the hides or skins are not neutralized within 30 days after being so packed.

II. DISINFECTION OF HIDES AND SKINS AFTER ARRIVAL IN THE UNITED STATES

Hides and skins required by regulation 1, Section 2, and regulation 2 of said Joint Order No. 2 to be disinfected on arrival at a United States port of entry shall be moved to an approved warehouse at such port or in sealed cars or containers to an establishment having proper facilities for their sanitary control and disinfection. They shall be stored and handled prior to disinfection in compartments set aside for that purpose, and all hides and skins stored or handled in such compartments shall be treated in accordance with the following rules:

1. All dust, litter, or waste arising from sorting, cutting, handling, or moving said hides or skins prior to soaking, shall be burned or disinfected by exposure to a temperature of not less than 100° C. (212° F.) moist heat for not less than 15 minutes.

2. The hides and skins shall be subjected to disinfection by one of the following methods:

(a) By immersion for not less than 20 hours in a solution containing 2 per cent absolute hydrochloric acid (hydrogen chloride) and 10 per cent sodium chloride.

(b) By immersion for not less than 40 hours in a solution containing 1 per cent absolute hydrochloric acid (hydrogen chloride) and 10 per cent sodium chloride.

(c) By immersion for not less than 24 hours in a solution containing 1 per cent formic acid, and mercuric chloride in the proportion of 1 part to 2,500 parts of the solution. Hides or skins treated by this process shall be held for two weeks following the treatment before neutralization.

(d) By immersion for not less than 48 hours in a 1 to 1,000 bichloride of mercury solution.

(e) By immersion for not less than 6 days in a 1 to 5,000 bichloride of mercury solution, plus not less than 5 days in lime of the usual strength for dehairing.

or, in lieu of disinfection by one of the foregoing mentioned

processes, the effluent shall be subjected to treatment by one of the following methods:

(f) Heat the effluent from soak vats, mill-drums, breaking machines, or other similar equipment, to a temperature of 100° C. (212° F.) and maintain at that temperature for at least one minute.

(g) Treat the effluent from soak vats, mill-drums, breaking machines, and other similar equipment with chlorine, in such manner and in such amount (not less than 250 parts per million) as to secure efficient disinfection.

(h) Subject the effluent from soak vats, mill-drums, breaking machines, and other similar equipment, to filtration, the effluent from the filters to be treated with chlorine in sufficient amount and in such manner as to secure efficient disinfection, provided, however, that in this method of treatment the sludge which collects on the filters shall be subjected to disinfection by heating at a temperature of not less than 100° C. (212° F.) for not less than 15 minutes.

(i) Treat the effluent from soak vats, mill-drums, breaking machines and other similar equipment with 50 parts of chlorine per 1,000,000 parts of effluent and heat at not less than 80° C. (176° F.) for not less than 30 minutes.

(j) In the case of sheep skins and goat skins, until further notice, by immersion for not less than 12 hours in a solution of milk of lime containing the equivalent of 5 per cent of calcium oxide (CaO).

III. DISINFECTION OF GLUE STOCK

All fleshings, hide cuttings, and parings or glue stock shall be moved from the port of entry to an establishment having proper facilities for the sanitary control and disinfection of such materials, in cars or approved containers sealed with either department of agriculture or customs seals, and upon arrival at the establishments, disinfected before removal therefrom by one of the following methods:

1. By heating in water at a temperature of 100° C. (212° F.) for not less than 15 minutes, or by heating in water at a temperature of not less than 82° C. (180° F.) for not less than 4 hours.

2. By soaking in milk of lime or lime paste for not less than 24 hours.

3. By soaking in water containing not less than 2 per cent of absolute hydrochloric acid for not less than 20 hours.

4. By soaking in water containing not less than 1 per cent of absolute hydrochloric acid for not less than 40 hours.

IV. DISINFECTION OF BONES

1. All hornpiths and porous bones classed as glue stock must be moved from the port of entry to an establishment having proper facilities for their sanitary control and disinfection, in cars or approved containers sealed with either department of agriculture or customs seals, and upon arrival at the establishment, disinfected before removal therefrom, as provided for glue stock.

2. Bones with pieces of hide or tendons attached and all other bones not otherwise provided for shall be moved from the port of entry to an establishment having proper facilities for their sanitary control and disinfection, in cars or approved containers sealed with either department of agriculture or customs seals, and upon arrival at the establishment, disinfected before removal therefrom by one of the following methods:

(a) By heating in water at a temperature of 100° C. (212° F.) for not less than 15 minutes.

(b) By heating in water at a temperature of not less than 82° C. (180° F.) for not less than 4 hours.

V. DISINFECTION OF HOOFS AND HORNS

Hoofs and horns shall be moved from the port of entry to an establishment having proper facilities for the sanitary control and disinfection of such materials, in cars or approved containers sealed with either department of agriculture or customs seals, and disinfected before removal from the establishment by heating in water at a temperature of not less than 74° C. (165° F.) for not less than 15 minutes. Bones removed from horns and hoofs that are required to be disinfected, shall be handled as provided for glue stock.

VI. DISINFECTION OF CONTAINERS OF GLUE STOCK, BONES, HOOFS AND HORNS

Containers of glue stock, bones, hoofs and horns, which under the provisions of regulations III and IV of Joint Order

No. 2, are required to be disinfected, shall be handled as follows:

(a) The containers shall be burned, or

(b) The containers shall be subjected to moist heat at a temperature not less than 100° C. (212° F.) for not less than 15 minutes.

. . . . . .

J. R. MOHLER,
Chief of Bureau of Animal Industry.

FIG. 12

Normal calfskin

A—Corneous layer
B—Malpighian layer
C—Papillary layer
D—Reticular layer
E—Hair shaft
F—Hair follicle
G—Sebaceous
H—Hair
I—Inner root sheath
O—Outer root sheath

FIG. 13

Calfskin after soaking 42 hours in water

A—Corneous layer
B—Malpighian layer
C—Papillary layer
D—Reticular layer
F—Hair follicle
G—Sebaceous gland
H—Hair
O—Outer root sheath
S—Shrinkage space

FIG. 14

Calfskin after 24 hours in lime water

B—Malpighian layer
C—Papillary layer
D—Reticular
F'—Degenerating hair follicle

FIG. 15

Calfskin after 66 hours in lime water

C—Papillary layer
D—Reticular layer
F'—Degenerating follicle
F''—Empty follicle
G—Gland

FIG. 16

Calfskin unhaired after 120 hours in lime water

C—Papillary layer
D—Reticular layer
F′—Degenerating hair follicles
F″—Empty hair follicles

FIG. 17

Calfskin after bating

C—Papillary layer
D—Reticular
P—Papillae

CHAPTER III

The Analytical and Constitutional Chemistry of Gelatin

In the following we shall first briefly describe the principal qualitative reactions of use in characterizing a gelatin, both positively, as proper to purified gelatin, and those which should for such a gelatin give a negative or nearly negative reaction. Next, the more important quantitative methods by which gelatin may be integrally determined will be noticed, as well as certain quantitative methods for approximate analysis of either characteristic impurities or characteristic components of a given gelatin. Finally, the relation of gelatin to the general group of the proteins will be discussed.

PRINCIPAL QUALITATIVE REACTIONS

From a purely empirical point of view the most convenient subdivision of these reactions for gelatin, as for other proteins, is into color reactions and precipitation reactions. A side classification, which can not be regarded as exact, but which is helpful from the theoretical standpoint, is:

(1) Colloid reactions, depending upon molecular indetermination and upon change of dispersity and colloid solution state—in general, therefore, associated with change in the interfacial tension between dispersed phase and dispersion medium. Naturally, this applies particularly to the precipitation reactions:

(2) Alkaloid reactions, some of which are probably to be reckoned among the colloid reactions, while others properly belong in the category following. They are supposed to depend upon the intra-molecular arrangement of the nitrogen atoms;

(3) Group chemical reactions, due to specific radicles or finite reacting groups of atoms.

Reference to this latter interpretation will be given only in a collateral column, the first noted division being of primary practical significance.

COLOR REACTIONS

No.	Name of Reaction	Reagent and Reaction	Result and Significance	Remarks
1	Biuret, Rose and Wiedeman	Add excess of KOH aq. or NaOH aq., then few drops $CuSO_4$ aq. dilute.	Positive for gelatin. A blue to violet color is held to indicate a native albumin, a rose color a primary dissociation product. Schiff believed the reaction was due to the amino-carbonyl group, $-HN \diagdown \diagup CO$ $-HN$ but it is less specific than this.[1]	May indicate a very specific type of colloid chemical protective action, in which there is continuity between chemical complex formation and adsorptive protection or dispersion of copper oxides.
2	Xanthoproteic.	HNO_3 conc., and heat. The yellow color is intensified by ammonia or Na_2CO_3 to orange or reddish brown.	Usually positive. The color is due to formation of nitro-derivatives of aromatic radicles in the protein.	The positive nature of this reaction with commercial gelatins is not to be taken as absolute evidence of aromatic residues in the protein proper, but may be caused by phenolic antiseptics, such as phenol, thymol, methyl salicylate, etc.
3	Millon's	Solution of mercurous nitrate $Hg(NO_3)_2$ in nitric acid; prepared by boiling Hg with strong HNO_3 till liquid gives no precipitate, with NaCl.	Usually positive. Given by aromatic derivatives with one OH substitution (oxyphenyl group.	The only oxyphenyl compound in proteins is tyrosin (see p.178) contained in all albumins except gelatin. The positive reac-

[1] Kober, P. A., and Suguira, K. (J. Biol. Chem. **13**: 1. 1912–13) conclude that the biuret reaction with proteins seems to be nothing more than ring formations of the copper with the nitrogen groups, and that the copper complexes of amino-derivatives and other similar substances can be divided, according to color, into three classes:

1 Blue—In each complex two nitrogen groups so placed that by forming stable (four, five, or six-membered) rings, they can combine with the copper (dipeptides);
2 Purple—(semi-biuret)—In each complex three such groups (tripeptides);
3 Red—In each complex four such groups (tetrapeptides).

No.	Name of Reaction	Reagent and Reaction	Result and Significance	Remarks
3		This gives, cold or with heat, rose-pink color with solution or dark red with solid.		tion usually obtained is often due to antiseptics added in manufacture.
4	Vogel's Sulphide Reaction	Solution is boiled with a lead salt (nitrate or acetate) and soda solution. Black precipitate or black-brown color.	Usually negative. Reaction indicates presence of organic sulphur from the $-SH$ group derived from cystine (see p. 127).	Desirable that reaction should be negative for photographic gelatins.
5	Molisch	Few drops of α-napthol in alcohol, followed by strong H_2SO_4; violet color passing to yellow by ether or potash solution. If thymol is used, carmine red, which water turns green.	Reaction depends upon strong H_2SO_4 converting carbohydrate groups to furfurol. This would indicate a carbohydrate group, and is usually negative (but cf., Mann, G., Chemistry of the Proteids, p. 563).	One of so-called furfurol reactions.
6	Adamkiewiez, Hopkins and Cole.	Protein is degreased with ether, dissolved in glacial acetic acid, then concentrated H_2SO_4 added; at junction of liquids, red, green and violet rings. Hopkins and Cole showed reagent is actually glyoxlyic acid ($COOH-CHO$), prepared by treating oxalic acid with Na-amalgam or magnesium. This is added, followed by strong H_2SO_4, giving bluish-violet color.	Reaction due to tryptophane (see p. 106) and is usually negative.	

Aromatic antiseptics may be tested for separately. Phenol and thymol may be detected by the odor developed on heating with sulphuric acid. Salicylic acid may be detected as follows: Precipitate the gelatin from aqueous solution with an excess of alcohol or acetone—three or four volumes—and concentrate the filtrate to a small bulk. Then shake with ether, separate the ethereal layer, treat with dilute sodium hydroxide solution and faintly acidify with hydrochloric acid. A violet coloration on the addition of ferric chloride solution indicates the presence of salicylic acid. Methyl salicylate (oil of wintergreen) is readily detected, on warming the solution, by its characteristic odor.

Other characteristic color reactions for proteins, such as the Liebermann-Cole reaction,[1] the diazo-reaction of Ehrlich and Pauly,[2] and the glucosamin-test of Ehrlich,[3] are of minor importance for gelatin, and where positive effects are obtained the presence of aromatic antiseptics is to be suspected.[4] It will be seen that the majority of the color reactions of the proteins are due to groups belonging to aromatic bodies which probably are not native to gelatin.[5]

Precipitation Reactions. The precipitation reactions of gelatin, as of other proteins, are of a very various and variable nature. In principle some of them are relatively simple precipitations, due to change of solvent but greatly influenced by acidity, alkalinity or salinity. Others are precipitations (as, for instance, those by concentrated salt solutions), which may depend upon dehydration or upon the amphoteric nature of gelatin, and which are affected by acidity or alkalinity.

While the color reactions of the proteins are for the most part definitely chemical reactions for specific groups or radicles, in the precipitation reactions the general colloidal characteristics of the proteins are displayed. They are,

[1] Liebermann, L., cited by Mann, G., l. c., p. 9; Cole, S. W., J. Physiol. **30**: 311. 1903.

[2] Ehrlich, P., Zeits. klin. Medizin **5**: 285. 1882; Pauly, H., Zeits: physik. Chem. **42**: 508. 1904.

[3] Ehrlich, P., cited by Mann, G., Chemistry of the proteids, p. 10.

[4] Johns, C. O., and Jones, D. B., in a review of work on Folin and Macallum's colorimetric method for the estimation of tyrosine (J. Biol. Chem. **36**: 319. 1918) state that they "tested a number of high-grade samples of gelatin and did not find one which did not respond to the test for tyrosine with Mellon's reagent. Even gelatin prepared from carefully cleaned cartilaginous rings of ox trachea gave a decided test for tyrosine." Gortner and Holm (J. Amer. Chem. Soc. **42**: 632, 821, 1678. 1920; **39**: 2477. 1917) have shown that proteins apparently contain some unknown factor, perhaps an aldehyde, which induces humin formation. This unknown factor was present in their samples of gelatin. Hence the possibility of tyrosine and tryptophane occurring in gelatin must be considered, but only when aromatic antiseptics have not been added. Dr. Gortner states (in a private communication) that he does not believe that any gelatin has ever been prepared from which tryptophane or tyrosine are entirely absent. From the small amount of tryptophane present normally, it is doubtful that it belongs to the gelatin molecule. See also Salkowski, E., Zeits. physiol. Chem. **109**: 32. 1920.

[5] On the limits of sensitiveness of color reactions for albumins, see Rakuzin, M. H., Brando, E. M., and Pekarskaya, G. F., Chem. Abst. **10**: 1655. 1916.

therefore, affected by a variety of auxiliary physico-chemical conditions, and consequently will receive fuller treatment in Volume II.

PRECIPITATION REACTIONS

No.	Name of Reaction	Reagent and Reaction	Result and Significance	Remarks
1	Alcohol precipitation.	Ethyl alcohol, Methyl alcohol, Acetone and higher alcohols of fatty series.	Positive, in absence of excess acid or alkali. Least alcohol required at the isoelectric point $P_H = 4.8$	More complete, the greater the excess of alcohol. In alcohol-water and similar mixtures, suspensoids and suspensions are formed.
2	Ether	Ethyl and other ethers. Higher alcohols immiscible with water. Benzene. Benzene and light paraffins. When shaken up with dilute gelatin solutions the gelatin separates at the interface, collecting mostly in the non-aqueous layer.	Positive for gelatin. The maximum is again at the isoelectric point.[1]	This reaction overlaps with (1). It appears to be a typical colloid or capillary chemical reaction, depending upon alteration of surface or interfacial tension between the gelatin and its solvent or dispersion medium.
3	Heat coagulation	Aqueous solutions of albuminous substances heated to a certain temperature coagulate, as for instance, white of egg. Reaction complete only for slightly acid solutions; excess alkalinity or acidity prevents it, as does dialysis of the salts present.	Negative for gelatin.	Although this reaction is negative, use is sometimes made of it in clarification of gelatins by means of albumins. (cf. p. 30).

[1] Recent investigations by the writer show that the interfacial tension between gelatin sols and toluol (probably also ether, etc.) is a maximum at $P_H = 4.8$, the isoelectric point, where the solubility is least.

No.	Name of Reaction	Reagent and Reaction	Result and Significance	Remarks
4	Tannin reaction (Alkaloid reaction).	Tannin (tannic acid) in water solution, gives a leathery precipitate, resoluble in excess of either component or by heating. Salt-free gelatin said to give no precipitate. (For quantitative uses see p. 131).	Positive, and relatively very sensitive.	This reaction is used for quantitative determinations. It may be regarded as an adsorption reaction, the mutual precipitation of oppositely charged colloids, or as a partly chemical reaction due to the basic character of gelatin. (See Vol. II.)
5	Phosphotungstic and phosphomolybdic acids (Alkaloid reaction).	50 gms. phosphotungstic acid and 30 cc. concentrated sulphuric acid are dissolved in water to make 1 liter. Similarly for phosphomolybdic, but using nitric acid. White curdy precipitate with phosphotungstic; cream colored, phosphomolybdic.	Positive and relatively sensitive.	The precipitate of peptones (break-down products of albumins) with phosphotungstic acid is (incompletely) soluble in acetone and alcohol.
6	Ferrocyanic acid (Alkaloid reaction).	Usually employed as acetic acid plus potassium ferrocyanide; whitish precipitate.	Formerly stated to be negative, but Mörner found that under 30° C. a precipitate may be formed in very dilute solutions, soluble in excess of either gelatin or ferrocyanic acid. Further, the precipitation is prevented by salts, organic acids or bases, and urea. Gelatoses give no precipitate. This reaction, as in the	Insufficient attention appears to have been paid to presence of calcium compounds in gelatin, which give precipitates of calcium ferrocyanide. Experiments in this laboratory show that a calcium content considerably less than that due to saturated $CaSO_4$ (.25 gms. per 100)

No.	Name of Reaction	Reagent and Reaction	Result and Significance	Remarks
6			foregoing, may be regarded as formation of an insoluble salt of the base gelatin, or a colloid adsorption.	gives a strong reaction with ferrocyanic acid.
7	Picric acid (Alkaloid reaction)[1]	2–5% picric acid in water, or Esbach's reagent (10 gms. picric acid 20 gms. citric acid in 1 liter of water). Yellowish precipitate.	Reaction is positive but precipitation is very incomplete, a gelatinous coagulum being formed which dissolves on heating and reappears on cooling.	
8	Tri-chloracetic acid (Alkaloid reaction).	100 gms. dissolved in water to 1 liter. White precipitate.	Positive; precipitate redissolves in excess acid.	
9	Chromic acid	Concentrated chromic acid solution in water. Coagulum on standing.	Reaction very incomplete, coagulum dissolves on heating, reappears on cooling.	Compare further action of bichromates in light, Chap. I p. 18.
10	Gold chloride	$HAuCl_4$ aq. concentrated solution. Coagulum. Reduction of gold to colloidal gold also occurs.	Positive—is probably partly a tanning reaction, i. e., adsorption of hydrolyzed auric oxide or hydrous oxide. Precipitate soluble at boiling temperature, reappears on cooling.	This reaction is to be reckoned with the colloid ones. The dissolution on heating is probably a peptization.
11	Platinic chloride	$HPtCl_4$ aq. concentrated solution. Coagulum.	As with gold.	*v. supra.*

[1] A physico-chemical study of the purification of proteins by alkaloid reagents has been made by Hanzlich (Hanzlich, P. J., J. Biol. Chem. **20**: 13. 1915), chiefly with reference to hydrogen-ion concentration, salt effect, and the iso-electric point. His results will be dealt with in Volume II, as also those of J. Loeb and others.

No.	Name of Reaction	Reagent and Reaction	Result and Significance	Remarks
12	Stannous chloride	$SnCl_2$ aq. plus HCl diluted.	*v. supra.*	*v. supra.*
13	Silver nitrate, ammoniacal.	Equal parts of 1 or 2% gelatin solution and 10% ammoniacal silver nitrate solution are mixed and placed in the dark.	Reduction in less than 30 minutes denotes poor quality gelatin.	The specific working of this test must be adjusted to practical requirements.

Certain other "tanning" or coagulation reactions with metal salts, halogens, and formaldehyde, and with quinonoid oxidation products of phenolic reducing agents are of especial importance in photographic practice, and will be dealt with later.

PROTEIN REACTIONS OF COMMERCIAL GELATIN

The protein reactions of some twenty different commercial gelatins as determined in the Eastman Research Laboratory, were as follows:

	Test	Result	Remarks
1	Biuret	All positive	No difference in intensity
2	Xantho-proteic	All positive	Small differences
3	Millon	All positive	Some differences
4	Molisch	Very faint or indeterminate	
5	Sulphide	One positive, rest negative	
6	Ammoniacal silver nitrate	Generally faint	Considerable differences, especially on heating
7	Tannin	All positive	
8	Phosphotungstic	All positive	
9	Tri-chlor-acetic	Generally positive	Some differences
10	Esbach	All positive	

QUANTITATIVE METHODS

The quantitative analysis of gelatin may be undertaken from either of two standpoints. For technical purposes, relatively rapid methods are necessary, varying somewhat according to the use—culinary, photographic, or otherwise—for which the gelatin (or glue) is intended. In such an analysis gelatin is regarded as a proximate chemical unit, and there are required certain definite proportions of gelatin, water, and foreign matters.

On the other hand, from the standpoints of organic and biochemistry, gelatin may be fractionally analyzed and decomposed. The principal object here, as with other proteins, is the determination of chemical constitutional factors, and, correlated with these, the vital functions of proteins in life processes.[1] The very specialized research in this direction lies outside the scope of this monograph, but certain features and results are of interest and importance for the technologist, and will be discussed briefly in a later paragraph.

Technochemical methods will be considered first, with especial reference to the tests for photographic gelatins, and those which are important for gelatins intended for use in paper-making.

TECHNOCHEMICAL ANALYSIS

1. Moisture determination.

As already stated, air-dry commercial gelatins contain from six to twenty per cent of moisture. In determining the amount of moisture, it is important to bear in mind that it is very difficult to determine the water by heating at a given temperature without either heating too high, and initiating decomposition, or heating insufficiently to overcome adsorption of the water. Practically, therefore, the determination of the moisture content of gelatin, as of similar bodies, must be based on definite conventional conditions. The moisture content is defined as the amount of water removed by heating a sample of the finely divided air-dry material to constant weight at 100-110° C.

A. *Method of determining moisture content, using powdered gelatin.*

The usual method consists in mechanically granulating or powdering the gelatin, using some type of drug mill. About 2 gms. of powdered gelatin or glue are dried in an oven at 100-

[1] Plimmer, R. H. A., The chemical constitution of the proteins. Part I. Analysis.

105° C. for one hour, cooled in a dessicator, and weighed. It may then be dried again for another hour and re-weighed, and this repeated until sufficient approximation to "constant weight" is reached.

As this "anhydrous gelatin" powder is hygroscopic, closed weighing bottles should be used.

B. *Method of determining moisture content, using a solution and drying in vacuo.*

Another useful method is one similar to that used in milk analysis. One or two grams of a ten per cent solution of the gelatin is weighed out in a tared flat covered dish, either a Petri dish or, better, an aluminium vessel, and evaporated in a vacuum oven at 105-110° C. This method is rapid (taking two or three hours) and, where a suitably heated vacuum oven (40 mm. at least) is available, is very satisfactory.

C. *Estimation of moisture as acetylene by decomposition of carbide.*

This method, which is useful for determining moisture in materials such as leather, gelatin, etc., which decompose if overheated, consists in mixing the finely divided material with purified sand and calcium carbide, and heating the mixture at a fixed temperature. The tube containing the sample is fastened to an inclined reflux condenser and connected with a gas-measuring burette. It is heated in a toluene-containing jacket, so that the temperature remains constant at 110° C. The analysis is complete when the volume of acetylene generated ceases to increase (usually after about two hours). The volume of acetylene at *n.t.p.*[1] is then readily converted to grams water. We have found this method very rapid and satisfactory.

In any case, these methods are to be regarded only as conventional approximations. Benedict and Manning[2] have shown that materials such as gelatin, ossein, elastin, etc., dried in a high vacuum at room temperature for two weeks, regained water when kept five hours in an air-bath at 100° C., losing it again in a high vacuum. Heating in an air-bath at 110° C. may give an error of one per cent, causing an error of 0.5 per cent in carbon, and an appreciable error in nitrogen.

More recently the precise determination of moisture in amorphous and colloidal organic materials has been very fully investigated by Hulett and his collaborators.

[1] *N.t.p.* = normal temperature and pressure.

[2] Benedict, F. G., and Manning, C. R., Amer. J. Physiol, **18**: 213. 1907.

D. *Moisture content by method of G. A. Hulett.*

For exact work the method developed by Hulett[1] and his collaborators for coal and cereals should be employed. They point out that the "hygroscopic" moisture adsorbed on the surface (including inner pore space of gels) of colloids has a vapor pressure much lower than water in bulk, and if adsorbed as a monomolecular layer can not be removed by dessicating agents *in vacuo*. To distinguish decomposition from release of adsorbed moisture they heat the material in a very high vacuum for definite periods of time. The moisture liberated is condensed quantitatively in a small tube cooled with carbon dioxide and weighed. This is done for a series of temperatures, and the moisture-temperature curves plotted. These indicate by "breaks" the temperature at which decomposition becomes measurable when the sample is heated for three hours or more. It does not necessarily show the total amount of moisture that is actually present in the material, but does show the time and temperature to which it may safely be heated. By extrapolation of the flat part of the curves beyond the breaks an idea of the total amount of moisture present may be obtained. For a full description of the method and apparatus reference should be made to the papers cited.

2. Determination of Ash and Total Inorganic Matter.

The inorganic or mineral constituents of gelatin are determined as ash by ignition, using the anhydrous gelatin from the moisture determination. One to two grams of this are incinerated in a fairly large crucible, precautions being taken that, in the early stage of giving off volatiles,[2] no solid material is mechanically carried off, and, in the later stage, that complete oxidation of the carbon is effected. (This is assisted by the addition of a little nitric acid.)

It is commonly stated[3] that the ash from bone gelatin or glue will, because of the phosphates present, fuse in a bunsen flame, and give an acid or neutral reaction to litmus, and that hide gelatin or glue gives an alkaline reaction and is infusible, owing to the presence of lime. However, as pointed out by Alexander,[4] the character of the ash is determined almost as much by the treatment in manufacture as by the source of the raw stock.

[1] Mack, E., and Hulett, G. A., Amer. J. Sci. **43**: 89. 1917; ibid. **45**: 174. 1918; and Nelson, O. A., and Hulett, G. A., J. Ind. Eng. Chem., **12**: 40. 1920.

[2] A preliminary determination of "volatile matter," as for fuel, etc., may be made, but has little general interest.

[3] Cf. Kissling, R., J. Soc. Chem. Ind. **6**: 565. 1887.

[4] Alexander, J., in Allen's Commercial Organic Analysis.

A number of analyses of specimens of hide and of bone gelatin, made in this Laboratory, showed that no relation between the phosphate content and the stock exists. This result is in agreement with similar analyses by Dr. E. T. Oakes.[1]

The ash figure varies considerably for different products. Good gelatins should contain not more than two per cent ash, while glues may have as high as six or eight per cent. The nature of the ash is in many respects more important than the amount, in view of the relation of electrolyte content to colloid condition. This will be dealt with more fully in Volume II.

Ash constituents. Complete analysis of the ash is seldom attempted or necessary. Requirements vary, however, depending upon the special purpose for which the gelatin is intended. The mineral substances to be tested for in gelatins destined for photographic uses and directions for the tests will be considered in a later paragraph. (See p. 117).

3. Determination of Acidity or Alkalinity.

If the gelatin gives a marked acid reaction, 10 gms. may be dissolved and made up to 200 cc.—(*ca.* 5 per cent solution)—and the total acidity determined by titration of an aliquot part with N/5 to N/2 alkali, using litmus paper as indicator. Volatile acids are then removed by evaporating a measured portion, and their amount determined by dissolving the residue and titrating the solution thus obtained against the alkali used in the first determination. The difference between the amounts of acid found in the two determinations gives the amount of volatile acids present.

Sulphurous acid may be determined directly at this time by boiling or steam-distilling with phosphoric acid, passing the distillate in a current of carbon dioxide into standard iodine solution, and titrating back with thiosulphate.

Poetschke[2] calls attention to the precautions necessary for accurate determination of small amounts of sulphur dioxide in gelatin. Error due to the presence of other reducing substances may be eliminated by replacing titration with thiosulphate by gravimetric precipitation of the sulphate formed. Also, precautions should be taken against casual admission of sulphur-dioxide from burners.

Of samples from all sources examined in 1912:—

43% contained 100 to 500 parts SO_2 per million;
3.3% contained over 1,000 parts SO_2 per million;
48% contained less than 100 parts SO_2 per million.

[1] Private communication.

[2] Poetschke, P., J. Ind. Eng. Chem. 5: 980. 1913.

Hydrogen peroxide was found in many of the samples containing less than 10 parts per million, having been added to destroy the sulphur dioxide.

A very complete discussion of the occurrence and determination of sulphurous acid in gelatin was given in 1904 by W. Lange,[1] who points out that its use in manufacture as a bleaching agent and as an extractive assure its almost constant presence in commercial gelatins. For qualitative detection, the distillation with phosphoric acid may be used, with a piece of potassium iodate-starch paper as an indicator. A transient blue color, due to liberation of iodine, is given by as small amounts as .002 per cent sulphurous acid. Lange attempted to replace the somewhat laborious distillation process by direct titration of the gelatin-sulphurous acid solution with 1/10 normal iodine solution. But, although pure gelatin to which different amounts of sulphurous acid had been added gave a sharp end-point with starch in this way, commercial gelatins gave more or less indefinite end-points, the starch-iodine color not persisting definitely.

Fairly consistent results, agreeing reasonably with those obtained gravimetrically by the distillation process, have been obtained by taking as a measure of the sulphurous acid content that amount of iodine consumed up to the point when two drops of a 1/10 normal iodine solution give a definite blue coloration lasting at least ten seconds. Generally, this iodine number is a useful value, giving fairly accurate results for high amounts of sulphurous acid. Some gelatins, however, evidently contain something beside sulphurous acid which combines with iodine,[2] not always formaldehyde.

Volatile fatty acids may be determined by the method of Duclaux,[3] preferably as modified for steam distillation by Dyer.[4] The probability of their occurrence is not considerable,[5] although Lange, in his work on sulphurous acids just discussed, states that most brands of gelatin contain more or less small amounts of soluble and insoluble volatile fatty acids, which come off in the distillation with phosphoric acid. In determining sulphurous acid in photographic gelatins we have found some indications of this kind in this laboratory, viz., solid fatty acids of soaps.

[1] Lange, W., Die schweflige Säure und ihre Verbindungen mit Aldehyden und Ketonen (Part II., p. 168), from the "Arbeiten aus den Kaiserlichen Gesundheitsamte, Vol. 21, 1904."

[2] See later section on the action of halogens, p. 154.

[3] Duclaux, E., Traitê de microbiologie. III, p. 394. 1900.

[4] Dyer, D. C., J. Biol. Chem. 28: 445. 1916–1917.

[5] They may occur either from treatment, or as the result of putrefactive, etc., changes.

In our investigation of sulphurous acid determination we find that hydrochloric acid is preferable, in distillation, to phosphoric, since foaming is rapidly eliminated. At the same time, it is then necessary to determine the sulphurous acid, not by titration of residual iodine (which is always uncertain), but by precipitation of the sulphuric acid formed as $BaSO_4$, and either gravimetric or nephelometric determination of this. The direct iodine number has a certain value but is not simply a measure of sulphurous acid[1] in gelatins.

The estimation of amino-acids and other organic acids and bases derived from protein breakdown is considered later.[2]

Alkalinity may be due to excess of lime or to putrefactive decomposition, the latter being readily detected by odor. Alkalinity, like acidity, may be determined by titration, except in the case of volatile alkalies such as ammonia, where the determination is made by distillation or aeration processes.

Although the direct titration method is sufficient for usual technical requirements, the influence of acidity or alkalinity,—i. e., in physico-chemical terminology, hydrogen-ion concentration,—on the properties of gelatin is so great that more refined methods are necessary where it is desired to determine the neutral point of a gelatin and its actual hydrogen-ion concentration. These will be discussed in Volume II.

4. Determination of Organic Matter.

Determination of volatile (organic) matter and of fixed carbon may be interpolated in the procedure for ascertaining the amounts of moisture and of ash, described above. The crucible containing the anhydrous gelatin from the moisture determination is weighed, then lightly covered and heated four or five minutes over a bunsen burner and the same length of time in a blast flame. Next it is cooled and weighed, the loss in weight indicating the amount of volatile matter present in the gelatin.

Ignition is then continued until all the carbon is burned, when the crucible is again cooled and weighed, the loss in weight here representing the amount of fixed carbon, the weight of matter remaining, the ash, since air-dry gelatin = moisture + volatile matter + fixed carbon + ash. According

[1] Gutbier, A. Sauer E., and Brintzinger, H., (Koll. Zeits. **29**: 130. 1921) state that direct iodine titration can be used for bone glues but not for hide glues. For bone gelatins we find, however, considerable discrepancy between the SO_2 evaluated from the iodine number, and by distillation, the former being much larger; this discrepancy is of the same order as for hide gelatins.

[2] See p. 174.

to W. A. Scott (*l. c.*), the larger the amount of fixed carbon present, the stronger and more viscous the gelatin or glue,—i. e., the more "body" it will have.

It must be noted that such determinations of volatile matter and fixed carbon require, as in coal analysis, very rigorous conventions in working for concordant and comparable results.

TESTS OF ESPECIAL IMPORTANCE FOR GELATINS FOR PHOTOGRAPHIC USES

Apart from physical and colloid-chemical characteristics (to be considered later), there are certain special requirements for photographic gelatins.[1] They should give clear and nearly neutral solutions; the moisture content should not be above twenty per cent, and lower for "soft" gelatins; and the ash should not exceed two per cent.

The ash should be dissolved in nitric acid and tested for:

METALS AND BASES	ACIDS
Sodium	Phosphates
Calcium	Sulphates
Zinc	Chlorides
Lead	Borates
Iron	Sulphites
Copper	
Aluminium	
Nickel	

Bases and heavy metals should not be present in more than mere traces, as they are harmful to photographic emulsions. Sulphates and chlorides should also be low. A delicate test for chlorides given by Lüppo-Cramer is as follows: Some 6 to 8 per cent gelatin solution is coated on a small glass plate, a drop of 10 per cent $AgNO_3$ solution placed on it, and the plate exposed to light. If chlorides are present the drop becomes surrounded by rose to purple-colored rings. This is due to the silver nitrate diffusing into the surrounding chloride-containing gelatin and forming Liesegang rings of silver chloride (see Vol. II), which pass to photochloride in light.

Gelatins containing much chondrin (see p. 141) are objectionable as they are liable to gel in hot solution, especially when alum is present. They may be distinguished by adding a saturated solution of chrome alum to a hot ten per cent solution of gelatin.

Detection of phenolic antiseptics and heavy metals has already been discussed.

[1] Wandrowsky, H., Phot. Ind. 1916: 4 and 22. 1916.

Formaldehyde, even in small quantities, tends to make the gelatin insoluble. To detect this, heat about 10 gms. of gelatin and 15 to 20 cc. of water to about 105° C. on an oil-bath and distill with steam. Add to the distillate a few drops of dilute phenol solution followed by concentrated sulphuric acid (see Molisch's test, p. 105). Rose color indicates the presence of formaldehyde.[1]

Where dyes are to be employed the presence of sulphites or sulphur dioxide is objectionable and should be determined as directed above.

The chemical test of most general importance for photographic gelatins is silver reduction, for determining which ammoniacal silver nitrate is used. The reagent is made by adding ammonia to a 10 per cent silver-nitrate solution until the precipitate is just redissolved. Equal quantities of this and some 1 or 2 per cent gelatin solution are mixed, the mixture left in the dark for a definite time, and any darkening or precipitation observed.[2]

THE GRAVIMETRIC ANALYSIS OF THE INORGANIC (ASH) CONSTITUENTS OF GELATINS

The following scheme for quantitative analysis is suitable for gelatins for photographic purposes and for paper making. This was worked out in this Laboratory by the writer and Mr. H. Hudson, and is based on a system described by Sindall and Bacon[3] for the examination of gelatins in respect of their suitability for papermaking. Our object was to secure a reliable procedure covering the quantitative analysis of all the normal mineral constituents, which, though not in itself suitable for control and routine work, being too lengthy, could, however, be used as a reliable standard in developing a method or methods of rapid analysis not involving gravimetric practices. (See p. 121.)

The outline flow-sheet of the procedure is given in Fig. 21. The details of the estimations are as follows:

A weighed sample B of dry gelatin is ignited in a weighed porcelain crucible over a bunsen burner. Since the gelatin swells and spatters till all the volatiles are driven off, care must be exercised to prevent loss. After driving off the volatile matter a cover is placed on the crucible, leaving sufficient opening for decarbonization. The ignition takes about one

[1] Cf. Acree, S. F., J. Biol. Chem. **2**: 145. 1906-7.

[2] Exact conditions for this test can not be given, since the specifications will vary according to the particular photographic use of the gelatin

[3] Sindall, R. W., and Bacon, W., Analyst **39**: 20. 1914.

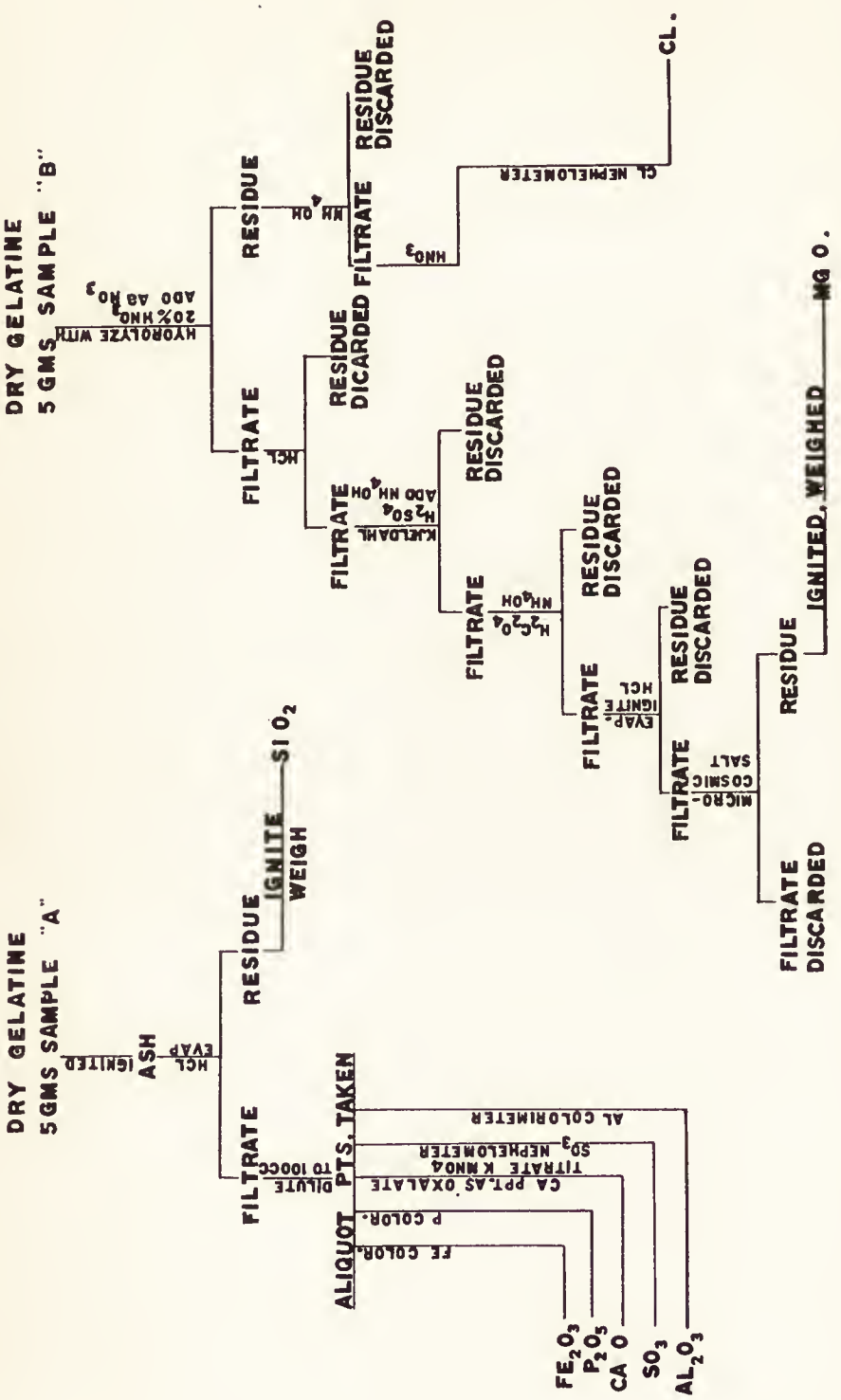


FIG. 21
Flow sheet for gravimetric analysis of ash of gelatin

hour. Crucible and contents are cooled in a dessicator and weighed, giving the ash content of the gelatin.[1] The ash is then dissolved with concentrated hydrochloric acid, the solution evaporated to dryness on a steam bath, remoistened with hydrochloric acid, and again evaporated to dryness. The beaker containing the residue is heated in an oven for ten or fifteen minutes at 110-120° C., the residue taken up with a little hydrochloric acid and water, and the insoluble matter filtered off on quantitative paper and weighed. This gives SiO_2. The filtrate is then made strongly alkaline with ammonia and boiled, and the precipitate filtered off, ignited, and weighed. This consists of Al_2O_3, Fe_2O_3, P_2O_5 and a trace of CaO.[2] (This may not contain all the P_2O_5). After acidifying the filtrate with hydrochloric acid a solution of oxalic acid is added, and the liquid brought to the boil. Then ammonia in small quantities is added until the reaction is alkaline and a precipitate forms, after which the mixture is boiled a few minutes. After standing about one hour, the precipitate of CaC_2O_4 is filtered off, ignited, and weighed as CaO.

The filtrate from the calcium precipitate is evaporated to dryness, ignited in a small evaporating dish to drive off ammonium salts, and the residue thus obtained taken up with hydrochloric acid and water. This is then filtered to remove carbonaceous matter, after which a microcosmic salt solution is added to the filtrate in an erlenmeyer flask. This solution is boiled and ammonia added. As soon as the flask has cooled it is stoppered and shaken for about ten minutes, after which it is allowed to stand two or three hours, and the precipitate then filtered off, ignited, and weighed as $Mg_2P_2O_7$, giving MgO.

A second sample (A) of five grams is ignited and the ash determined to check sample (B). The ash is taken up as before and SiO_2 determined after dehydration as in (B). Ammonia is added to the filtrate and the precipitate which forms is filtered, washed, and redissolved in a little hydrochloric acid. This solution is made up to a definite volume (100 cc.), and aliquot portions taken. In one portion iron is determined colorimetrically, and calculated as Fe_2O_3. To a second part ammonium molybdate and nitric acid are added, precipitating the phosphorus as ammonium phosphomolybdate, which is filtered, gently ignited, and weighed as $24MoO_3.P_2O_5$, from which P_2O_5 is calculated. The Al_2O_3 is determined by difference. The filtrate a_2 from the iron : alumina separation is acidified, barium

[1] Although chlorides are liable to be vaporized in this way, we have found little actual loss. Leaching out from the carbonized material was not found satisfactory. We prefer the independent determination without ashing.

[2] Practically negligible.

nitrate in hot solution added, and the solution boiled. After standing one hour the precipitate formed is filtered off, ignited and weighed as $BaSO_4$, from which SO_3 is calculated. Ammonium molybdate and nitric acid are added to the filtrate and the precipitate of ammonium phosphomolybdate ignited and weighed as before, the calculated P_2O_5 being added to the previous amount to give total P_2O_5.

SUPPLEMENTARY GRAVIMETRIC DETERMINATIONS FOR CHLORIDES AND ALKALI METALS

Chlorides. Chlorine is determined by deproteinization with 20 per cent nitric acid, precipitation with silver nitrate, filtration, re-solution in strong aqueous ammonia to free from humin and similar substances, reprecipitation, ignition, and weighing as silver chloride.

Alkali Metals. Sodium and potassium are determined as follows: Organic matter is destroyed by wet combustion with nitro-sulphuric acid, and the residue is taken up with hydrochloric acid. Barium chloride is then added to remove sulphates, and barium hydrate to precipitate magnesia. Calcium and barium oxides are removed as carbonates. The filtrate is treated twice with $(NH_4)_2CO_3 + NH_4OH$ aq., and then taken up with hydrochloric acid. Total alkali chlorides may be determined by evaporation to dryness.[1] These may be separately estimated as usual if required.

THE RAPID ANALYSIS OF ASH CONSTITUENTS

As already stated, the gravimetric analysis described above was worked out and used as a standard for comparing more rapid methods. For the latter we have applied colorimetric and nephelometric methods as far as possible, using the very satisfactory and convenient Kober nephelo-colorimeter. The methods used are not individually novel.

Procedure. The flow-sheet (Fig. 22) gives the outline of the operations. For sample A, 5 grams of air-dried gelatin are ignited as before in a weighed crucible, and the ash determined. The ash is then treated as in the gravimetric determination to separate silica, which may be determined gravimetrically if desired. The filtrate is diluted to 100 cc. in a volumetric flask and aliquot portions removed for the following determinations. The acidity of the reference standards should be nearly the same as that of the (aliquot) solutions under examination.

[1] See Abderhalden, l. c., Arbeitsmethoden. Vol. V., Pt. I, p. 460.

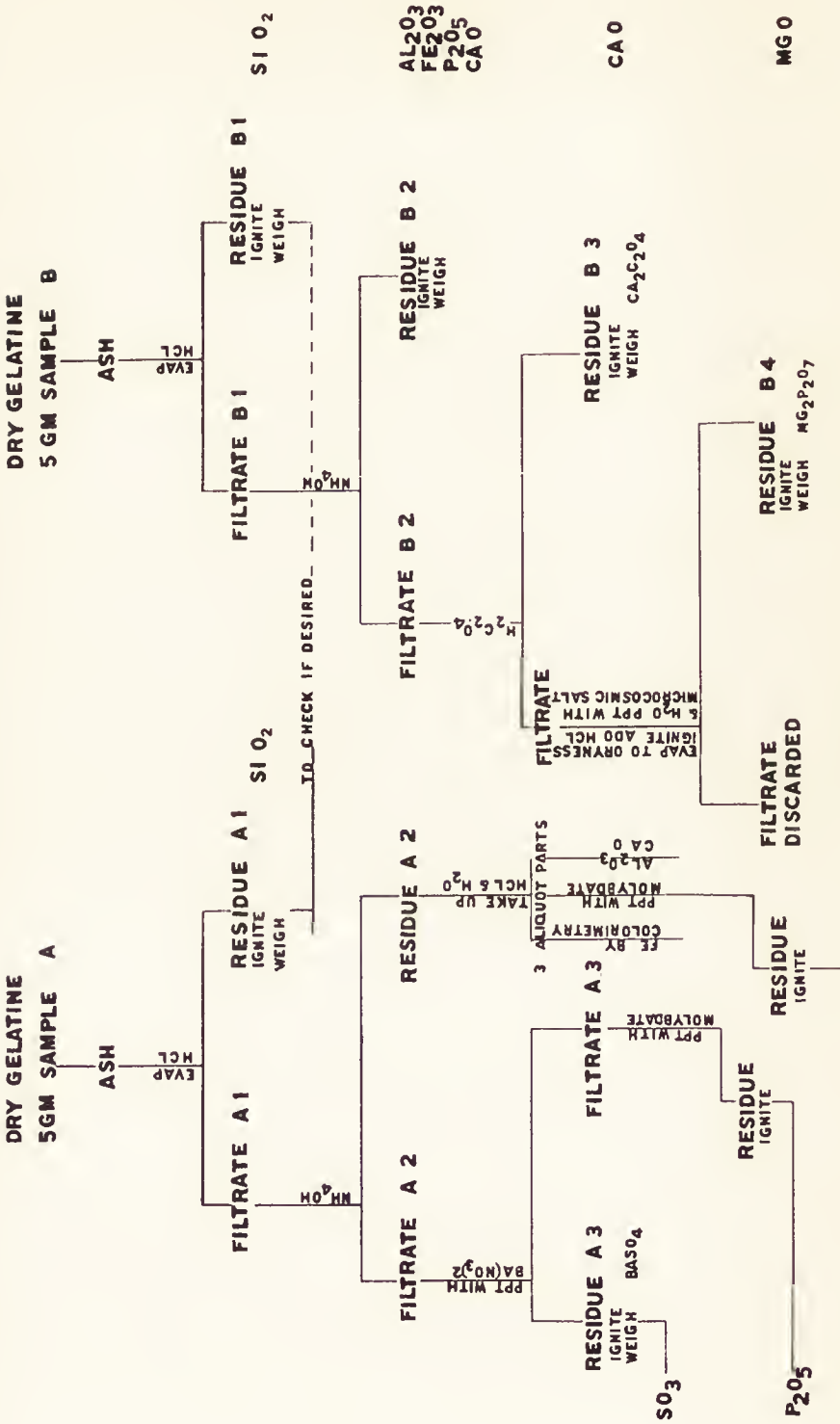


FIG. 22
Flow sheet for rapid analysis of mineral part of gelatin

IRON is determined colorimetrically as ferric-ferrocyanide (Prussian blue). 20 cc. of the solution for analysis are put into a 50 cc. Nessler tube and .5 cc. of a one per cent potassium ferrocyanide solution added. The reference color standard is prepared by pipetting 2 cc. of an iron solution containing 0.100 mgms. iron per cc. into a 50 cc. Nessler tube, treating with standard acid till the acidity equals that of the unknown, and then adding the potassium ferrocyanide. After standing five minutes the solutions are made up to 50 cc. and comparisons made in the Kober-Dubosc colorimeter. The unknown solution is placed on the right side and the setting kept constant at 100 mm., the match being made by adjusting the height of the column of known solution (reference standard) on the left hand side. From a calibration curve (see Fig. 23), the mgms. of iron in the 50 cc. tube of unknown can be read directly from the scale readings in mm. of the known solution matching it. ALUMINIUM can be determined by difference, subtracting the iron found as above from the combined iron, aluminium and phosphorous oxide of a second 5 gram sample B. (See diagram, Fig. 22.)

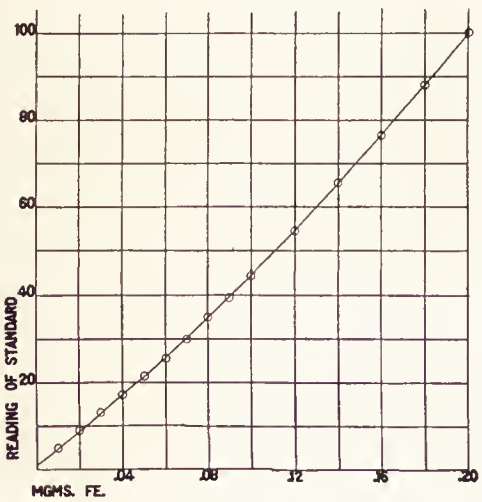


FIG. 23
Calibration curve for reading iron content

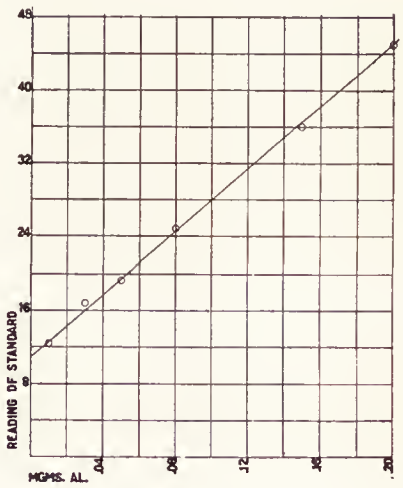


FIG. 24
Calibration curve for reading aluminium content

A colorimetric method proposed by Atack[1] is being investigated with a view to applying it to determinations of aluminium in gelatin. The reagent used is a one per cent solution of Alizarine (Red) S, the sodium salt of alizarine mono-sulphuric acid. Atack claims that by carefully controlling the produc-

[1] Atack, F. W., J. Soc. Chem. Ind., **34**: 936. 1915.

tion of the color—due to a lake—with aluminium, the method can be used for colorimetric analysis, and will readily detect one part in ten million. The procedure is as follows: The solution for analysis and a standard containing a known amount are placed in 50 cc. tubes and acidified with hydrochloric or sulphuric acid, and 10 cc. of glycerin and 5 cc. of the one per cent alizarine red solution are added. The solutions are diluted to about 40 cc. and made slightly ammoniacal. After standing five minutes, they are acidified with acetic acid, the alizarine S acting as indicator, until no further change in color occurs. The solutions are then compared in the colorimeter (after making up to 50 cc.), the unknown being placed on the right, with setting at 45 mm. The known (standard) solution is placed on the left and adjusted to match. The scale reading for the standard gives, through a calibration curve, mgms. of alumina (Al_2O_3) in 50 cc. (See Fig. 24.)

However, contrary to Atack's statements, we find that moderate amounts of iron, calcium, chromium and phosphate interfere with the coloration, while citrate destroys it entirely. A large excess of acetic acid also destroys the red color and it appears that further research is necessary before this attractive method can be used satisfactorily.

Phosphorus as P_2O_5. 5 or 10 cc. of the solution for analysis are pipetted into a 50 cc. colorimeter tube, and treated according to the method of Bell and Doisy.[1] A standard monopotassium phosphate solution containing 0.10 mgms. phosphorus per cc. is used. 4 cc. of this standard are pipetted into a 50 cc. colorimeter tube, and the acidity made equal to that of the unknown. Then 5 cc. of an ammonium molybdate solution containing 50 gms. ammonium molybdate per liter of N/1 H_2SO_4 are added to each tube. Following this 5 cc. of a hydroquinone solution, containing 20 gms. of pure hydroquinone and 1 cc. of concentrated H_2SO_4 per liter, are added. After standing 5 minutes, 25 cc. of a carbonate-sulphite solution[2] are added

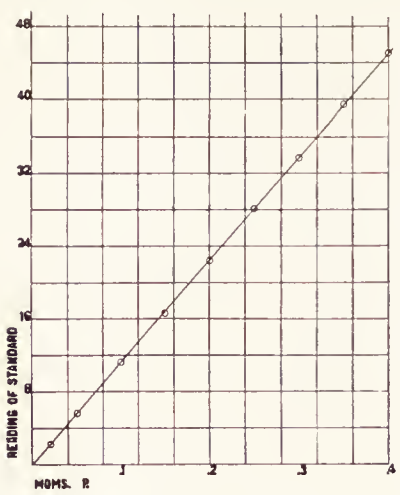


Fig. 25
Calibration curve for reading phosphorus content

[1] Bell, R. D., and Doisy, E. A., J. Biol. Chem. **44:** 55. 1920.

[2] Prepared as follows: 75 gms. of sodium sulphite in 500 cc. of water are added to 2 liters of 20 per cent sodium carbonate.

and the tubes filled to the 50 cc. mark with water. A blue color is developed and a color match made as before, the unknown being placed on the right side and set at 45 mm., and adjustment made to color match with the standard on the left. The reading then gives mgms. phosphorus per 50 cc. unknown, from a calibration curve. (See Fig. 25.) The phosphorus value can be converted to P_2O_5.

Calcium. The calcium is precipitated as oxalate by adding oxalic acid to a 5 cc. portion of the solution for analysis, and boiling. Ammonia is then added in small quantities to an alkaline reaction, and the calcium precipitated. Ammonium oxalate solution is added and the mixture boiled. After cooling, the precipitate is filtered off and washed until free from soluble oxalates. Then it is transferred to a beaker, dissolved in dilute sulphuric acid, and titrated with N/10 or N/1 potassium permanganate. From the CaC_2O_4 thus determined, calcium is calculated as oxide. We have also tested the nephelometric method of estimating calcium as stearate, but at present the volumetric method given seems preferable.[1]

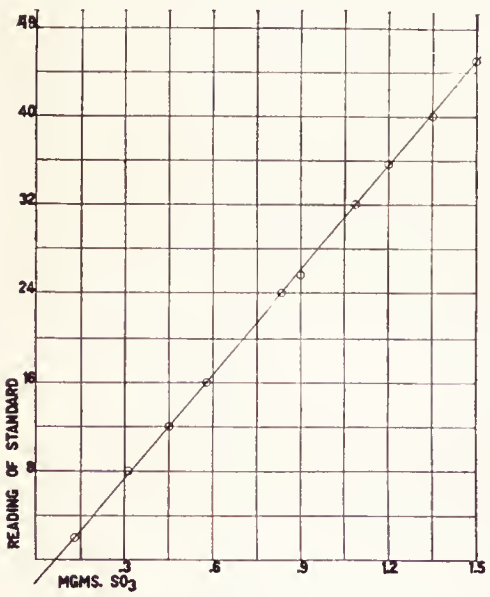


Fig. 26
Calibration curve for reading sulphate content

Sulphates. A 5 cc. portion of the solution for analysis is delivered into one 50 cc. tube containing 25 cc. peptizing solution made in the proportions of 20 gms. citric acid and 2 gms. potassium citrate per 100 cc. For the standard suspension 5 cc. of a standard ammonium sulphate solution, containing 0.30 mgms. SO_3 per cc., are delivered into the other tube, and to each tube are added, with stirring, 10 cc. of 10 per cent $BaCl_2$ solution. The acidity of the two solutions should be the same. The contents of the tubes are diluted to the 50 cc. mark, and the mixtures digested at 40° C. for

[1] For the determination of calcium, magnesium, sodium, and potassium in gelatin and cognate materials, the following may be consulted: Marriott, W. McK., and Howland, J., J. Biol. Chem. **32**: 233. 1917. [Decolorization of ferric thiocynate by oxalates and phosphates]. Halverson, J. O., and Bergeim, O., J. Biol. Chem. **32**: 159. 1917. [Deproteinization, precipitation as oxalate, titration with permanganate]. Lyman, H., J. Biol. Chem. **21**: 551. 1915; ibid. **29**: 169. 1917. [Nephelometrically as calcium soap]. McCrudden, F. H., and Sargent, C. S., J. Biol. Chem. **33**: 235. 1918. Kramer, B., and Tisdall, F. F., J. Biol. Chem. **46**: 339. 1921. [With sodium cobalt nitrite]. Ibid. **48**: 223. 1921.

30 minutes. The unknown solution is then placed in the nephelometer on the right side, and set at 45 mm., and a photometric match made by adjusting the height of the standard in the left. From a calibration curve the scale reading in mm. gives mgms. SO_3 per 50 cc. (See Fig. 26.)

Chlorides. A second 5 gram B of gelatin is hydrolyzed with 20 per cent nitric acid for 30 minutes, a solution of silver nitrate added, and the mixture boiled. After cooling, the precipitated silver chloride, contaminated with humic substances and detritus from the gelatin, is filtered off on paper, washed, and the silver chloride dissolved in about 10 cc. of 20 per cent ammonia. The solution and washings are transferred to a 50 cc. colorimeter tube and acidified with nitric acid. The suspension of silver chloride is digested at 40° C. for 30 minutes, and then matched in the nephelometer with a standard silver chloride suspension. This latter is prepared by pipetting 5 cc. of a potassium chloride solution containing 0.20 mgms. potassium chloride per cc. into a 50 cc. tube, adding 2 cc. N/10 silver nitrate, dissolving the precipitate in the same amount of ammonia as used for the unknown, reprecipitating with the same amount of nitric acid, making up to 50 cc., and digesting as before. The unknown is placed on the right side of the nephelometer, set at 45 mm., and the photometric match made by adjusting the standard. Fig. 27 shows the calibration curve prepared for this estimation.

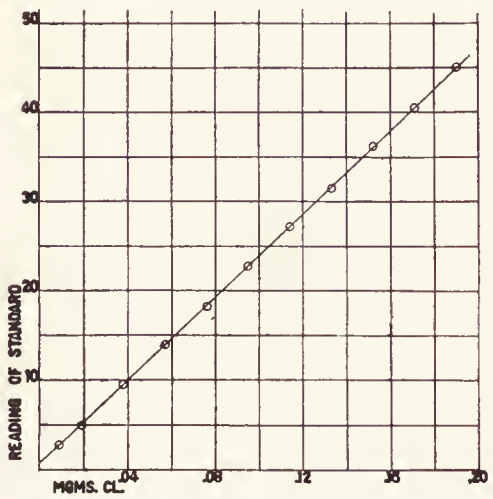


Fig. 27
Calibration curve for reading chlorine content

Total Oxides and Alumina. The filtrate from the chloride precipitation is now treated with excess of hydrochloric acid and the silver chloride precipitate washed well. The filtrate and washings are transferred to a Kjeldahl flask and decarbonization effected by boiling with 40 cc. concentrated sulphuric acid. The solution is transferred to a beaker, ammonia added, the solution boiled, and the precipitate filtered off and ignited. It is weighed as $Al_2O_3 + Fe_2O_3 + P_2O_5$. By subtracting the

amounts of Fe_2O_3 and P_2O_5 as found in the A-determination, Al_2O_3 is obtained as difference.

MAGNESIUM. The filtrate from the above is acidified, treated with oxalic acid solution, and brought to the boil, and ammonia added in small quantities until the solution is alkaline. Then it is again boiled, cooled, and the precipitate filtered off, washed and rejected. The filtrate and washings are evaporated to dryness in a porcelain dish and ignited to drive off ammonium salts. The residue is taken up with hydrochloric acid and filtered, and microcosmic salt solution and ammonia added to the filtrate. This is shaken for ten minutes and cooled. The ammonium-magnesium phosphate is then filtered off, ignited to magnesium pyrophosphate, weighed, and the MgO calculated.

The presence of sulphide sulphur is detected by boiling with alkali and adding lead acetate. It may be due either to cystine itself, or to sulphides indirectly formed from cystine in the liming, or added directly.

Where a determination of sulphur is necessary, it can be obtained by Eshkar's method.

The following table gives typical results obtained for ash analysis of photographic gelatins:

——	American Commercial	Calfskin	Hide	Hide	German
	%	%	%	%	%
Moisture	11.04	13.88	12.67	12.17	15.19
Ash	1.08	.70	1.36	.69	1.00
	Percentage	composition of ash			
SiO_2	3.40	1.40	1.62	1.43	.....
Al_2O_3	2.57	.97	1.20	.62	2.69
Fe_2O_3	.31	.76	.79	1.05	
P_2O_5	8.27	3.89	6.48	2.15	6.55
CaO	47.06	45.71	43.36	41.67	52.59
MgO	13.10	7.55	6.40	4.10	4.10
Cl	2.09	2.00	1.45	1.50	1.33
SO_3	24.08	40.28	45.11	50.51	34.80
Total..	100.88	102.56	106.41	103.03	102.06

Sodium and potassium present in traces, also traces of copper.

The following table shows typical analyses of the ash of samples of gelatin for technical and culinary use, by courtesy of Dr. E. T. Oakes.[1]

——	Hide	Ossein	Bone		
			X	XX	XXX
	%	%	%	%	%
Silicia (SiO_2)	0.56	0.18	1.48	0.15	0.15
Chloride (Cl)	2.87	1.27	12.36	7.58	11.73
Iron Oxide (Fe_2O_3)	3.57	2.97	2.43	3.25	3.43
Phosphate (PO_3)	13.78	9.87	5.23	7.54	5.50
Calcium Oxide (CaO)	32.33	28.70	18.17	25.49	28.67
Sulphate (SO_3)	32.36	32.01	30.83	32.48	32.15
Sodium Oxide (Na_2O)	14.85	16.56	17.55	22.08	19.54
Potassium Oxide (K_2O)			1.27		
Aluminium	Present	Present	Present	Present	Present
Nickel	Trace	Trace	Trace	Trace	Trace
Total	100.32	91.56	89.32	98.57	101.17

Other tests of importance for these gelatins are considered below.

a. Chlorine and Chlorides. Chlorine is sometimes used as an antiseptic in the preparation of gelatin, but its value as a preservative is more than offset by the fact that it unites with gelatin to form loose combinations termed chloramines (see p. 154), which affect the physical properties of gelatin, especially in storage. Also, chlorides lower the strength of gelatin, both as jelly and when dry; and, being hygroscopic, render sized paper more subject to putrefactive changes. Hence the examination of gelatin for chlorine and chlorides is important.

To test for the presence of chlorine and chlorides, use potassium iodide, aqueous, and starch, and acidify with acetic acid. (It is well to run a blank.) The chlorides may be estimated as follows:

Heat 10 gms. of gelatin with dilute HNO_3 on a water bath, and filter from insoluble matter if necessary. Add $AgNO_3$ aq., boil the solution, and allow it to stand over night. Then decant off the liquid, add distilled water and enough NH_3 aq. to redissolve the precipitate, and boil. The AgCl is then reprecipitated with HNO_3. This treatment frees the precipitate from humic substances.[2]

[1] Laboratory of the National Biscuit Co.

[2] For the determination of chlorides in albuminous liquors, see Gazetti, G., Chem. Abst. 9: 2392. 1915.

Chlorides may also be determined volumetrically by Volhard's method, after destroying the gelatin with HNO_3. (See also the method of rapid analysis by nephelometer, p. 126.)

b. Copper and Mercury. Copper in the form of copper salts is to be regarded as an objectionable impurity in both culinary and photographic gelatins. Hart[1] reported that culinary gelatins sometimes contained as much as 56 mg. copper per 1,000 grams gelatin.

The method of determination of copper recommended by the Association of Official Agriculture Chemists (Methods of Analysis 1920, p. 147), has not worked so satisfactorily in this laboratory as the following: 50 grams of the gelatin are ignited and ashed in a platinum dish and the ash digested on the steam bath with concentrated nitric acid. After fifteen minutes the contents of the dish are cooled, diluted and filtered. This removes part of the insoluble calcium sulphate. After washing the residue thoroughly the filtrate and washings are evaporated down with 2 cc. of concentrated sulphuric acid. This removes the excess of nitric acid and also throws out of solution some more calcium sulphate. The residue in the dish is washed on to a filter with about 100 cc. of water (or sufficient to carry through all the copper sulphate or nitrate). The filtrate is then electrolyzed, the copper being plated on to a platinum dish, using 4 volts and about 5 amperes per 100 square centimeters cathode surface; time—about twenty to thirty minutes at room temperature.

It may also be necessary to test photographic gelatins for *mercury* (also a desensitizer), which may be used as an antiseptic at some stage in the manufacturing process (cf. Chapter II.), and *lead*, coming from water.[2]

c. Determination of Fats. After being dried in powder form, the anhydrous gelatin is soaked over night in dry ether and extracted in a Soxhlet for 8 hours. Because of the very small quantities of fat present (often below .05 per cent), it is best to weigh the flask against another tared flask, and to re-extract the ethereal extract with two or three small quantities of ether after drying the flasks. This avoids any error due to mechanical carrying over of non-fatty substances.

When the extraction is completed, the flask is cooled and weighed, the difference in weight giving the amount of fat present in the gelatin.

[1] Hart, W. B., J. Soc. Chem. Ind. **28**; 739. 1909.

[2] See Winkler, L. W., Zeits. angew. Chem. **26**: 38. 1913.

d. Test for Chondrinogen.[1] Solutions of many gelatins, quite clear or merely opalescent, give a certain amount of coagulum after being heated some time with 5 per cent HNO_3 aq. After filtering off and washing this coagulum, it is dissolved in N/10 NaOH aq., when the solution becomes a deeper yellow in color (see p. 104). It is then treated with acetic acid in slight excess to obtain complete precipitation.

(A large excess redissolves the precipitate.) The precipitate ("chondrinogen") has not the slimy character of mucin, though, like this substance, it reacts with alum.

Ordinary gelatins used by paper-makers contain from .54 per cent chondrinogen in the low grades, down to .09 per cent in the better grades. Alum gives a precipitate with the samples higher in chondrinogen.

In commenting on the method just described, Dr. Fernbach approved it as giving evidence of the presence of "unhydrolyzed stock." His own practice was to digest the sample for a short time with sulphate of alumina.

THE DIRECT DETERMINATION OF GELATIN IN COMMERCIAL GELATINS, GLUES, ETC.

Methods for the direct determination of gelatin necessarily depend on the separation and isolation of gelatin as at least equivalent to a definite single chemical entity. In point of strict fact, we have to deal with gelatin as a group of organic radicals of animal origin (cf. Chapter II), defined by the series of operations isolating it from a given physiological material (tissue). In this regard it resembles the group cellulose.

There exist normally a large number of variates,[2] and it is probably desirable to have defined a normal typical gelatin to which these varieties may be referred. Such a norm has been defined, in the case of cellulose,[3] as the resultant of a certain series of operations on cotton, but complete agreement upon a convention of this character has not been reached for gelatin[4].

In any case, however, a given gelatin or glue may contain, in addition to accessory and mineral "impurities" (from the nature of its origin, by reason of imperfect purification or of over-hydrolysis in extraction), any or all of the following types of proteins:—

[1] This term is applied with reservation, owing to uncertainty as to the exact nature of the product.

[2] Reichert, E. T., Amer. J. Botany **3**: 91. 1916.

[3] Cross, C. F., and Bevan, E. J., Cellulose; Beltzer, J. G., and Perzoz, J., Les matières cellulosiques.

[4] For preparation of de-ashed (demineralized) gelatin, see Vol. II.

a. Albumin, from blood;
b. Globulin, from blood serum;
c. Fibrinogen and fibrin, from blood;
d. Myo-proteins, nucleo-proteins, mucins and mucoids, from flesh, cartilage, and membranes;
e Proteoses and peptones, hydrolytic breakdown products of gelatin or any of the foregoing proteins;
f. Lipines and lipo-proteins;
g. Haemoglobin and derivatives (traces).

In the course of proper manufacture the foregoing classes of material should, in general, be removed as completely as possible. There always exists, however, the possibility of a certain amount being carried through, and, as will be seen, analytical processes for estimating gelatin independently of other proteins are neither simple nor completely satisfactory.

The following approximate methods have been proposed:—

1. Determination of total nitrogen by the Kjeldahl method and calculation of gelatin by multiplying by 5.5;

2. Determination of total nitrogen by distillation with soda lime;

3. Precipitating by tannin, and either measuring the nitrogen in the precipitate by the Kjeldahl method, or titrating the excess of tannin in the liquid. For this, a normal gelatin is taken as a standard, and made up to a standard solution of 10 grams per liter. This is titrated with a solution of tannin (10 grams per liter) until no further precipitate is formed. A weighed quantity of the sample is then titrated against the previously calibrated tannin solution;

4. Precipitation by chlorine and determination of nitrogen in the precipitate;

In respect of all these methods it was pointed out by Trotman and Hackford in 1904[1] that peptones and non-gelatin nitrogenous substances are determined concurrently.

5. Separation from non-gelatin constituents by precipitating with alcohol, filtering and weighing. This method is very uncertain and imperfect.

Trotman and Hackford proposed the following method:

a. Determination of total nitrogen by the Kjeldahl method;

b. Determination of "albumoses" by determining nitrogen in the precipitate thrown down by zinc sulphate, using 5.33 as a multiplying factor (N = 18.7 per cent);

c. Estimation of lower nitrogenous bodies either by precipitation with bromine or as the difference between a and b.

[1] Trotman, S. R., and Hackford, J. E., J. Soc. Chem. Ind. **23**: 1072. 1904.

The procedure used was as follows:—One gram of finely powdered gelatin or glue (or an equivalent of solution) is dissolved in not more than 20 cc. water. While hot, zinc sulphate crystals are added until the solution is saturated. It is then well stirred, filtered through glass wool, and washed with saturated zinc sulphate solution. The nitrogen in the wool and precipitate is determined by the Kjeldahl method, and the amount of nitrogen multiplied by 5.33 is assumed to give the proteose content.

Instead of filtering the coagulum, using excess of zinc sulphate crystals, it may be removed directly on a stirring rod, washed with saturated zinc sulphate solution, and the nitrogen determined by the Kjeldahl method. The filtrate may be rejected; or, if the content of peptones and lower breakdown products is required, the peptones may be determined by bromine (Allen's method)[1] and nitrogen determined in the residue by the Kjeldahl method. Trotman and Hackford concluded that in a good gelatin or glue the proteose figure should be practically equal to that of the total nitrogen.

This method has been criticised by Watson.[2] While representing an advance, it can not be considered as completely satisfactory.

Greifenhagen, König and Scholl[3] have investigated various methods proposed, and recommend the combined precipitation of gelatin and gelatose by zinc sulphate. This precipitate is redissolved in water and treated with mercuric chloride, and the nitrogen in the precipitate thus obtained determined by the Kjeldahl method. If this latter precipitate contains as much nitrogen as the zinc sulphate precipitate, gelatin is absent. The presence of gelatin indicated by difference in the nitrogen content of the two precipitates may be confirmed by precipitating a solution of the zinc sulphate precipitate with mercuric iodide dissolved in acetone or alcohol. If this precipitate has a higher nitrogen content than the zinc sulphate precipitate, gelatin is present. The separation of gelatin from proteoses by these precipitants is only approximately quantitative.

Vanvakas finds that, on adding Nessler's reagent to which tartaric acid has been added to a ten per cent gelatin solution, there is formed, slowly in the cold, but immediately on boiling, a precipitate which is lustrous in concentrated but dull gray in dilute solutions. Gelatin can be separated from gums and

[1] See p. 154.

[2] Watson, H. J., J. Soc. Chem. Ind. **23**: 1189. 1904.

[3] Greifenhagen, W., König, J., and Scholl, A., Biochem. Zeits. **35**: 217. 1911; J. Soc. Chem. Ind. **30**: 1174. 1911.

sugars in this way, but not from other nitrogenous substances, such as peptones.

Berrar[1] gives the following procedure for separating gelatin from other proteins, proteoses, peptones, mucins and casein:— To one volume of the gelatin solution add two volumes of a mixture of one part picric acid solution plus four parts 96 per cent alcohol. The gelatin only remains in solution, and after filtration may be quantitatively precipitated by adding about one and one-half volumes of saturated picric acid and allowing the mixture to stand twelve hours at 10° C. Gelatin admixed with milk or egg albumin can be accurately estimated in this way.

The gelatin-picric acid precipitate is washed with a solution of potassium-mercuric iodide until the washings are no longer yellow, and the gelatin in the residue is calculated from the nitrogen content determined by the Kjeldahl method. Berrar states that the procedure described may be used for the quantitative detection of gelatin in the presence of other proteins. After precipitating these with the alcoholic picric acid solution and filtering, the presence of gelatin in the filtrate, even in concentrations as low as one part in 100,000, may be shown by the yellowish-white opalescence produced with aqueous picric acid—(best as a ring test).

COLLAGEN AND THE ISOLATION OF GELATIN

In connection with the estimation of gelatin the following considerations are pertinent.

Physiological and biochemists have discussed various methods of preparing pure collagens (the parent protein of gelatin), and the gelatins or glutins derived therefrom. Thus, collagens may be obtained from bones, sinews, or hide by cold hydrolytic treatment with dilute hydrochloric acid, dilute caustic soda, or tryptic fermentation (thus removing the albumins and mucins), followed by extraction with alcohol and ether to remove the fats. But the collagens thus obtained are not necessarily identical. Sadikoff, a prominent worker in this field, distinguishes at least two kinds of collagen:—

A. The hyaline collagen of bones and gristle (cartilage), a translucent elastic mass of little cohesion or plasticity. The sub-types from bones and cartilage differ in respect of gelatinization (yielding gelatin on treatment with hot water), bone collagen offering greater resistance,

B. The fibrous collagen of sinews and hide, a white opaque plastic mass of great cohesion and great resistance to gelatinization by boiling water.[2]

[1] Berrar, M., Biochem. Zeits. **47**: 189. 1912.

[2] Sadikoff, W. S., Zeitz. physiol. Chem. **48**: 130. 1906.

Fish collagen belongs more to the hyaline type and gives a gelatin when treated with water at 40° C. Sadikoff terms such easily gelatinizing collagens "glutogens." However, the same property is said to be conferred on other collagens by allowing them to stand a week or more in alcohol or ether.[1]

Collagens, especially the fibrous type, swell greatly in dilute hydrochloric acid, but shrink in concentrated acid. They also swell in quite weak alkali solutions. They are decomposed in the cold by four or five per cent solutions of caustic alkali with evolution of ammonia, but ten per cent alkali carbonate solutions at 40° C. cause neither swelling nor decomposition. Heating in weak acid or alkali destroys the gelatin-yielding complex, but concentrated neutral solutions do not affect collagens even at 40° C.

It would follow from this that collagen is a name for a group of bodies having similar properties, but varying considerably, even in composition. Since gelatin and glutin are derived from collagen by treatment with boiling water, there should exist as many gelatins as parent collagens. Gelatinization of the collagen may be accelerated by acids, especially sulphurous acid, by heating under pressure in the presence of alkalies, or by concentrated ammonia solution.[2]

Methods for the purification and isolation of gelatin will be discussed in detail in Volume II, but a brief notice seems desirable here, as indicating the lines on which convention as to a normal standard gelatin may be reached. The methods involved depend generally (1) upon extracting the collagen with dilute alkali to remove coagulable proteins, mucins, etc., followed by gelatinization with boiling water and precipitation of the glutin from its aqueous solution with alcohol, or (2) upon the behavior of glutin toward concentrated salt solutions, such as twenty per cent magnesium sulphate, in the heat and cold respectively.

Selective enzyme hydrolysis of tissue has also been employed. Thus by tryptic digestion of sinew glutin Sadikoff has obtained two glutin types or groups, which he terms "trypsin-glutin-A" and "trypsin-glutin-B." The A-type glutin is difficultly soluble in cold water, but readily in warm; its reaction is neutral; the solution jellies at room temperature, and, if acidified with acetic acid, remains clear; it dissolves very slowly in 0.25 per cent caustic potash, the solution becoming turbid if neutralized with acetic acid and heated.

[1] Tebb, M. C., J. Physiol. **27**: 463. 1901–1902.

[2] See Abderhalden, E., Biochemisches Handlexikon, Vol. IV., p. 179.

The B-type glutin dissolves easily in cold water, gives a neutral reaction, and its solutions form very weak jellies even at 0° C. From this description the B-type glutin would seem to consist of more fully hydrolysed collagen.

Sadikoff[1] further distinguishes between *glutins* and *gluteins*. The cartilage glutins (gluteins) differ from sinew glutin and from bone and hide glutins (gelatins) in having a lower carbon and nitrogen content and a higher sulphur content. They give uncertain values for nitrogen by the Kjeldahl method, and show the following characteristic reactions: (a) After hydrolysis by acids they reduce alkaline copper solutions very slightly; (b) They give a reaction with phloroglucinol.

After treating gluteins with 0.2 per cent hydrochloric acid and precipitating with alcohol, the addition of a few drops of concentrated salt solution forms a substance having a higher carbon and a lower nitrogen content than the original.

Sadikoff's *gluteins* appear to be equivalent to the substances termed chondrin and chondrosin in works on the manufacture of gelatin.

THE USE OF SALT SOLUTIONS IN SEPARATION AND ANALYSIS

The use of saline solutions in the analytical fractionation of proteins antedated the physico-chemical study of the subject. Sadikoff, following the earlier work of Pascheles, Pauli, Levites, and others, found that glutins from purified gelatins and sinews were insoluble in concentrated solutions of potassium chloride, nitrite, and cyanide, but that gluteins were soluble in these solutions and also in cold saturated sodium chloride solution.

Sadikoff's results may be tabulated as follows:—

Protein	Concentrated Solution of				
	Potassium cyanide	Potassium nitrite	Potassium chloride	Sodium chloride	Magnesium sulphate
Glutin from purified gelatin.	Insoluble	Insoluble	Insoluble	.....	Soluble, but precipitated on acidifying.
Glutin from sinews.	Insoluble	Insoluble	Insoluble	Insoluble	
Gluteins.	Soluble	Soluble	Soluble	Soluble	
Commercial gelatins.	Partly soluble	Partly soluble	Partly soluble	Partly soluble	Partly soluble even on acidifying.

[1] Sadikoff, W. S., Zeits. physiol. Chem. **39**: 396 and 411. 1903; **41**: 15. 1904. Abderhalden, E., l. c.

Sadikoff considers that different samples of commercial gelatins which have a different history and different thermal pretreatment behave differently toward these salt solutions according to the amount of transformation and decomposition products which occur in the course of gelatinization (hydrolysis) of the collagen with water. These products are unstable, passing into each other readily in the sense of progressive decomposition (hydrolytic degradation); but up to a certain stage the reactions are thermally reversible (condensation possible). At a later stage of the hydrolytic process the condensation capacity with respect to water and salt solutions is lost, though the jelling capacity and the elementary composition remains.[1]

Sadikoff divides commercial gelatins into three constituents, according to their behavior toward salt solutions:—

a. a part insoluble in salt solutions;
b. a part soluble in salt solutions, but precipitated by acid;
c. a part soluble in acid salt solutions.

He gives the following reactions for glutin as proper to gelatins which have undergone no hydrolytic decomposition:—

i. soluble in saturated salt solutions in the cold or on warming, particularly in magnesium sulphate solution;
ii. precipitated from these solutions on acidifying;
iii. soluble in 70 per cent acid alcohol;
iv. precipitated from this solution on neutralizing.

Although very small variations (influence of water, salts, acids, alkalies, heat) may condition a negative result of these reactions, Sadikoff considers that they may be not only employed to characterize pure gelatins, but applied to their purification. Although some of these conventions appear doubtful, the application of "salting out" methods both to the analysis and in the technology of gelatin merits consideration. The use of salting out as a method of fractional separation and purification of proteins has undergone a considerable evolution, developing into methods for obtaining crystallizable proteins.

As the progress in this field has been connected mainly with the development of exact physico-chemical methods, it will be considered at greater length in Volume II; but it may be pointed out here that purification of such a material as gelatin is relative to the purpose in view. For the technologist, purity corresponds to the fulfilment of practical specifications, with certain permissible tolerances defined by the purpose for which the material is intended. For the organic chemist,

[1] Sadikoff, W. S., l. c., and as cited by Schroeder, P. von, Zeits. physik. Chem. **45**: 75. 1903.

purity implies satisfaction of the criteria of chemical singularity. That crystallizable substances can be obtained does not in itself solve the problem here, as will be noted later.

That insufficient attention has been paid to criteria of purity and individuality is very evident in the field of protein chemistry, where a large amount of "multiplication of entities beyond necessity" has gone on. Haslam,[1] in an important paper on the separation of proteins, points out that even the fundamental principle of practical chemistry, that in the separation of one substance from others such separation can not be regarded as complete unless definite proof be afforded, has been much neglected. He proposes two methods for testing purity, which are applicable to the processes of fractionation and salting out:—

1. Where it is to be proved that the protein precipitate is freed from the substances of the filtrate. The precipitate is dissolved in water and made up to a given volume; the requisite salt (or alcohol) is added; the mixture allowed to stand twenty-four hours, then filtered. Organic nitrogen in the filtrate is determined by the Kjeldahl method. Absence of protein in the filtrate gives the required proof.

If protein is present, the precipitate is redissolved, made up to the original volume, and treated as above. If the amount of nitrogen in the second filtrate is the same as that in the first, it is clear that this is due to small amounts of the precipitate soluble in that medium; hence the precipitate is free from all nitrogenous matter of the filtrate, and is "pure" as regards this particular separation. On the other hand, if the nitrogen value of the first filtrate is greater than that in the second, evidently undesirable nitrogenous matter is present, and further precipitations are needed.

2. Where the substance to be purified is in the filtrate, and the proteins being removed are in the precipitate—e. g., serum albumin, to be freed from and tested for globulin. This is less simple than it appears. Taking the example of serum albumin and globulin, the solubility of globulin in the half-saturated solution,—e. g., of ammonium sulphate,—should first be considered. This solubility is greatly increased by the presence of serum albumin and other constituents of serum; hence in the filtrate containing the albumin there is a notable quantity of globulin. That the same holds for peptones and albumoses may be readily shown as follows:—The globulins are precipitated from the serum by an equal volume of saturated

[1] Haslam, H. C., J. Physiol. **32**: 267. 1905.

ammonium sulphate. Saturated ammonium sulphate is added to the clear filtrate until there is a faint precipitate, which is filtered off and redissolved in water. On adding to this an equal volume of saturated salt solution, the appearance of a precipitate shows the persistence of globulin in the original filtrate. Haslam shows that such globulin (or albumose, etc.,) can be removed by a process of fractional precipitation, using the process just outlined to test through fractions and subfractions the complete precipitation of the globulin (or proteose).

Finally, it must be noted that criteria satisfying the analytical chemist may fail when subjected to the higher criticism of physical chemistry, which will enquire how far the putative chemically singular entities can be accepted as "independent components" in the sense of the Phase Rule. Not much has been done in this direction, but Sörensen's work[1] may be noted as conforming to these conceptions.

Reference may also be made to the work of Lippich,[2] who concludes that, in the precipitation of proteins by zinc sulphate, compounds of a stoichiometric character are formed—e. g., ZnO-protein-$ZnSO_4$,—in definite proportions.

In this laboratory, from work described in Volume II, we find that the following procedure yields a very fairly reproducible material from any good grade commercial gelatin; (a) de-ashing, to .10 to .01 per cent, and (b) precipitation from aqueous solution by grain alcohol. From this work it appears doubtful if there is any advantage in the conception of different pure "gelatins" or "glutins;" there may or may not be an individual protein corresponding to this, but probably the combination of this with varying amounts of hydrolyzates and impurities is sufficient to account for the observed differences in gelatins.

CONGENERS OF GELATIN

The uncertainty as to composition, constitution, and both physical and chemical definitiveness which characterizes practically all the proteins is perhaps even more evident for the bodies designated by the terms mucins and mucoids.[1] Physically, or rather physiologically, they have in common a slimy viscid texture from which the generic name is derived. Chemically, they are regarded as gluco-proteins,—i. e., albuminous substances, among the dissociation products of which is found a carbohydrate or the derivative of a carbohydrate.

[1] Sörensen, S. P. L., Zeits. physiol. Chem. **103**: 1. 1918.

[2] Lippich, F., Zeits. physiol. Chem. **90**: 236. 1914.

However, the setting up of a distinct class of gluco-proteins, due to Hammersten[1] originally, is of very doubtful significance, since actually the greater number of proteins give varying amounts of such a carbohydrate radicle.

Further, these so-called gluco-proteins differ—in degree, if not in character,—from the nucleo-proteins and from haemoglobin in that this auxiliary or prosthetic group is not readily split off from the albumin group proper. The carbohydrate is set free only by boiling with mineral acids or by strong alkalies, both processes causing simultaneous degradation of the albumin to peptones or amino-acids. Mann concludes therefore that "it is doubtful whether we are justified in making a special group of gluco-proteins. It is quite possible that we are only dealing with a group of albumins in which one of the dissociation products, namely the sugar radicle, is present in larger amounts than in ordinary albumin".

The difficulty with which mucins and the cognate mucoids are separable from albuminoids like gelatin, particularly when occurring naturally or physiologically combined therewith, the widespread occurrence in different parts of the animal organism, and, finally, their yielding a relatively reactive (reducing) carbohydrate, makes some account of their occurrence and properties desirable. The differences between gelatins from different stocks, supposing the treatment in preparation to have been the same, are very probably due in large measure to the extent to which mucins and mucoids are present in the final product.

Mucins and *mucoids* have in common the following properties. Physically, they occur as slimy, strongly hydrated masses, which, even when greatly diluted, possess high viscidity. They are acid compounds, contain no phosphorus, and yield a reducing substance on being boiled with an acid.[2] Their percentage composition is notable for the low carbon and nitrogen and high oxygen content, due of course to the carbohydrate rich in oxygen. They also contain considerable sulphur, present as a conjugated sulphuric acid. They give a violet biuret reaction like ordinary albumins, also the xanthoproteic and lead sulphide reactions.[3] They are not coagulated by heat, and are denatured by acids, alkalies, and by alcohol and other precipitants. This denaturing, as with the simple albumins, is irreversible.

[1] See Mann, G., l. c., p. 530; Abderhalden, E., l. c., pp. 137-156.

[2] Occurrence of such reducing compounds might of course be a cause of fogging in photographic emulsion making.

[3] Dissociable sulphide or hydrogen sulphide might also be a cause of fogging.

Mucins and mucoids are precipitable by acids stronger than themselves, such as acetic acid, but are only slightly soluble in excess of acetic acid, thereby differing from globulins and nucleo-albumins. They are precipitated by mineral acids, but readily dissolved in excess. They are readily soluble in solutions of alkalies, alkali carbonates, and ammonia.[1]

The separation of the mucoids from the mucins is quite arbitrary. They differ either in respect of physical properties, or in not being precipitated by acids. (These differences, however, very probably depend partly on the degree of colloid "protection" afforded by associated proteins.) They occur both in solution, as in blood serum, and in solid tissue, along with collagens.

Although there are no essential differences between mucins and mucoids, from a physiological chemical standpoint, Cohnheim has regarded it as preferable to reserve the name mucins for the substances secreted by epithelia, mucoids for the similar substances derived from connective tissue.

In the technology of gelatin the mucoids from tendons or sinews, bones, and cartilage are the most interesting.[2] Thus the mucoid from tendons has the following compositions:—

C	H	N	S	O
48.26	6.49	11.51	2.31	31.43

being chiefly notable for a rather low nitrogen content, and for relatively high oxygen and sulphur contents. From this tendo-mucoid a substance very similar to chondro-sulphuric acid has been separated. When tendo-mucoid is hydrolyzed with acids or in an autoclave, a carbohydrate of high molecular weight is split off, which is dextro-rotary and non-reducing, but by more intense acid hydrolysis gives a reducing carbohydrate.

Tendo-mucoid resists the action of alkalies more strongly than do the mucins. It may be prepared by extracting tendons with half-saturated lime-water. In the presence of acids, as for instance sulphuric, hydrochloric, or acetic, it is said to form relatively stable compounds with alkali albuminates, acid-albumins, proteoses, gelatin, and the water-soluble albumins of muscle, tendon, blood serum, and egg-white.[3] These new compounds have an acid reaction, are but little soluble in dilute acids, and behave like mucoids toward pre-

[1] The mucins, etc., are therefore presumed to be removed by the lime treatment in gelatin manufacture, (cf. Chapter II), but it is evident that this treatment may be inadequate, and that the lime solution should be thoroughly renovated before the material is extracted.

[2] Mann, G., l. c.

[3] Posner, E. R., and Gies, W. J., Amer. J. Physiol. **11**: 494. 1904.

cipitants. They contain more nitrogen than the mucoid itself, and when boiled with dilute hydrochloric acid give rise to gluco-thionic acid and a reducing substance.

It was formerly supposed that cartilage was composed of a uniform substance, chondrigen, giving rise to chondrin on boiling with water, in a manner similar to the derivation of gelatin from collagen.[1] That chondrin when boiled gave rise to a reducing substance was shown by Fischer and Boedeker and by de Bary.[2] It was then found by Morochowetz and confirmed by Krukenberg that chondrin is a mixture of glutin (gelatin) and mucin. The investigations of Mörner[3] led him to the following differentiation of the constituents of cartilage:—

i. chondro-mucoid;
ii. chondroitin sulphuric acid, its dissociation product, a normal constituent in small quantities;
iii. collagen;
iv. an albuminoid in old, but not in young tissue.

The tissue of old cartilage consists of albumoid plus collagen, while the enclosed or dispersed "chondrin balls" are composed of collagen plus mucoid. The two constituents stain differently. Cartilage treated with dilute acids at 40° C. yields a mixture of gelatin and chondro-sulphuric acid, while when boiled in an autoclave a mixture of gelatin plus mucoid plus chondro-sulphuric acid is obtained. It is this latter mixture which in general represents the chondrin of the older observers; it differs from the gelatin obtained from bone or hide in not being precipitated by tannin, since the chondro-sulphuric acid interferes with the precipitation of the gelatin portion.

Chondro-mucoid shows the usual reactions of the mucins and mucoids, dissolving in alkalies to form a thick, viscous fluid from which it is reprecipitated by acids. Most salts of the heavy metals precipitate it, but alkaloid reagents do not. The color-reactions are all positive, and it is salted out by ammonium sulphate. In composition it corresponds to the mucins, being notable for its high sulphur content, 2.42 per cent, of which 1.8 per cent is due to chondro-sulphuric acid. It is hydrolyzed by acids, and more readily by alkalies, to albuminate, albumose, peptones, a reducing carbohydrate, and chondro-sulphuric acid. The latter substance, sometimes termed chondroitin-sulphuric acid, is a colloid of unknown composition. If boiled a short time with acids sulphuric acid is split off and the remainder, free from sulphur, is termed chondroitin. Chondroitin-sulphuric acid is therefore assumed

[1] Müller, J., Ann. Chem. [Liebig's] **21**: 277. 1837.
[2] Mann, G., l. c., p. 542.
[3] Mörner, C. T., Zeits. physiol. Chem. **23**: 311. 1897.

to be a partial or substituted sulphuric acid, similar to ethyl or methyl sulphuric acid. Chondroitin, a gum-like body, is further hydrolyzed by acids to "chondrosin," a polysaccharide containing amino-groups.[1] The empirical composition of chondro-sulphuric acid is given as:—

C	H	N	S	O	by
35.28	4.68	3.15	6.33	50.56	Mörner
37.1	4.83	2.71	5.5	50.14	Schmiedeberg

It has a strongly acid reaction and unites with metals to form neutral salts which are usually soluble. Amorphous copper, iron, and "copper oxide" salts have been prepared.[2] The acid, readily soluble in water, is precipitated by stannous chloride, basic lead acetate, mercurous nitrate, ferric chloride, and uranium nitrate, but not by alkaloid reagents. It does not reduce, but keeps copper oxide and other oxides in solution. This is attributed to the formation of soluble salts, but may be largely a peptization. The aqueous solutions are laevo-rotatory.

With proteins such as gelatin chondro-sulphuric acid forms "insoluble salts which behave like nucleic acid, for they become hydrolytically dissociated in the absence of excess of acid."[3] Since the alkali salts of chondro-sulphuric acid do not precipitate proteins, the mixture of alkaline or alkaline earth chondro-sulphate and gelatin which is obtained by digesting cartilage in an autoclave is precipitated only after addition of acids, and the precipitate is redissolved by excess of mineral acid.

Of more recent important work on mucoids that of Posner and Gies, Levene,[4] and Schmiedeberg should be noted. Posner and Gies[5] find that the glucothionic acids (chondroitin-sulphuric acid) obtainable from the mucoids have the property of combining with proteins when their salts are dissolved with the latter and the mixtures acidified. The products are very similar to typical tissue mucoids. Further, Posner and Gies have found that mucoids vary in composition even in the same tissue, the variability consisting in fluctuations of the proportions of protein and glucothionic acid radicles. They also found that when neutral or alkaline mucoid solutions are mixed

[1] There is considerable probability that a carbohydrate or some radicle resembling a carbohydrate is contained in or associated with all albumins (proteins). The so-called gluco-proteins differ only in the amount of this carbohydrate radicle. Cf. Mann, G., l. c., pp. 154–6.

[2] However, some of these are liable to be considered adsorption compounds.

[3] Cf. Mann., G., l. c., p. 544. Like "gelatin tannate," these compounds may be adsorption compounds rather than true chemical combinations.

[4] Levene, P. A., and López-Suárez, J., J. Biol. Chem. **36**: 105. 1918.

[5] Posner, E. R., and Gies, W. J., l. c.

with neutral or alkaline solutions of various common proteins and the mixtures acidified, heavy precipitates are formed, which may be several times as bulky and heavy as the mucoid separable by acidifying control blanks containing no protein. It is suggested that the glucothionic radicle acts as amboceptor to unite mucoid with any of various proteins. Gelatin is readily precipitated.

The precipitates, which resemble hydrous alumina, dissolve in dilute lime water forming clear solutions which are stable on boiling, but in which acid immediately causes a precipitate as highly resistant to acids as are mucoids themselves. As tendo-mucoid forms these complexes with gelatin, proteose, alkali and acid albuminates, serum albumin, and globulins, it appears that the earlier work on mucoids was really performed on protein-mucoid compounds.

CONSTITUTIONAL ANALYSIS OF GELATIN

CLASSIFICATION

While the technochemical analysis of gelatin is directed principally to the estimation of supposed impurities and foreign substances, a constitutional analysis envisages the determination of the elemental composition and constitution of gelatin,—i. e., an ideal organic integer, free from mineral and other foreign ingredients. Considering gelatin as a colloid, as an actual and individual physico-chemical complex, it is very possible that certain of the inorganic constituents, at present considered impurities by analytical chemists, are actually essential. This question will be dealt with more fully later.

Gelatin, if distinguished by origin and physiological affinities, is classified by physiological and bio-chemists as a sclero-protein, like elastin, collagen, and keratin. If distinguished by its relation to the typical protein albumin (as insoluble in neutral solvents and yielding only α-amino-acids on hydrolysis —see p. 144), it is reckoned as an albuminoid.

However, the classification of proteins and *a fortiori* of albuminoids on the basis of chemical constitution is very difficult, if not impossible.[1] For one thing, in all but few cases the ordinary criteria of chemical purity and individuality, such as distillation and conversion to the vapor or to the crystalline state, fail. The result is (as stated above), that the failure of clear-cut concepts in this branch of chemistry has been eked out with a plethora of names, for the most part analytical labels tacked

[1] Cf. Alexander, J., l. c.

on to arbitrary sections of processes of incomplete separation and purification.

Hence the classifications at present exhibit an ambiguity or vacillation between distinctions based on biological and physiological grounds of occurrence (as for instance vegetable and animal proteins), and partial differentiations based on physico-chemical behavior (as albumins and globulins, albuminoids and albumoids).

The classifications into various protein groups, adopted (A) by the English Chemical Society, and (B) by the American Chemical Society, are as follows:

A—(English Chemical Society).

I. Protamines; e. g., salmine, clupeine, etc.
II. Histones; e. g., thymus histone.
III. Albumins; e. g., ovalbumin, serum albumin, plant albumins.
IV. Globulins; e. g., serum globulin, fibrin, etc.
V. Glutelins; e. g., glutenin in wheat (soluble in very dilute alkali).
VI. Gliadins; e. g., wheat-gliadin, zein (soluble in 70-80 per cent alcohol).
VII. Phospho-proteins; e. g., caseinogen.
VIII. Sclero-proteins; e. g., keratin, gelatin, elastin.
IX. Conjugated proteins:
  a. Nucleo-proteins; nucleic acid with protein;
  b. Chromo-proteins; chromogenic substance with protein;
  c. Gluco-proteins; carbohydrate in combination with protein, e.g., mucin.
X. Derivatives of proteins:
  a. Meta-proteins, as acid-albumin, alkali-albumin;
  b. Proteoses; e. g., gelatose;
  c. Peptones; e. g., fibrin-peptone;
  d. Polypeptides; e. g., glycyl-alanine, leucyl-glutamic acid.

B—(American Chemical Society).

I. Simple proteins; that is, yielding only α—amino—acids on hydrolysis:
  a. Albumins; *water-soluble*, coagulated by heat.
  b. Globulins; insoluble in water, soluble in neutral salts.
  c. Glutelins; insoluble in water or neutral salt solutions, soluble in very faintly acid or alkaline solutions.
  d. Alcohol soluble; soluble in 70-80 per cent alcohol, as zein.
  e. Albumoids; insoluble in solutions of neutral salts, weak acids or alkalies or alcohol-water.
  f. Histones; soluble in water, insoluble in NH_3 aq., precipitated with other proteins.
  g. Protamines; equivalent to simpler polypeptides; soluble in water, not coagulated by heat, strong bases.
II. Conjugated proteins; contain simple protein combined with some other molecule, not as salt.
  a. Nucleo-proteins; with nucleic acid.
  b. Gluco-proteins; with sugar molecule.
  c. Phospho-proteins; containing organically combined phosphorus.
  d. Haemoglobin; with haematin.

III. Derived proteins:
  1. Primary cleavage products:
     α Proteids, insoluble in water, produced by hydrolysis.
     β Meta-proteins, acid-albumin, etc. (acid or alkali proteins).
     γ Coagula by heat or alcohol (denatured proteins).
  2. Secondary cleavage products.
     α Proteoses; first hydrolysis, precipitated by $(NH_4)_2SO_4$, ½ saturated, or $ZnSO_4$ saturated.
     β Peptones; water soluble, not precipitated by salts.
     γ Peptides; condensations of two or more amino-acids.

A tentative scheme for the further subdivision of the albuminoids, based chiefly on relative ease of hydrolysis, has been proposed by Alexander (l. c. 113) as follows:

	Characteristic
A. Collagens or jelly-forming albuminoids ossein, collagen (and gelatin) from bones and skin.	Dissolved more or less readily by boiling water. Solutions gelatinize on cooling. Little or no sulphur. (But see p. 117, on chondrin.)
Chondrigen (and chondrin) from permanent cartilage.	
Isinglass: from swimming bladders of fish.	
Serine (silk gum, from silk).	
B. Fibroids	Not acted on by boiling water, by very dilute boiling alkali, nor by dilute acids. Stronger alkali dissolves. No sulphur.
Elastin: from ligaments.	
Fibroin: from silk, spiders' webs, etc.	
C. Chitinoids: external skeleton of invertebrates.	Not acted on by boiling water nor by alkalies.
Conchiolin: shells of mollusks.	
Spongin: from sponges.	Contains no sulphur.
D. Keratins:	Not acted on by boiling water. Dissolved by boiling with dilute caustic alkali.
Keratin; from hoofs, horns, feathers, hair, wool, etc.	
Neurokeratin: from brains.	Contains sulphur.

ELEMENTARY COMPOSITION

The ultimate empirical composition of gelatin, i. e., purified gelatin taken ash-free and anhydrous, has been determined, with fairly concordant results, to be as follows:

Gelatin from	Bone	Isinglass	Bone	Connective Tissue
Observer	Mulder	Mulder	Freary	Chittenden
Carbon	50 40	50.76	49.81	49.38
Hydrogen	6.64	6.64	7.34	6.81
Nitrogen	18.34	18.32	17.32	17.97
(Sulphur)	.....	.....	.....	0.71
Oxygen	26.64	24.69	25.67	25.13
Total	100.02	100.41	99.4	100.0

as stated, these figures are for ash-free gelatin.

It is questionable whether any sulphur is to be regarded as an integral constituent,—that is, organically combined,—although, as will be noted later, it is readily brought into more or less intimate combination. Again, attention should be called to the fact that the values given depend upon the somewhat doubtful convention that only and all the water retained at 100-105° C. is organically combined.

Hofmeister, on the basis of such empirical analyses, has given the following formula and equation for the production of gelatin from collagen:

$$C_{102}H_{149}N_{31}O_{38}+H_2O=C_{102}H_{151}N_{31}O_{39}.$$

But not very much weight can be attached to this in the absence of definite molecular weight determinations.

MOLECULAR AND COMBINING WEIGHT

The physico-chemical methods of determining molecular weight by depression of freezing point and elevation of boiling point are inapplicable to substances such as gelatin, which form colloid solutions. Although certain measurements have been made on albumin, etc., by Sabanéeff[1] and others, it is doubtful that they have any validity, since traces of saline electrolytes would have an effect of the same order as that observed. Measurements of the osmotic pressure by Biltz,[2] (subject to similar criticism), gave values as follows:

Kind of gelatin	Per cent ash	Molecular weight
Photographic dry plate	1.4	10,900
Purified gelatin	.06—.62	5,500—15,500
Technical gelatin	1.55—2.40	5,650—18,500

Assuming Paal and Procter's combining weight 900, it is supposed that these molecular weights mean that aggregates

[1] Sabanéeff, A., and Alexandoff, N., in J. Russ. Phys. Chem. Soc., cited by Lloyd, D. J., l. c.

[2] Biltz, W., Bugge, G., and Mehler, L., J. Soc. Chem. Ind. **36**: 297. 1917.

of some ten "molecules" are present. It is concluded that the technical value increases with the aggregate; similarly, the "gold number" diminishes as the aggregate increases.[1]

The combining weight of approximately 900 was deduced by Procter from his work on the swelling of gelatin in acids. (See Volume II.) On the other hand, ultra-microscopic observations, viscosity data, and other colloid-chemical evidence make it doubtful whether much significance can be attached to *molecular* weight determinations here, while leaving the question of equivalent or combining weight unaffected.

Summing up, the physical and chemical equivalence methods give values of the molecular weight of gelatin as follows:—

Schützenberger and Bourgeois (1876)	1836;
Paal (1892)	(990)x;
Berrar (1912)	(823)x;
Procter (1914)	(839)x;
Biltz (1916)	5,500—31,000

Miss Lloyd[2] states that considerations based on the most accurate estimations of individual amino-acids lead to a reacting weight of the order of 10,000. Van Slyke's estimations of the distribution of nitrogen in the gelatin molecule give:—

	Per cent of total nitrogen
Amide-nitrogen	2.25
Melanine	0.07
Cystine	0.00
Arginine	14.7
Histidine	4.48
Lysine	6.32
Mono-amino	56.3
Non-amino	14.9
	99.02

To reduce these percentages to nitrogen equivalents or atoms per molecule Miss Lloyd uses an arbitrarily determined factor, as given below. Since histidine contains three atoms of nitrogen, arginine four, and lysine two, the atomic ratios must have these values or a multiple thereof. Since histidine is present in very small amounts, it may be used to give the unit. If only one unit of histidine is assumed, $4.48 \times 3 = 6.65$, but this factor gives only half molecules of ammonia and arginine. Taking two histidine units, the factor is 1.33, whence we have:—

[1] But see Volume II.

[2] Lloyd, D. J., Biochem J. **14**: 166. 1920.

Amide nitrogen.....	$2.9 = 1 \times 3$	approximately;
Arginine nitrogen.....	$19.7 = 4 \times 5$	approximately;
Histidine nitrogen.....	$6.0 = 3 \times 2$	approximately;
Lysine nitrogen.....	$8.4 = 2 \times 4$	approximately;
Mono-amino nitrogen.....	$75.2 = 1 \times 76$	approximately;
Proline + oxyproline.....	$20.0 = 1 \times 20$	approximately.
	$132.2 = 133$	

Hence it may be assumed that one gelatin molecule contains (133) nitrogen atoms distributed among:—$3x$ amide groupings, $5x$ arginine groupings, $2x$ histidine, $4x$ lysine, $76x$ mono-amino, and $20x$ proline + oxyproline groupings. In absence of further evidence, x may be taken as unity.

It does not appear that any great weight can be attached to this argument, since some 56 per cent of the nitrogen is allotted to mono-amino acids as a whole. The amide-nitrogen figure is actually quite variable; hence the real basis of this argument is the relative proportions of the three "hexone bases" of Kossel,[1] which do not form a preponderant part of the complex,—are, in fact, but a small part. Therefore the value deduced by Miss Lloyd for the lowest weight of gelatin which can act as a chemical individual, viz., 10,300, can not be regarded as substantially founded.

The osmotic properties of gelatin solutions will be referred to in greater detail later (Volume II). Their variability and the indeterminateness of molecular weights of colloids as deduced therefrom has been specially treated by Moore,[2] who suggests that the range of "solution aggregates" indicated by these vapor pressure and osmotic pressure results points to a physical rather than a chemical complexity of the protein molecule.

The possibility of the determination of significant molecular weights of proteins by osmotic pressure, etc., methods, may be regarded as revived by the important work of Sörensen on the crystallization and isolation of egg-albumin.[3] Although this will be considered more fully under the physical chemistry section (Volume II), certain conclusions are of great importance in the present discussion. Sörensen's general contention is that emulsoid colloids such as gelatin, albumin, etc., are better defined by differences in degree of reactivity of the dispersoid (dissolved) phase with its dispergent (solvent), than by dispersity (mechanical subdivision). Hence emulsoid solutions

[1] Cf. Mann, G., l. c., p. 20.

[2] Moore, B., and Parker, W. H., Amer. J. Physiol. 7: 261. 1902; Moore, B., and Roaf, H. E., Biochem. J., 2: 34. 1906.

[3] Sörensen, S. P. L., Compt. rend. trav. lab. Carlsberg **12**: 1. 1915. Zeits. physiol. Chem. **103**: 1. 1918.

resemble true solutions in their behavior, which follows the Phase Rule when the number of the components is rightly chosen. The successful outcome with egg-albumin is no doubt due largely to the possibility of purification by recrystallization, as it is nearly ash-free,[1] and free from certain less determinate congeners (mucin, con-albumin).

On the other hand, the exact determination of the conditions of crystallization with this colloid can probably be extended to other proteins.[2] An important result with regard to osmotic pressure is the following. An egg-albumin solution of a given composition has a constant well-defined osmotic pressure, the magnitude of which is dependent upon the egg-albumin concentration, the ammonium sulphate concentration, and the hydrogen-ion concentration, according to definite laws. The osmotic pressure of an emulsoid solution thus definitely defined is as definite a quantity as that of a crystalloid solution of definite composition.

These conclusions are not necessarily incompatible with the solution-aggregate hypothesis. The definite osmotic pressure, in the foregoing, would correspond to a definite solution-aggregate, but the magnitude of this aggregate would be determined by definite chemical (valency) forces, rather than indefinite physical forces—such as those implied by the vague concepts capillarity, internal pressure, etc. Again, crystallization is not necessarily the expression of chemical individuality. If the electrostatic neutralization of charged ions can form a regular space lattice, a crystal will be formed, but need not correspond to chemical molecular integration.[3] This question will be taken up again in discussing the physical chemistry of the gel condition, as also will be the theses of Pauli, Procter, and Loeb[4] in regard to the stoichiometric nature of gelatin reactions with acids, bases, and salts, and their dependence upon hydrogen-ion concentration. Provisionally, the most probable "combining weight" of gelatin may be taken as 840,[5] while the "molecular weight" is uncertain.

PROXIMATE GROUP COMPOSITION

Analysis of an organic compound for proximate groups of atoms or finite reacting radicles gives an initial view of its probable constitution in terms of certain partial configurations.

[1] Small amounts of phosphorus pentoxide from the phosphorus of the albumin excepted.

[2] The important work of Grübler (Grübler, G., J. prakt. Chem. **23**: 97. 1881) and of Osborne (*The Vegetable Proteins*) on proteins from seeds should be noted in this connection.

[3] Langmuir, I., Liquids and solids, l. c.

[4] Pauli, W., Kolloidchemie der Eiweisskörper. Procter, H. R., J. Chem. Soc. (Trans.) **105**: 313. 1914; Loeb, J., J. Biol. Chem. **34**: 77. 1918.

[5] In a recent publication, Wintgen and Vogel (Koll. Zeits. **30**: 45. 1922) find 885, for combination with HCl.

It is, in fact, analogous to setting up the mathematician's partial differential equations. The integral view of the constitution of a given compound obtained from analysis must then be confirmed by a synthesis in agreement therewith. It is, however, not entirely certain that bio-colloids such as gelatin, cellulose, etc., can be assigned either a perfectly definite and invariable elementary composition or a fixed molecular constitution.[1] This uncertainty arises not only from the difficulty of isolating the dispersed phase of these colloids from the dispersing medium and stabilizing electrolytes without denaturing it, but from the continuity in the change of their physical and chemical properties with changes of temperature, pressure, etc. True chemical compounds necessarily represent equilibrium states, whereas complex colloids of this type are perhaps better represented as impermanent stages of a process.

However, the conception generally though provisionally accepted is that for the carbohydrate colloids, such as dextrin, starch and cellulose, we have either polymers of a simple carbohydrate unit $(C_6H_{10}O_5)_n$, or anhydrides of such a polymer, $(C_6H_{10}O_5)_n - mH_2O$. Similarly, proteins are regarded as compound polypeptides, these latter being condensations of two or more amino acids, as in the following structure:—

$$NH_2.CHR.CO—(NH.CHR.CO)_x.\ NH.CHR.COOH.$$

For a detailed account of progress in the analytical resolution of proteins into recognizable compounds and in the synthesis of polypeptides, reference should be made to the literature cited.

DECOMPOSITION PROCESSES

The general processes of decomposition are:

i. Dry or destructive distillation;
ii. α Oxidation and β halogenization;
iii. Hydrolysis;
iv. Fusion with alkali;
v. Bacterial and enzyme action;

(i) DRY OR DESTRUCTIVE DISTILLATION. Some progress in the study of proteins has been made as a result of dry distillation.

Gelatin gives by this method: water, ammonium carbonate and carbamate, glycocoll (amino-acetic acid), possibly other ammonium salts of organic acids, amides, free ammonia, an oil containing pyridine, picoline, quinoline, aniline, lutidine, methylamine, etc.; in sum, a mixture of aliphatic amines and heterocyclic bases.[2] It appears probable that further theoret-

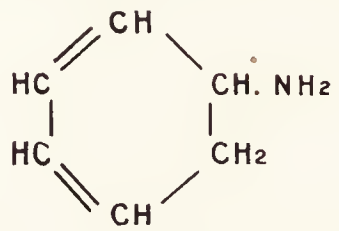
[1] Cf. Cross, C. F., and Bevan, E. J., l. c.
[2] Watts' Dictionary of chemistry, Vol. IV.

ical progress may be made by applying distillation under reduced pressure to proteins, as has been done by Pictet for coals, celluloses, and albumins.

In more recent work on the distillation of concentrated egg-albumin under pressure of 20-22 mm., and temperatures ranging from 70-350°C., Pictet and Cramer[1] obtained the following:—

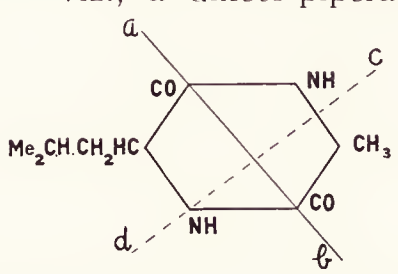
Water.....30 per cent;
Organic distillate, soluble and insoluble in water..15 per cent;
Coke.....32 per cent;
Gases and loss.....23 per cent.

The organic distillates were entirely soluble in ether, from which they were separated into acid, basic, and neutral fractions. Among the acids were acetic, propionic, butyric, and succinic. Distillation of the basic portion yielded fractions smelling of pyroline. One fraction appeared nearly homogenous, giving a copious precipitate with picric acid (needles, having a melting point of 185°). This is tentatively regarded as the primary base dehydroaniline, having the constitution



and as being the source of aniline and benzene in bone oil, etc. (Dippel's animal oil.)

The neutral fraction gave on distillation chiefly crystalline substances, notably iso-butyl acetamide (melting point 120°). Iso-butyl aceto-nitrile has been identified as one of the main constituents of animal oil, but was formerly attributed to the action of ammonia on fats. The present work shows that it is a direct though secondary product of albumin decomposition through the amide. Thus, iso-butyl acetamide ($Me_2CH.CH_2.C_2.CO.NH_2$) and leucine ($Me_2CH.CH_2.CH.NH_2COOH$) show a certain analogy, and are probably derived from a common source — viz., a diketo-piperazine combination.



[1] Pictet, A., and Cramer, M., Helvetica chim. acta 2: 188. 1919.

Hydrolysis gives leucine. Thermolysis gives iso-butyl acetamide.

Johnson and Daschavsky[1] have distilled silk fibroid in vacuo, (25-27 mm. pressure), obtaining 43 per cent as a red oil, 41 per cent as coke, and 16 per cent volatile and gaseous products absorbed by caustic soda and sulphuric acid. In the red oil, phenol was found, presumably from the tyrosine known to be a normal hydrolytic product of fibroin.

(ii-α) OXIDATION. Complete oxidation is, of course, identical with the determination of elementary composition by combustion. Partial oxidation in aqueous solution has yielded different products, according to the oxidizer used and the extent and intensity of the treatment. Oxidation here is always accompanied by hydrolysis.

With nitric acid the principal product is oxalic acid, and it has been reported that saccharic acid is obtained when hot nitric acid is used. The general absence of nitro-proteins (xantho-proteins) in gelatin indicates the absence of any aromatic residue such as is found in other proteins. Care must be taken that gelatin free from aromatic antiseptics is used.[2]

In sulphuric acid solution various volatile fatty acids, such as formic, acetic, butyric, and capronic are obtained, also aldehydes, benzoic acid, ammonia, and nitriles. These compounds are obtained as distillates, and organic bases, such, for instance, as guanidine, tend to accumulate in the solution.

Alkaline permanganate[3] has been used in many studies on the oxidation of proteins[4]. Maly described the initial oxidation product with this reagent as oxy-proteic acid or peroxy-protacid,—regarding it as a primary oxidation product of the unresolved protein,—but later work has shown that albumoses and other decomposition products are formed simultaneously. On further treatment ammonia is evolved, leading to so-called desamino-protein acids, which yield mixtures of amino-acids on hydrolysis. Schryver[5] is of the opinion that insufficient data exist to draw any very definite conclusions as to the course of oxidation of the proteins.

It is generally considered that Hofmeister's suggestion is reasonable:—Supposing the proteins to be built up of polypeptide groupings of the type

[1] Johnson, T. B., and Daschavsky, P. G., J. Amer. Chem. Soc. **41**[2]: 1147. 1919.

[2] It is to be concluded that the formation of aniline on dry distillation is due either to phenylalanine or to secondary processes in the vapor state. See dry distillation in vacuo. See also Mörner, C. T., Zeits. physiol. Chem. **95**: 263. 1915; Chem. Abst. **10**: 482. 1916.

[3] Cf. Schryver, S. B., The general characters of the proteins, p. 59.

[4] Mann, G., l. c., pp. 240–248.

[5] Schryver, S. B., l. c.

$$-NH-\underset{\displaystyle R}{\underset{|}{CH}}\,.\,CO-NH\,.\,\underset{\displaystyle R}{\underset{|}{CH}}-CO-,$$

the first stage of oxidation would be to

$$-NH-\underset{\displaystyle COOH}{\underset{|}{CH}}\,.\,CO-NH\,.\,\underset{\displaystyle COOH}{\underset{|}{CH}}-CO-$$

from which, by scission of CO_2, the grouping

$$-NH-CH_2\,.\,CO-NH\,.\,CH_2\,.\,CO-$$

would be formed, yielding on further oxidation

$$-NH\,.\,CO-CO-NH\,.\,CO\,.\,CO-$$

and from such a group oxalic acid and ammonia would be obtained by hydrolysis. However, this hypothesis is insufficient to account for all the facts, since the amount of amide nitrogen which can be eliminated with nitrous acid does not correspond to the amount of oxalic acid obtained.[1]

Hydrogen Peroxide and Ozone. While Maly's oxyproteic acids appear to be derived from a scission product of the protein complex, substances termed oxy-proteins have been obtained by the action of hydrogen peroxide and ozone in neutral solution. These oxy-proteins seem to be simple (primary) oxidation products, not differing much in percentage composition from the original protein. They are of interest in connection with the action of hydrogen peroxide and of ozone on photographic emulsions.

By more intense action, e. g., with iron or copper salts as catalysts, Blumenthal and Neuberg have obtained acetone from gelatin and hydrogen peroxide.

(ii-β) Halogenization. When chlorine is passed into a solution of gelatin, a precipitate is produced. Many such chlorproteins have been separated but the possible reactions are so complex and the products so variable in composition that they have not been generally used in the characterization of individual proteins.

The nature of these halogen compounds is of interest technically, however, because of the frequent use of chlorine bleaches and also because, in the photographic process, considerable exposure of gelatino-halide emulsions involves a decomposition of the halide with release of halogen.

[1] Oxalic acid on an average equals about thirty per cent. See Mörner, C. T., l. c.

The researches of Raschig and of Cross and Bevan on the action of chlorine on amino-bodies throw some light here. Raschig has shown that chlorine and alkaline hypochlorites act upon ammonia with the formation of chloramines—

$$NH_3 + M.OCl = NH_2Cl + M.OH.$$

These chloramines behave as if containing available chlorine and react with iodides, liberating iodine.

Proteins seem to undergo a similar reaction and Cross and Bevan[1] have suggested that such a reaction may serve to measure the reactive amino-groups in the protein complex. In any case, the initial state of combination of the halogen appears to be a loose one, so that it reacts readily. This agrees with certain facts relative to the reversal of the image in photographic emulsions upon intense or prolonged exposure. Cross and Bevan note that the product first formed by the action of chlorine from hypochlorite is impermeable to this solution, but permeable to water. Similarly, when chlorine is passed into a gelatin solution, each gas bubble becomes surrounded with a sheath of tanned chlor-gelatin impermeable to chlorine.

Allen and Tankard[2] state that, in brominating gelatin with bromine water, variable results are obtained, ranging from complete precipitation to very incomplete, the amount varying with the history of the gelatin. The nature of the coagulum is complex. On treating gelatin in solution with bromine water, the color does not disappear at first, but is slightly weakened. On digestion, the color disappears and a variable amount of white coagulum is produced. With silver nitrate the solution gives a copious white cloud, which, however, is not precipitated, but peptized. There is then a reaction for the bromide ion, showing production of hydrobromic acid by substitution. Further, if starch-potassium iodide be added, the blue starch-iodide is immediately produced, in approximate equality to the bromide ion reaction. Both coagulum and residual solution react for "free" bromine. The denser coagulum yields part of its bromine to alcoholic potash, (as in substituted organic acids), and part only by oxidation with nitric acid in sealed tubes (Carius' method). Hence, it is evident that there is a deep-seated substitution of halogen.

The halogenization of proteins is, then, a reaction similar in many respects to the vulcanization of caoutchouc, the sul-

[1] Cross, C. F., and Bevan, E. J., J. Soc. Chem. Ind. **27**: 1187. 1908.

[2] Allen, A. H., and Tankard, A. R., in Allen's Commercial Organic Analysis, 2nd edition, Vol. IV, pp. 320 and 470.

phuretting of gelatin (cf. p. 186), starch, and cellulose (xanthocellulose reaction), and the iodine reaction of starch. In all of these, we have a connected chain of reactions varying from initial adsorption and formation of an absorption compound to deep-seated chemical reaction and decomposition, accompanied by marked changes in the dispersity and physical state. In the case of starch-dextrin-iodine complexes, the behavior is that of iodine in various stages of dispersity down to iodion and organically combined iodine.

It appears as if, in the absorption stage, the element or radicle in question were assimilated by the colloid,—i. e., underwent a chemical induction to a chain of conditions of continuously varying dispersity, thus:

$$\begin{cases} \text{Iodine} \rightarrow I_m \ldots + I_{m-n} + I_2 + \bar{I}\ \text{(iodion)} \\ \text{Starch} \rightarrow (X)_m) + X_{(m-n)} + X \\ \text{Bromine} \rightarrow (Br)_m + Br_{m-n} + Br_2 + Br \\ \text{Gelatin} \rightarrow Y_m + Y_{m-n} + \ldots . Y \end{cases}$$

with a variable entail of substitution (decomposition) reactions and side-reactions (oxidations and hydrolyses). The early adsorption is then regulated partly by physical conditions, partly by formation of addition compounds (according perhaps to some principle of coördination); and the subsequent chemical decomposition proper by the chemical conditions of reaction with simpler molecular units (dextrose groups or amino-acids, etc.).

Siegfried and Reppin[1] have used the action of bromine as a method of following up the hydrolysis of proteins. They determine the ratio for N/Br under exactly comparable conditions before and during hydrolysis. For gelatin before hydrolysis the value of N/Br is 15.85, after hydrolysis 20.4, which indicates that gelatin has a greater bromine absorption than its products.

Herold prepares halogen combinations of gelatin, which he considers solid solutions or adsorption compounds, by treating dry or swollen gelatin with free halogen, alone or in solution, and in absence of a substance which combines with acid. The product is freed from the liquid used to swell the gelatin, and is used as an antiseptic.[2]

Oswald has prepared colorless iodo-proteins from alkaline solutions at 0° C., using KI-I_2 and dialysis.[3]

[1] Siegfried, M., and Reppin, H., Zeits. physiol. Chem. **95**: 18. 1915; Chem. Abst. **9**: 2905. 1915.

[2] Herold, J., J. Soc. Chem. Ind. **32**: 622. 1913.

[3] Oswald, A., Zeits. physiol. Chem. **95**: 351. 1915.

C. Lange considers that the reaction of iodine with proteins depends upon the presence of aromatic nuclei, and that the determination of an "iodine number" would be useful. He proposes a method based on decolorizing standard starch-iodine mixtures.[1] In this connection reference should be made to the previously cited work of W. Lange, on the determination of sulphurous acid (p. 115).

(iii) Hydrolysis. Decomposition by hydrolysis is the process which has been used most extensively and with the most fruitful results in breaking down proteins to recognizable chemical entities. It is owing to the fact that the various nitrogenous colloids termed proteins, which are isolated by processes described elsewhere (p. 144), differ one from another both qualitatively and quantitatively in their yield of amino-acids on hydrolysis that the theory of the chemical plurality of proteins has been founded. Early investigators, e. g., Liebig and Mulder, held the view that in spite of differences in physical properties and appearance of the nitrogenous colloids from plants and animals, only one protein existed. This idea of the unity of protein has faded from exact science, to remain as one of the metaphysical props of speculative biology in the shape of the unproven assumption of a unitary physical basis of life.

The hydrolysis of proteins, including gelatin, involves a splitting off of relatively simple molecules by taking up the elements of water. It may be effected (α) by heating in solution under pressure, (β) by boiling with acids, (γ) by boiling with alkalies, or (δ) by the action of enzymes. This last will be considered separately (see p. 159).

(α) This method, while extensively used practically, has been little used in analytical work.

(β) Of the acids, hydrochloric, sulphuric, and hydrofluoric have been most frequently employed; and it is generally considered, in consequence of the investigations of Abderhalden, Funk, and others, that complete hydrolysis is effected by boiling for sufficient time with either concentrated hydrochloric or 25-33 per cent sulphuric acid. For the former six to twelve hours', with the latter twelve to twenty hours' boiling is usually sufficient, the completion of hydrolysis being determined by a negative result of the biuret test. The length of time varies greatly with different proteins and is of primary importance.

[1] Lange, C., Biochem. Zeits. **95**: 46. 1919.

Results also depend upon the strength of the acid (the stronger the acid, the greater the amount of complex polypeptides), as dilution promotes more complete hydrolysis, but requires a longer time.

Andersen[1] finds that the products of complete acid hydrolysis contain considerable ammonia. The ammonia in proteins is bound partly as amide nitrogen, possibly in part as uramido-acids.

Levene and Birchard[2] have investigated Siegfried's hypothesis that partial hydrolysis of a protein gives a "kyrine" fraction which resembles natural protamines. Using gelatin they found polypeptides of arginine, lysine, glutaminic acid, etc.

Van Slyke considers that acid hydrolysis is complete when the amino-nitrogen reaches a maximum, whether hydrolysis be effected at 100 or 150° C. About twenty-four hours at 100°C., using twenty per cent hydrochloric acid, is generally sufficient.

(γ) Alkaline hydrolysis, which appears to be more complete than acid, gives different products. In particular, certain di-amino-acids are further decomposed, with evolution of ammonia. Cystine, a sulphur-containing amino-acid, is also decomposed by alkali. A further noteworthy fact is that hydrolysis by alkalies is accompanied by racemization,—i. e., diminution of optical activity. This racemization is generally not complete. Thus, Dakin[3] found that, when gelatin and casein[4] were digested at low temperatures with dilute alkali, rapid racemization occurred, but the optical activity was not entirely destroyed. From Dakin's work and from the researches of Kossel and Weiss[5] it appears probable that certain of the protein units (amino-acid groups) undergo racemization much more readily when bound up or condensed as protein than when free. Thus active arginine is not affected in this way by dilute alkali. In explanation of his earlier observations on the different behavior of hydrantoins and uramido-acids, Dakin advances the hypothesis that racemization occurs when the peptide linking exists, because then there is the possibility of keto-enol isomerism, of which free amino-acids are incapable (but see p. 159 on

[1] Andersen, A. C., Biochem. Zeits. **70**: 344. 1915.
[2] Levene, P. A., and Birchard, F. J., J. Biol. Chem. **13**: 277. 1912–13.
[3] Dakin, H. D., J. Biol. Chem. **13**: 357. 1912-13.
[4] Dakin, H. D., and Dudley, H. W., J. Biol. Chem. **15**: 263, 1913.
[5] Kossel, A. and Weiss, F., Zeits. Physiol. Chem. **59**: 492, 1909; **60**: 165, 1910.

the formation of aldehyde: ammonia). Thus the keto-enol equilibrium for a peptide grouping of two amino-acid units would be represented as:

$$R-\underset{\alpha}{CH}.CO.NH.CHR.CO_2H \rightleftharpoons R.\underset{\alpha}{C}:C(OH).NH.CHR.CO_2H.$$

When, by alkali, the enol form is induced to a small extent, racemization must necessarily follow, because asymmetry of the α–carbon atom is abolished. Further, of the two amino-acids supposed to be condensed (peptided), only that containing the CH . CO group could show keto-enol isomerism,—i. e., undergo racemization The one with free carboxyl would not be changed by alkali. Such terminal groups of a complex peptide chain are protected (by neutralization and ionization), and hydrolysis of a racemized protein should give both active and inactive amino-acids, the former from the terminal or side-chain groups, the latter from the central or closed peptide linkages. Dakin found that, when gelatin is racemized with dilute alkali, then hydrolysed by acid, glutaminic acid and lysine are obtained active, although they are obtained inactive from casein. Hence it appears that their relations are not the same in the gelatin and casein protein complexes. Later, Dakin and Dudley[1] demonstrated that racemized casein is unattacked by pepsin, trypsin and erepsin. Hence no peptide linkages in the protein remain in their original condition. As a result of their experiments, Dakin and Dudley question the possibility of the synthesis of a natural protein by existing methods, for in all these syntheses, treatments of peptide compounds with alkali occur, hence racemization is probable.[2] Although in some respects a protein may be regarded as a reservoir of relatively simple diffusible amino-acids, it is evident that the reversibility of the process,

protein + water $\rightleftharpoons$ amino-acids, is still beyond our control.

Moreover, it is important to note that the state of amino-acids in solution, complex enough in view of their amphoteric character and possibly pseudo-acid or pseudo-basic nature, is apparently still further chemically complicated by the discovery of Dakin and Dudley[3] that all amino-acids in solution undergo dissociation into α -ketonic aldehydes and ammonia, thus;

$$R.CH.NH_2.CO_2H \rightleftharpoons R.CO.CHO + NH_3.$$

[1] Dakin, H. D., and Dudley, H. W., J. Biol. Chem. **15:** 271. 1913.

[2] See also Hopkins, F. G., J. Chem. Soc. (Abst.) **106:** 1014. 1914. Physiological Chemistry p. 195.

[3] Dakin, H. D., and Dudley, H. W., J. Biol. Chem. **14:** 555. 1913.

This is supported by the production *in vivo*, as the first stage of the breakdown of amino-acids, of the corresponding keto-acid by oxidation. The production of aldehydes in this way may also be significant in regard to the reducing action of gelatins on silver salts, and the occurrence of emulsion fog. Further, it lends support to the solution-aggregate theory of proteins to be mentioned later.

For an account of the action of alcoholic caustic soda solutions on albumin and gelatin reference may be made to papers by Fahrion,[1] and by Paal and Schilling[2].

(iv) Fusion with Alkali. Direct fusion of solid proteins with caustic alkali produces a very far-reaching decomposition, products similar to those formed by bacterial putrefaction being obtained. References to work on this subject will be found in Mann's *Chemistry of the Proteids*, p. 91.

(v) Decomposition by Bacteria and Enzymes. Although the synthesis of proteins from amino-acids *in vivo* is supposed to be accomplished by enzymes, it is pointed out by Cohnheim[3] that no case is yet known in which the conversion of amino-acids in solution has been effected after destroying the structure and vitality of the cell.[4] On the other hand, the decomposition and hydrolysis of proteins is readily accomplished by certain enzymes, and important conclusions have been drawn from the specificity of these reactions.

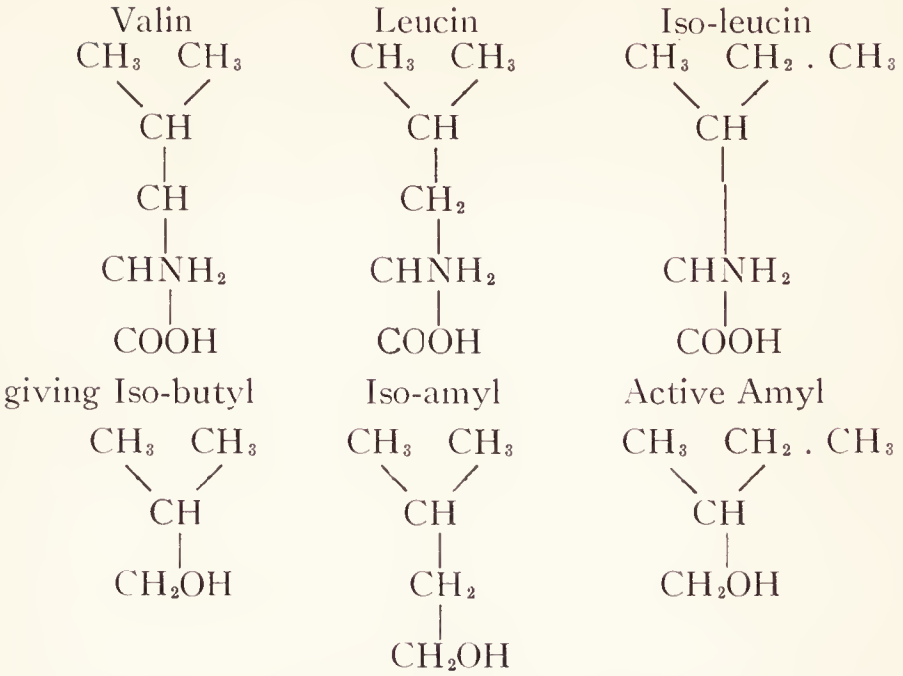
For the action of yeasts, molds and putrefactive bacteria, reference may be made to the works of Cohnheim, Mann and others. One of the interesting features of the action of yeast cells is the production of the alcohols of fusel oil (iso-butyl, iso-amyl, and active amyl alcohol) from the corresponding amino-acids, thus:

[1] Fahrion, W., Chem. Ztg. **19**: 1000. 1895; Chem. Centrbl. IV. **7**: 227. 1895.

[2] Paal, C., and Schilling, W., Chem. Centrbl. IV. **7**: 537. 1895.

[3] Cohnheim, O., Chemie der Eiweiss Körper, p. 56.

[4] On reversible enzyme reactions for polysaccharides see Bourquelot, E., J. pharm. chim. **10**: 361 and 393. 1914; Chem. Abst. **9**: 1339. 1915; and on reversibility in relation to protamines, see Robertson, T. B., J. Biol. Chem. **5**: 493. 1908–9; also Taylor, A. E., J. Biol. Chem. **3**: 87. 1907.



a result of great importance in fermentation chemistry.

In the decomposition of proteins by bacteria the first stages are similar to those of hydrolysis by boiling acids or by separate enzymes—i. e., the production of proteoses, peptones, and amino-acids. Since α-amino-acids form the best nutriment for bacteria, however, the process goes further, in that (i) as with alkalies or oxidizers, ammonia may be split off and acids produced; (ii) carbon dioxide may be split off, and bases produced; or (iii), as with yeasts, both ammonia and carbon dioxide are eliminated, and alcohols, acids and oxy-acids formed.

Of the proteoclastic enzymes or ferments the most important are:

Enzyme	Obtained from	Acts in	Produces
Pepsin	Animal stomach (gastric juice)	Acid (HCl) solution	Proteoses and peptones from proteins.
Trypsin	Pancreas	Neutral to alkaline solution	Amino-acids, from peptones and some proteins.
Erepsin	Small intestine	Neutral to faintly alkaline	Amino-acids, from peptones and proteoses.

A fairly rapid conversion of gelatin to gelatose is possible by the action of trypsin.

It has been found that the exact hydrogen-ion concentration is of great importance for enzyme hydrolysis. Thus Palitzsch and Walbum,[1] using a boric acid-sodium hydroxide mixture as a hydrogen-ion buffer, found that the optimum hydrogen-ion concentrations for trypsin are:

Temperatures	Hydrogen-ion concentration	(PH)
30°C.	$10^{-9.9}$	9.9
37°C.	$10^{-9.7}$	9.7
45°C.	$10^{-9.1}$	9.1
55°C.	10^{-8}	8.0

While a gelatin sol free from boric acid sets more rapidly in neutral then in alkaline solution, no such difference is observed in the presence of boric acid; in fact, one German gelatin, partly decomposed by alkali, and containing boric acid, set more rapidly when alkaline than after neutralization.

The great mass of biochemical work on enzyme hydrolysis of proteins can not be discussed here. It may be noted, however, that Andersen,[2] following the action of pepsin, of trypsin and of erepsin finds that enzyme hydrolysis is never complete; and that Selitrenny[3] isolated the following bacterial decomposition products of gelatin after anaerobic action of *B. liquefaciens magnus:*—

Distillate	Residue
Methyl mercaptan (slight)	glycocoll
Skatol, indol, phenol—absent	(peptones)
Phenylpropionic acid (chief product)	

After aerobic decomposition, phenyl acetic acid was also found.

NITROGEN CONTENT AND DISTRIBUTION IN PROTEINS

The total nitrogen in different proteins, while not varying very considerably, does so more than the carbon and hydrogen. It is generally two to three per cent higher in the plant globulins than in animal proteins. In gelatin, as stated above, it is about eighteen per cent. On decomposition by hydrolysis a number of amino-acids are obtained, as well as ammonia and small quantities of complex nitrogen-containing substances termed humins. The general distribution of nitrogen in the protein may be referred to:

Ammonia, termed amide-nitrogen;
Mono-amino-acids, termed mono-amino-nitrogen;
Di-amino-acids, termed di-amino-nitrogen;
Humins, termed humin-nitrogen.

[1] Palitzsch, S., and Walbum, L. E., J. Soc. Chem. Ind. **31**: 1140. 1912.
[2] Andersen, A. C., l. c.
[3] Selitrenny, L., Monatsh. Chem. **10**: 908. 1889; J. Soc. Chem. Ind. **9**: 401. 1890.

The di-amino-acids are differentiated from the mono-amino-acids by precipitation with phosphotungstic acid. Hausmann[1] was the first to devise a systematic scheme for determining the percentage distribution of the nitrogen in these groups, and the figures are often known as the Hausmann numbers. The method as originally devised by Hausmann is as follows:

i Hydrolysis of the protein with hydrochloric acid;
ii Determination of the amide nitrogen by distillation of the diluted product with milk of magnesia, by which the ammonium salts are decomposed and the ammonia collected in standard acid;
iii After distillation, di-amino-acids are precipitated from the residual liquid with phosphotungstic acid, and nitrogen in the precipitate determined by the Kjeldahl method;
iv The nitrogen in the filtrate from (iii) is determined, giving mono-amino-nitrogen.

Various objections to this scheme have been raised,[2] notably as to the dependence of the amide-nitrogen on the strength of the hydrolyzing acid and the duration of hydrolysis. A modified method has been developed from the work of Osborne and Harris[3] and Gümbel,[4] in which the process is as follows:

i About 1 gm. of protein is boiled with 100 cc. of 20 per cent HCl aq. in a 500 cc. round-bottomed flask with reflux until a biuret reaction is no longer obtained—(usually 7 to 10 hours). The solution is then concentrated in vacuo at 40° C. to 2 or 3 cc., the greater part of the HCl being thus removed.

ii This is then diluted with 300 cc. water, and cream of magnesia, freed from all NH_3 by long boiling, is added in slight but definite excess. The solution is then distilled in vacuo at 40° C., the distillate being collected in an excess of standard acid, until about half the liquid is distilled off. Titration of the acid gives the amide-nitrogen.

iii The residual solution is filtered through nitrogen-free paper, and the residue washed thoroughly with water. The nitrogen in this precipitate is estimated by the Kjeldahl method and taken as the humin nitrogen.

iv The filtrate is concentrated to 100 cc., cooled to 20° C., and 5 gms. of H_2SO_4, followed by 30 cc. of a solution containing 20 gms. of phosphotungstic acid and 5 gms. H_2SO_4 per 100 cc., are added.

v After standing 24 hours, the precipitate is filtered off and washed with a solution containing 2.5 gms. of phosphotungstic acid and 5 gms. of H_2SO_4 per 100 cc. Washing is effected by rinsing the precipitate from the filter into a beaker and returning to the paper three successive times, each portion of the wash solution being allowed to run out completely before the next is applied—about 200 cc. fluid being used.

[1] Hausmann, W., Zeits. physiol. Chem. **27**: 95. 1899; **29**: 136. 1900.
[2] Plimmer, R. H. A., l. c. Part I., p. 64.
[3] Osborne, T. B., and Harris, I. F., J. Amer. Chem. Soc. **25**: 323. 1903.
[4] Gümbel, T., Beitr. Chem. Physiol. Path. **5**: 297. 1903–4.

vi The precipitate is transferred to a 600 cc. resistance glass flask and nitrogen estimated by the Kjeldahl method, the precipitate being digested with 35 cc. H_2SO_4 conc. for 7 to 8 hours. Potassium permanganate crystals may be added as catalyst. The amount of H_2SO_4 may be reduced when the precipitate is small, but enough must be used to prevent bumping.

vii The amount of the remaining nitrogen, due to the mono-amino acids, is ascertained by substracting the sum of the nitrogen previously found from the amount of total nitrogen as determined by the Kjeldahl method.

The results of such analyses for gelatin and certain other proteins phototechnically interesting are given below in percentages.

Protein	Amide Nitrogen	Di-amino Nitrogen	Mono-animo Nitrogen	Humin Nitrogen	Total Nitrogen
Egg Albumin .	1.3	3.2	10.4	.3	15.5
Gelatin[1].....	.3	6.45	11.3	....	18.0
Caseinogen...	1.6	3.5	10.3	.21	15.6

In a recent paper by Jodidi and Moulton[2] attention is drawn to certain inaccuracies in the Hausmann method. The proportion of acid-amide-nitrogen obtained by Osborne and Harris' modified method is constant, and independent of the quantity of magnesium oxide used in distillation. The percentage nitrogen in the magnesium oxide precipitate is higher the greater the amount of oxide taken, and vice versa. Conversely, the proportion of mono-amino and di-amino nitrogen is smaller, the larger the amount of magnesium oxide. To obtain uniform results and a minimum of humin-nitrogen, it is necessary to use the least quantity of magnesia sufficient to render the substance to be distilled alkaline.[3]

ACTION OF NITROUS ACID AND DISTRIBUTION OF NITROGEN IN SEVEN GROUPS

Nitrous acid reacts only with amino-groups, liberating nitrogen—

$$\begin{array}{l} CH \cdot NH_2 \\ | \\ COOH \end{array} + HNO_2 = \begin{array}{l} CHOH \\ | \\ COOH \end{array} + N_2$$

and in this way Schiff obtained products from proteins no longer giving the biuret reaction. These proteins he termed

[1] Different gelatins give values of amide nitrogen ranging from 0.3 to 1.4 per cent.

[2] Jodidi, S. L., and Moulton, S. C., Science **51**: 70. 1920.

[3] See also Denis, W., J. Biol. Chem. **8**: 428. 1910–1911.

"desamido-proteins." Later investigations have not confirmed Schiff's isolations of such bodies, but the use of nitrous acid for estimating amino-acids and amides has been made practicable by the work of Sachse and Kormann, Brown and Millar, and Van Slyke.[1] By means of this reaction it is possible to subdivide the products of hydrolysis of a protein into fractions containing amino-groups and those containing nitrogen in a heterocyclic combination. Therefore, a further refinement in the distribution of nitrogen in the protein becomes possible. For a fuller account of this, reference should be made to the literature cited.

The following scheme (p. 165) gives a general outline of the method worked out by Van Slyke, and used for pure amino-acids singly and mixed, as well as for typical proteins.[2]

RESULTS (VAN SLYKE)

Protein	Amide	Humin	Cystine	Argi-nine	Histi-dine	Lysine	Mono-Amino	Non-Amino	Total
	N	N	N	N	N	N	N	N	N
Gliadin from wheat	25 .52	.86	1 .25	5 .71	5 .20	0 .75	51 .98	8 .5	=99 .77
Gelatin[3]	2 .25	.07	0 .00	14 .70	4 .48	6 .32	56 .3	14 .9	=99 .02
Fibrin	8 .32	3 .17	0 .99	13 .86	4 .83	11 .51	54 .3	2 .7	=99 .58
Haemoglobin	5 .24	3 .6	0 .00	7 .70	12 .70	10 .9	57 .0	2 .9	=100 .04

[1] Slyke, D. D. van, Chem. Abst. **4**: 933. 1910; Ber. chem. Gesell. **43**: 3170. 1910; Ber. chem. Gesell. **44**: 1684. 1911; J. Biol. Chem. **9**: 185. 1911; Ber. chem. Gesell. **43**: 3170. 1910; J. Biol. Chem. **9**: 205, 1911; J. Biol. Chem. **10**: 15. 1911.

[2] On special precautions in determination of arginine and histidine, see Plimmer, R. H. A., l. c.

[3] From determinations on a large number of commercial gelatins we have found amide-nitrogen ranging from .70 to 1.25 per cent.

Protein → (Total N by Kjeldahl)
Hydro-lysis
Distillation at 40° C with magnesia
Distillate Amide N
Residue filtered
Insoluble residue
Humin N
Filtrate treated with phosphotungstic acid
Precipitate
Filtrate
Di-amino N
Mono-amino N
Amino-N by nitrous acid
Cystine by total sulphur of precipitate
Hexone bases (+ cystine) by total nitrogen of precipitate
Arginine, by boiling with alkali gives half its nitrogen
Arginine N (Arginine)
Total N by Kjeldahl
Amino N by Nitrous acid
Mono-amino N
Non-mono-amino N by difference
Total N of bases less total amino-N gives total non-amino N. This is D and is in arginine + histidine. Therefore, histidine N = 3/2 (D − 3/4 Arginine) = 1.500 D − 1.125 Arginine
Lysine N = Total N − (Arginine N + Cystine N + Histidine N)

Bogue[1] has made a number of analyses of hide and bone glues by this method, with results as follows:

HIDE GLUE ANALYSES

Figures show per cent of total nitrogen in each fraction.

	H_1	H_2	H_3	H_4	H_5	H_6	Average
Ammonia nitrogen	1.63	1.89	3.20	2.15	2.44	2.49	2.90
Melanin nitrogen	0.53	0.50	0.74	0.53	0.60	0.63	0.59
Cystine nitrogen	0.00	0.00	0.00	0.00	0.00	trace	0.00
Arginine nitrogen	13.27	16.28	13.76	13.72	13.50	12.87	13.90
Histidine nitrogen	1.31	1.30	3.19	3.31	2.45	1.59	2.19
Lysine nitrogen	8.17	8.50	8.58	7.40	8.00	7.22	7.97
Amino-nitrogen in filtrate	58.87	55.17	55.00	57.90	58.02	56.10	56.84
Non-amino-nitrogen in filtrate	17.00	15.53	15.58	15.26	15.24	15.20	15.63
Total regained	100.78	99.17	100.05	100.27	100.25	96.10	100.02

BONE GLUE ANALYSES

	B_1	B_2	B_3	B_4	B_6	B_9	Average
Ammonia nitrogen	4.43	4.49	4.57	4.49	4.48	5.04	4.55
Melanin nitrogen	0.74	1.18	1.03	0.82	0.76	0.95	0.91
Cystine nitrogen	0.00	0.00	0.00	0.00	0.00	trace	0.00
Arginine nitrogen	13.32	12.82	13.28	12.74	13.56	13.32	13.17
Histidine nitrogen	1.60	0.54	1.52	1.44	1.58	4.02	1.78
Lysine nitrogen	7.18	8.23	7.18	8.57	9.42	9.13	8.28
Amino-nitrogen in filtrate	56.90	58.15	57.30	57.58	54.30	53.40	56.27
Non-amino-nitrogen in filtrate	16.21	15.18	15.32	14.36	15.90	14.54	15.25
Total regained	100.38	100.59	100.20	99.80	100.00	100.40	100.21

ANALYSIS OF DOG HAIR AND GELATIN

	Dog Hair	Gelatin
Ammonia nitrogen	10.05	2.25
Melanin nitrogen	7.42	0.70
Cystine nitrogen	6.60	0.00
Arginine nitrogen	15.33	14.70
Histidine nitrogen	3.48	4.48
Lysine nitrogen	5.37	6.32
Amino-nitrogen in filtrate	47.5	56.3
Non-amino-nitrogen in filtrate	3.1	14.9
Total regained	98.85	99.02

[1] Bogue, R. H., Chem. Met. Eng. **23**: 154. 1920.

Bogue concludes from these results that the ammonia nitrogen increases as the grade decreases, and is decidedly higher in bone glues than in hide glues; that melanin (humin) nitrogen is higher in bone glues than in hide; that arginine shows no consistent variation with the grade, but is somewhat higher in the hide series; that histidine is irregular, generally high with hide, but highest with pressure tankage from bone. Lysine is fairly uniform in both series, increasing slightly in the lower grades of bone glue, while both amino- and non-amino-nitrogen show a tendency to decrease with the grade. Bogue points out that in lower grade glues hydrolysis may have extended to protein complexes other than collagen—e. g., keratin, chondridin, etc. Further, it appears that there is a sufficiently marked variation between hide and bone glues to justify the conclusion that they are derived from different original proteins—a conclusion which is shown most decisively by the higher ammonia-nitrogen figure for bone glues, indicating possibly a higher content of dicarboxylic acid, and which is confirmed by the physical differences of the reprecipitated proteins. "That from hide glue is white and forms very tough leathery masses which are not sticky, while the bone protein, although white at the moment of precipitation, very rapidly becomes brownish and is a sticky, soft, gummy mass."[1]

REMARKS ON THE FOREGOING SYSTEMATIC ANALYSIS

So far as the technical use of gelatin or other proteins, particularly in photography, is concerned, the foregoing analyses for nitrogen distribution have up to the present had no such importance as they possess for pure and applied biochemistry. But, although the more complete schemes would probably seldom be of use save in research, three at least of the methods used in such analyses are of great importance and value, even for control. These are:

i) Total Nitrogen Determination by the Kjeldahl Method. The well-known Kjeldahl method for the determination of organic nitrogen is described in most analytical textbooks. According to Bennett and Holmes,[2] some difficulty may be encountered in obtaining concordant results for the amount of nitrogen in gelatin. They carried out experiments to determine the shortest reliable time and the best method

[1] From recent experiments in this laboratory it appears that this statement requires some qualifications, as tests with bone and hide gelatins do not entirely agree with Bogue's results with glues.

[2] Bennett, H. G., and Holmes, N. L., J. Soc. Leather Trades' Chem. 3: 24. 1919; Chem. Abst. 13: 1164. 1919.

for obtaining complete decomposition, using the following conditions of digestion with sulphuric acid:—

a. No accelerator . . . . . Incomplete decomposition;
b. 2 grams $Na_4P_2O_7$ . . . . . Not so good as sulphate;
c. 5 grams $Na_4P_2O_7$ . . . . .
d. 10 grams K_2SO_4. . . . . Best results at 4-6 hours' digestion.

Care must be taken to ascertain complete decomposition (clearing of the solution is not necessarily an indication), and also not to prolong digestion unduly, as in the latter case low results, due to loss of ammonia, are inevitable.

Our practice in the Eastman Research Laboratory is to digest 0.5 gm. gelatin (air-dry sample), with 15-25 cc. of concentrated H_2SO_4 and one drop of mercury. This is heated gently until fuming begins, when the flame is removed and 10-12 gms. $KHSO_4$ added. The mixture is then digested till clear—usually for about thirty minutes. There is no advantage in prolonging the digestion over an hour when mercury is used as catalyst. After adding K_2S to break up mercury-ammonia complexes, the ammonia is distilled off the alkaline liquor as usual. Very concordant results have been obtained in this way.

Moisture- and ash-free samples of various gelatins gave percentages of total nitrogen as follows:—

A 18.09 per cent nitrogen—(hide, experimental)
B 18.13 per cent nitrogen—(hide, experimental)
C 18.05 per cent nitrogen—(hide, experimental)
D 18.22 per cent nitrogen—(commercial)
E 17.7 per cent nitrogen—(commercial)
F 17.78 per cent nitrogen—(pure, from calfskin)
G 18.66 per cent nitrogen—(from ossein)
H 17.2 per cent nitrogen—(glue)

ii) Determination of Ammonia and Amide Nitrogen. The determination of free ammonia and ammonia available by hydrolysis is also an important datum for photographic gelatins. Methods of determination have been noticed in passing, but fuller particulars are desirable. The modifications of the ordinary distillation of ammonia used are either (a) distillation in vacuo or (b) a method of aeration.

(a) The distillation in vacuo is accomplished by means of an apparatus similar to that shown in Fig. 28. A double-necked Claisen flask of one liter capacity and an ordinary distilling bulb of the same size are connected as shown, with a small guard flask of 200 cc. The solution to be distilled is placed in the Claisen flask, diluted to 200 cc., and 100 cc. of alcohol (to prevent frothing) and an excess of a 10 per cent suspension of

magnesium or calcium hydroxide (excess being shown by turbidity and alkaline reaction), are added. The stopcock serves to admit air if distillation starts too rapidly, and to release the vacuum on conclusion of distilling. Both receiving flasks contain N/10 acid, and the contents are titrated with N/10 NaOH, using preferably alizarin sulphate as indicator.

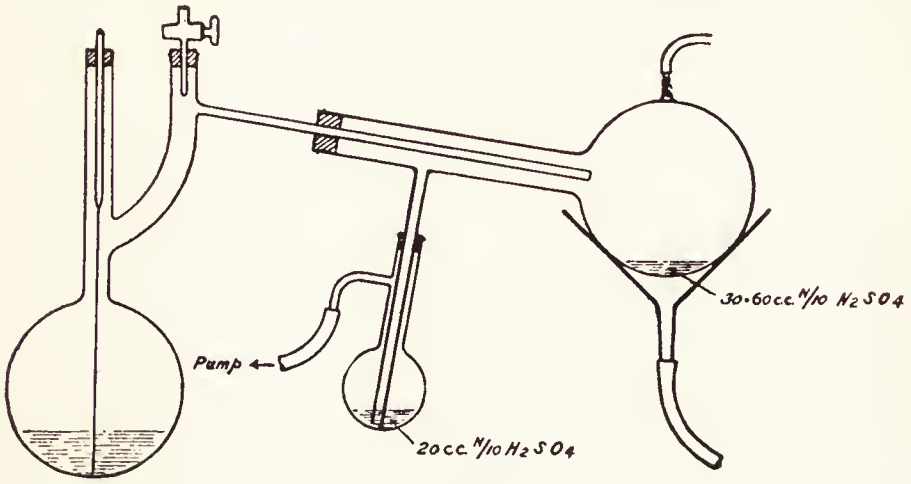


FIG. 28
Distillation apparatus

(b) Instead of distillation, the discharge of ammonia may be effected by aeration, as introduced by Denis[1] and Folin,[2] and elaborated by Kober.[3] The ammonia is removed by suction with a water-pump or by an air-blast. For particulars of the Folin-Denis micro-chemical method for determining nitrogen by direct Nesslerization, their papers, and also a criticism by Bock and Benedict, should be consulted.[4]

THE AMINO INDEX

Reference has been made to the decomposition of amino-acids by nitrous acid. The method was used by Brown and others to give an amino index (termed AoI) from hydrolyzed proteins, and the technique has been greatly improved by Van Slyke. The typical reaction is:—

$$\begin{matrix} R{-}NH_2 \\ | \\ COOH \end{matrix} + HNO_2 = \begin{matrix} R\ \ .OH \\ | \\ COOH \end{matrix} + N_2 + H_2O,$$

[1] Denis, W., J. Biol. Chem. **8**: 427. 1910–11.

[2] For Folin's micro-analytical methods see Plimmer, R. H. A., l. c.

[3] Kober, P. A., J. Amer. Chem. Soc. **30**: 1131. 1908; *ibid.* **32**: 689. 1910.

[4] Folin, O., and Denis, W., J. Biol. Chem. **26**: 473. 1916; **29**: 329. 1917. Bock, J. C., and Benedict, S. R., J. Biol. Chem. **20**: 47. 1915. See also Bang, I., Biochem. Zeits. **49**: 19. 1913; Bang, I., and Larsson, K. O., Biochem Zeits. **51**: 193. 1913; Kochmann, M. Biochem Zeits. **63**: 479. 1914; and Barnett, G. D., J. Biol. Chem. **29**: 459. 1917.

so that half the volume of nitrogen evolved corresponds to the amount of nitrogen in the amino-acid. The amino-index is then the ratio of $\frac{\text{Nitrogen by nitrous acid}}{\text{Total Nitrogen (by Kjeldahl)}}$. The apparatus consists of a reaction chamber for the reaction of the amino-body with nitrous acid, an absorption vessel to absorb nitric oxide, and a nitrometer. Fig. 29 shows Van Slyke's apparatus for this purpose.

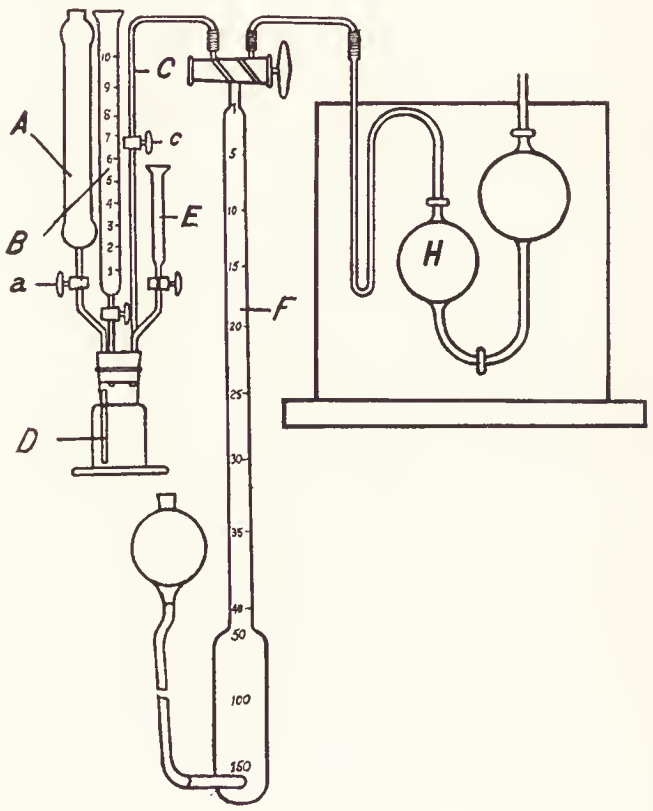


FIG. 29
Van Slyke's apparatus for nitrogen determination

In working with this apparatus, the writer has found it advantageous to substitute a Traver's gas absorption pipette[1] in place of the Hempel bulb. Instead of shaking the bulbs, the gas is circulated around the absorption pipette and thoroughly scrubbed.

THE FORMALDEHYDE REACTION (and alternative amino-index). A reaction for quantitative work on proteins, second in importance only to that of nitrous acid, is that with formaldehyde. From the presence of amino- and imino-groups a reaction is to be expected.

[1] Travers, M. W., The Experimental Study of Gases.

The action of formaldehyde in coagulating egg-albumin and in rendering gelatin insoluble was early noticed by Trillat and Hauser, and Schwarz showed that Blum's apparently discordant observation that small amounts of formaldehyde prevented coagulation on heating, depends on the concentration used. In dilute solutions of albumin coagulation is inhibited; in concentrated solutions increased turbidity is found, and coagulation can be effected by salts.

Benedicenti studied the reaction quantitatively by adding dilute (two per cent) solutions of formaldehyde to protein solutions and determining the remaining formaldehyde at intervals. For this he used hydroxylamine hydrochloride, which reacts as follows:

$$NH_2.OH.HCl + H.CHO = CH_2.N.CH + HCl.$$

Hence, by titrating the hydrochloric acid set free, using methyl orange as an indicator, the formaldehyde can be estimated. Since the proteins behave as bases to methyl orange at the start, an extra amount of acid is necessary to neutralize to this indicator before adding hydroxylamine. This basicity diminishes as the reaction proceeds, indicating that the formaldehyde is condensed with amino-groups, forming methylene derivatives.

The reaction with dilute solutions, which requires two to three weeks, is too slow to be of much use technically. Schiff has used concentrated formaldehyde solutions—forty per cent, commercial "formalin" strength—and then titrated to determine the acidity of the formalin-protein solution. Assuming the reaction to be the same as with amino-acids, the amino-groups react with aldehyde, forming methylene derivatives; hence the basicity due to these is eliminated and the acidity due to carbonyl groups set free. This can be titrated directly, using phenolphthalein. In this way it was found that one gram molar equivalent of potassium hydroxide neutralized 4.680 grams gelatin, after treating the solution with formaldehyde. Whether neutralization was effected directly after mixing or after long standing did not noticeably affect the results.

Sörensen,[1] considering that the reaction

$$\begin{array}{l} CH_3 \\ | \\ CH.NH_2 + HCOH \\ | \\ COOH \quad + KOH \end{array} \rightleftharpoons \begin{array}{l} CH_3 \\ | \\ CH.N.CH_2 + H_2O \\ | \\ COOK \qquad\quad H_2O \end{array}$$

[1] Sörensen, S. P. L., Zeits. physiol. Chem. **103**: 1, 1918; **103**: 15, 211, 267, 1918; **103**: 104, 1918.

is reversible, looked for an indicator giving its color change at such a high hydroxyl-ion concentration that the above reaction might be carried practically completely from left to right. Phenolphthalein, with titration to strong red color, is satisfactory, but thymolphthalein is better. In a case where not a differential increase (as in proteolysis), but the actual content of amino-acids is to be measured, allowance must be made for the presence and activity of other acids. For this it is necessary to adjust the solution first to a hydrogen-ion concentration such that equivalent amounts of acid and of basic groups are present in the solution. Then add formalin (neutralizing the amino-groups), setting free the acidity equivalent thereto. Usually carbonic and phosphoric acids or acid salts are the trouble makers, and these may be thrown out with baryta and barium chloride. As a P_H value for adjustment, $P_H = ca.\ 6.8$ is satisfactory,—e. g., a very weak acid reaction to sensitive litmus paper. Where dark-colored hydrolyzates have to be titrated, clarification may be effected by silver chloride precipitated in the solution, or by a metallic sulphide similarly formed. The use of animal charcoal is generally objectionable. To adjust to the desired initial neutral point, a comparison buffered solution (equal parts M/15 primary potassium phosphate and M/15 secondary sodium phosphate), which has the desired P_H, is used. The litmus paper should conform to the following standards:

$P_H = 6.47$ 3 parts secondary + 7 parts primary phosphate →weak acid reaction.

$P_H = 6.81$ 5 parts secondary + 5 parts primary phosphate →neutral reaction.

$P_H = 7.77$ 7 parts secondary + 3 parts primary phosphate →weak alkaline reaction.

For the actual titration, N/5 sodium hydroxide, which is preferable to N/10, is titrated against N/5 hydrochloric acid, using a solution of 5 gms. phenolphthalein in 50 cc. alcohol and 50 cc. water. The formalin solution should be prepared fresh for each series, 50 cc. of 30 to 40 per cent formalin being used with 1 cc. of the indicator, and N/5 sodium hydroxide added to a faint rose red tint. The quantity of organic fluid titrated should be 20 cc., which is approximately N/10 for nitrogen. For sharp end-point determinations, a comparison solution is used, which may be prepared as follows: To 20 cc. well boiled distilled water 10 cc. of the formalin solution are added, then about half as much sodium hydroxide as necessary in the actual estimation. This is titrated back with N/5 hydrochloric acid

(with frequent shaking) until it shows a faint rose color (first stage); then, with the addition of one drop of N/5 caustic soda, a definite red color is obtained (second stage). The solutions are titrated to this point by comparison; then to 20 cc. of the fluid 10 cc. of the formalin mixture are added, and N/5 caustic soda in excess. The back titration with N/5 hydrochloric acid is carried just beyond the control color, and then caustic soda added by drops until the color matches. After all the fluids have been titrated in this way (phenolphthalein, second stage), the control solution is made deep red with two drops of caustic soda and all the sample fluids are then titrated to the same point.[1]

As hydrolysis proceeds and the polypeptide groups are broken down, the number of free amino- and carboxyl-groups increases in a given volume, so that by treating the product at any stage with formaldehyde and titrating, the rate of proteolysis can be followed.

Obermayer and Willheim[2] have also applied the method to a determination of the relative number of free amino-groups in different proteins. They term the quotient of the total amount of nitrogen divided by the nitrogen of the terminal amino-groups the amino-index, and find its value widely different in different proteins. For euglobulin the mean value (21.5) is higher than for albumins (12.0). It is thus possible to show that the various protein fractions from serum are not homogeneous substances, also that the index of a definite fraction may be characteristic for definite classes of animals. It is evident from this and also from the writer's work that this amino-index can be used technically with advantage to determine the presence of gelatoses, mucins, etc., in gelatins, as well as whether the gelatin has been over-extracted. The use of the amino-index as a fundamental in routine analysis, for control of the lime-bath, indicated by Wood and Law, has been discussed in Chapter II.

It should be noted in this connection that Glagolev,[3] from investigations on glycine anhydride, lactimide, tyrosine anhydride, and glycyl-leucine anhydride, concludes that the diketopiperazines (the anhydride ring combinations), (see p. 189), do not react to formaldehyde.[4]

[1] Thymolphthalein may be used instead of phenolphthalein, in which case dissolve 0.5 gms. in one liter of 95 per cent alcohol, and add 5 cc. of this solution to 50 cc. of a 30 per cent formalin solution, with 25 cc. of alcohol. Then add alkali until there is a bluish opalescence (first stages). Two more drops of alkali produce a blue color (second stage).

[2] Obermayer, F., and Willheim, R., Biochem. Zeits. **50**: 369. 1913; abstract J. Chem. Soc. **103–4**: 668. 1913.

[3] Glagolev, P., Chem. Abst. **9**: 2918. 1915; Biochem. Zeits. **70**: 119. 1915.

[4] Cf. Clementi, A., On micro-titration with formaldehyde. Chem. Abst. **10**: 617. 1916.

Mention should also be made of a colorimetric method for the estimation of α-amino-acid nitrogen, developed by Harding and MacLean,[1] who found that the method can be used to follow the hydrolysis of proteins. It is claimed to be more sensitive that Sörensen's or Van Slyke's methods, but is inapplicable in strongly acid or alkaline solutions.

In a recent paper Foreman[2] has described certain rapid volumetric methods for the estimation of amino-acids, organic acids, and organic bases, which can be applied usefully in gelatin technology, particularly in dealing with hydrolyzates, lime liquors, and special extracts. The method is based on precipitation of all proteins, albumoses, peptones and other interfering substances, followed by titration of the alcoholic solution or filtrate. In this procedure Foreman makes use of Sutton's observation[3] that potash or soda will displace ammonia in equivalent quantities from ammonia salts in alcoholic solutions at ordinary temperatures, the ammonia forming, however, no compound with the phenolphthalein used as indicator. The acid in an ammonium salt can therefore be titrated against standard alkali in alcoholic solution. Foreman finds that the alcoholic extract should contain about 85 per cent alcohol, and that this may be titrated with N/10 alkali (caustic potash) using phenolphthalein. Under these conditions the amino- or imino-groups released from "internal salt" combination resemble ammonia in showing no basicity to phenolphthalein, and carboxyl groups can therefore be estimated quantitatively. Certain amino-acids, notably dibasic ones, give low results, but the addition of acetone or formaldehyde makes these give quantitative values also. The general method is divided into stages, viz.:

Stage 1—Titration in water;
Stage 2—Titration in alcohol;
Stage 3—Titration in alcoholic formaldehyde.

The results from stage 1 give useful information when dealing with the dibasic amino-acids, arginine, and salts of amino-acids. The stage 2 value includes the stage 1, and the stage 3 gives an idea of the character of the amino-acid, and indications of the amount of dibasic amino-acids present. Also, a rapid estimation of volatile bases and amino-acids becomes possible, superposed on stage 3. The total titration value of an aliquot portion of the "alcoholic extract"[4] determined by completing

[1] Harding, V. J., and MacLean, R. M., J. Biol. Chem. **20**: 217. 1915; **24**: 503. 1916.
[2] Foreman, F. W., Biochem. J., **14**: 451. 1920.
[3] Sutton, Volumetric Analysis, p. 38 (Eighth edition, 1900).
[4] Cf. older method described by Foreman and Graham-Smith (J. Hygiene **16**: 144. 1917).

stage 3 affords an exact measure of the amount of alkali necessary for liberating the bases from their salts. If no more than this be added, a rapid method of distillation at a higher temperature than previously used can be adopted, because decomposition of amino-acids is reduced to a minimum. The residual liquid becomes aqueous as the alcohol is removed, and the alkali salts of the amino-acids hydrolyse, so that an amount of free alkali equivalent to the amino-acids is found in the aqueous solution. Improvement in the titration of the distillate containing volatile bases is rendered possible by finding that a sharp end-point can be obtained in all concentrations of alcohol when alizarine is used as an indicator. For fuller details the original paper should be consulted.

NITROGEN DISTRIBUTION AND PHYSICAL PROPERTIES

The first comprehensive application of the analysis for nitrogen distribution to the technology of gelatin and glue is due to Bogue.[1] Bogue found but little correlation between the proportions of proximate constituents (water, total organic matter, ash) and physical properties. He therefore greatly developed and extended the salting out method used by Trotman and Hackford, using magnesium sulphate instead of zinc sulphate. According to the classification given on page 144, gelatin, as a primary cleavage product of collagen, will have associated with it, depending upon the degree of hydrolysis, various secondary cleavage products.

- α Proteoses, precipitated by $(NH_4)_2SO_4$, half saturated, or $ZnSO_4$ saturated, or half-saturated to saturated $MgSO_4$;
- β Peptones, not precipitated by salts;
- γ Peptides and amino-acids.

Bogue adopted the following analytical procedure: Each sample was weighed out and made up to a volume such that 50 cc. contained one gram of gelatin. Then

a)	50 cc. aliquot part →	Total nitrogen by Kjeldahl.
b)	50 cc. aliquot part saturated with $MgSO_4$ →	Protein + proteose nitrogen by Kjeldahl (precipitate)
c)	50 cc. aliquot part half-saturated with $MgSO_4$ →	Protein nitrogen by Kjeldahl (precipitate)
d)	Hence proteose nitrogen— b − c = d	
e)	Amino-nitrogen in filtrate from $MgSO_4$ precipitation →	Amino-nitrogen by Sörensen

[1] Bogue, R. H., l. c.

f) Peptone-nitrogen by difference a − (b + d) → Peptone nitrogen

Bogue found it necessary to adhere to a fixed temperature (25° C.) and fixed acidity of the precipitating mixture (½ cc. of 1 part in 4 sulphuric acid to 100 cc. of the precipitating mixture). For the formaldehyde titrations the procedure was as follows:—"The amino-acid solutions in saturated magnesium sulphate and the control were made faintly pink to phenolphthalein. The formaldehyde was treated with caustic soda until, on adding 25 cc. of it to 100 cc. of the control, the intensity of the original pink color remained unchanged. A drop more of the base would cause an intensifying of the color; a drop less a decrease or removal. The amino-acid solutions were then treated similarly and titrated back to a uniform, rather deep red with barium hydroxide in N/5 concentration." It was found best to use the entire filtrate from the precipitation by magnesium sulphate in all amino-acid determinations.

A large number of glues and gelatins (including standard glues, edible gelatin, Russian isinglass, fish glue, etc.), were analyzed by this method for their protein, proteose, peptone and amino-acid nitrogen.

The results of this important investigation show that very important relations exist between these nitrogen proportions and the jelly strength. These may be summarized as follows:

Jelly strength varies directly as the protein nitrogen;

Jelly strength varies inversely as the proteose and peptone nitrogen;

Jelly strength tends to vary inversely as the amino-acid nitrogen.[1]

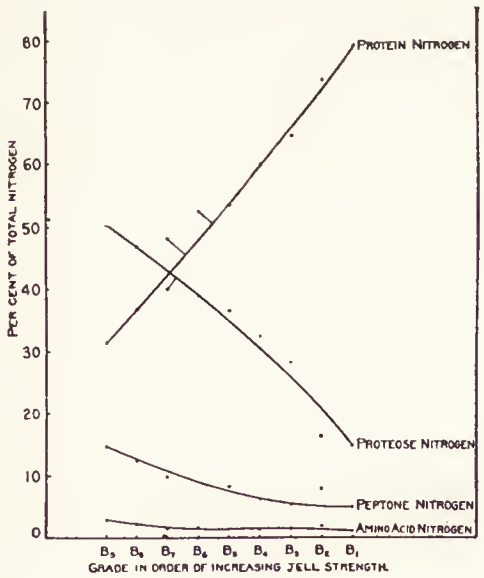


FIG. 30
Curve Showing Relation Between the Nitrogen Proportions and Jelly Strength

These results are given in Fig. 30.

On the other hand, no definite or consistent relation was found between the viscosity and the proportions of nitrogenous constituents. The author's con-

[1] It was observed that the amino-acid nitrogen is higher in bone glues than in hide glues or in fleshings.

clusions as to the influence of the size of the ultimate chemical molecule in any one group upon the physical properties will be discussed in the second volume.

In comment on the foregoing, it does not appear, from work done in the writer's laboratory, that the relation found by Bogue between "protein-nitrogen" and jelly consistency can be absolute. This is evident, on considering that many proteins, other than gelatin, have no jelly strength or consistency. On hydrolyzing a good grade animal glue, until the protein-nitrogen was approximately the same as in fish glue, the following result was obtained:

MOISTURE AND ASH-FREE SOLIDS

———	No. 1 Glue	No. 2 Hydrolyzed 4 hours at 240° F.	No. 3 Fish glue
	%	%	%
Solids	87.81	43.96	53.07
Moisture	12.19	56.04	46.93
Ash	1.50	1.50	1.13
Total nitrogen	17.10	16.17	16.81
Protein nitrogen	14.70	7.75	9.78
Proteose nitrogen	1.64	4.43	4.05
Peptone nitrogen	.30	2.75	.84
Amino-nitrogen	.48	1.24	2.12

On making solutions of all three to 10 per cent, No. 1 set to a stiff jelly at 26° C., No. 2 set to a stiff jelly at 10° C., No. 3 did not set at all. Yet No. 2 had the same viscosity as No. 3 at 25° C. It is, therefore, evident that when other proteins, as probably the case in fish glue, are present, that they may not give jelling power.

THE SEPARATION AND DETERMINATION OF THE AMINO-ACID UNITS FROM PROTEINS

As has been stated, the ultimate result of the hydrolysis of a protein is a complex mixture of amino-acids. Thus from gelatin there have been isolated and identified the following:

A—MONO-AMINO MONO-CARBOXYLIC:

Name	Formula	Per cent	
Glycine	$CH_2.(NH_2).COOH$	16.5	12.4[1]
Alanine	$CH_3.CH(NH_2)COOH$ α-amino-propionic acid	0.8	0.6

[1] After Skraup, Z. H., and Biehler, A. von, J. Soc. Chem. Ind. **28**: 947. 1909; Monatsh. Chem. **30**: 467. 1909.

Name	Formula	Per cent	
Valine	$(CH_3)_2.CH.CH(NH_2)COOH$ — α-amino-iso-valeric	1.0	
Leucine	$(CH_3)_2.CH.CH_2.CH(NH_2).COOH$ — α-amino-iso-capric acid	2.1	9.2
Iso-leucine	$\begin{matrix}CH_3\\C_2H_5\end{matrix}\!\!>\!CH.CH(NH_2)COOH$	....	
Phenylalanine	$C_6H_5.CH_2.CH(NH_2)COOH$	0.4	1.0
Tyrosine	$HO.C_6H_4.CH_2.CH(.NH_2)COOH$ — β-para-hydroxyphenyl — α-amino-propionic acid	0.0	
Serine	$CH_2(OH).CH(NH_2)COOH$ — β-hydroxy — α-amino-propionic acid	0.4	(trace)
Cystine	$HOOC.CH.(NH_2).CH_2.S$ — $HOOC.CH.(NH_2).CH_2.S$ — di (β-thio-α-amino-propionic acid)	....	

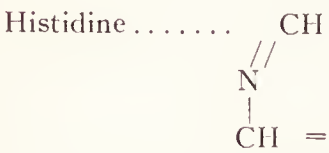
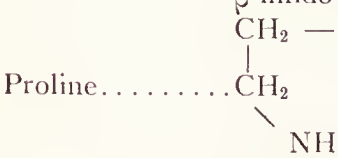
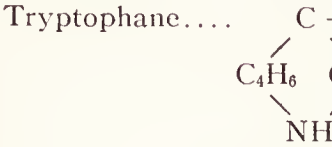
B—Mono-amino-di-carboxylic:

Aspartic acid	$HOOC.CH_2.CH(NH_2).COOH$ — amino-succinic acid	0.6	1.2
Glutamic acid	$HOOC.CH_2.CH_2.CH(NH_2)COOH$ — α-amino-glutaric acid	0.9	1.8

C—Di-amino-mono-carboxylic:

Arginine	$HN{=}C\begin{matrix}NH_2\\NH(CH_2)_3CH.(NH_2).COOH\end{matrix}$ — α-amino- δ-guanidine valeranic acid	7.6 to 9.3	9.3
Lysine	$H_2N.CH_2(CH_2)_3.CH(NH_2)COOH$ — α , ε-di-amino caproic acid	2.8 to 5	6.0

D—Heterocyclic Compounds:

Histidine	 — β-imido-azole — α-amino-propionic acid	0.4	0.4
Proline		7.7	10.4
	α-pyrolidine-carboxylic acid		
Oxy-proline	$C_5H_9NO_3$	3.0	3.0
Tryptophane	 — β-indole — α-amino-propionic acid	0.0	

E—AMINES:

Name	Formula	Per cent
Ammonia	NH_3	0.2 to 1.1

Methods of isolation based on fractional crystallization of the compounds themselves, or of their copper, silver or other salts are effective only when one or more occur in somewhat larger quantities than the rest.[1] Under suitable conditions 99 per cent of total nitrogen can be precipitated with edestin, but definite copper compounds are not isolated.

The discovery by Drechsel that proteins yield di-amino-acids as well as mono-amino was a great step in advance. In 1901 Emil Fischer introduced a new method of isolating and estimating the mono-amino-acids, known as the ester method, since it depends upon esterification and subsequent fractional distillation in vacuo. "While not yet really quantitative, it has enabled us to obtain a knowledge of some seventy per cent of the total products obtainable by hydrolysis, and it has shown that phenylalanine, serine and alanine, which were known to occur only in few, are present in all proteins, and that phenylalanine in its distribution is the principal aromatic constituent, for it often exceeds in amount that of tyrosine and occurs when the latter is absent."

Dakin[2] has quite recently shown that, by using butyl alcohol as a solvent, the products of hydrolysis of a protein may be separated into:

1) Mono-amino-acids, aliphatic and aromatic, insoluble in alcohol, but extracted by butyl alcohol;

2) Proline, soluble in alcohol and extracted by butyl alcohol;

3) Peptide anhydrides (diketopiperazines), extracted by butyl alcohol, but separated from di-carboxylic acids by phosphotungstic acid.

4) Di-carboxylic acids, not extracted by butyl alcohol;

5) Di-amino-acids not extracted by butyl alcohol, but separated from di-carboxylic acids by phosphotungstic acid.

The advantages of this method are:

a) The groups are composed of chemically similar individuals;
b) Practically all the products of the hydrolysis may be divided into five groups without serious loss, and each may be obtained in a solid form;
c) No indications of any racemization have been observed;
d) Higher yields of many amino-acids are obtained than by other methods.

[1] Osborne, T. B., and Leavenworth, C. S., l. c. See also Ritthausen, H., J. prakt. Chem. **5**: 215. 1872; with R. Rotl, *ibid.* **7**: 361. 1873; **15**: 329. 1877.

[2] Dakin, H. D., Biochem. J. **12**: 290. 1918.

Using this method Dakin has recently[1] separated and determined the amino-acids from the hydrolysates of gelatin:

Amino-acid	Per cent
Glycine	25.5
Alanine	8.7
Leucine	7.1
Serine	0.4
Phenylalanine	1.4
Tyrosine	0.01
Proline	9.5
Hydroxy-proline	14.1
Aspartic acid	3.4
Glutamic acid	5.8
Histidine	0.9
Arginine	8.2
Lysine	5.9
Ammonia	0.4

The sum of these is 91.31 per cent, whereas former analyses have given only from 40 to 70 per cent. It is noteworthy that traces of tyrosine were always found, although this amino-acid has hitherto been considered absent from the gelatin complex. The following amino-acids were not found in gelatin: valine, iso-leucine, amino-butyric acid, and hydroxyglutamic acid. Unidentified sulphur derivations were also found.

For the technique and results in this very specialized branch of organic chemistry reference should be made to the literature cited, where details concerning the chemical constitution of the units themselves are given.

The conclusion from this work is that a protein is built up of amino-acid units. Evidently the confirmation of this view depends upon the synthesis of a protein from such units. Although this has not been accomplished *de facto*, it is considered that the polypeptides synthesized by Fischer and his collaborators are such that "if found in nature they would be described as proteins," which amounts practically to a synthesis.

For a review of the evidence for polypeptide unions in the protein complex reference may be made to an article by Clementi,[2] in which he points out that the biochemical demonstration that amino-acids are bound in proteins in polypeptide form falls into two divisions:

1. Chemical isolation from the protein hydrolyzates of polypeptides synthesized chemically—(it may be objected here that the polypeptides found might be synthesized by the methods of isolation employed);

[1] Dakin, H. D., J. Biol. Chem. **44**: 449. 1920.

[2] Clementi, A., Chem. Abst. **9**: 2910. 1915.

2. Analogy of their action on the protein complex.

Fischer and Bergell's method of following hydrolysis was essentially chemical; Abderhalden and Koelker[1] used an optical method; and Euler[2] used an electrometric method; but none of these methods is quantitative. Clementi has utilized Sörensen's formaldehyde titration method to follow the typical polypeptide resolution

$$R.CONHR + H_2O \longrightarrow R.COOH + NH_2R—$$

which makes it possible to follow peptolytic hydrolysis quantitatively.

Thus Fischer's and Dakin's work gives knowledge of the amino-acids obtainable from gelatin by hydrolysis, data as to the distribution of nitrogen in various groups, and, as noted on page 146, an indication that the combining weight of gelatin is about 900. We may suppose that the amino-acid groups are conjugated as polypeptide chains (see page 189), but beyond that there is no certainty as to the structure of gelatin. We may note that Kober and Haw[3] concluded, from their spectrophotometric investigation of copper complexes in relation to biuret reactions, that no decomposition of the protein is thereby involved, and that the protein configurations are such as to permit only three nitrogen groups to form rings with copper; therefore, the protein molecule is aggregated, and not in the form of free chains or branches of peptides or conjugated amino-acids. This view they claim to be supported by other facts,—e. g., Kober and Suguira[4] found that native proteins give no test for free amino-acid. We may note here that Smith,[5] from a study of mutarotation in regard to change of temperature, considers that setting is due to a bimolecular polymerization of the gelatin "molecule." This view will be discussed in greater detail later.

It appears very difficult at present to discuss the constitution of gelatin, or any protein, apart from its physico-chemical behavior as an amphoteric electrolyte and an emulsion colloid. These subjects will be treated in the second volume of this monograph, and their bearing upon structure theories noted.

[1] Abderhalden, E., and Koelker, A. H., Zeits, physiol. Chem. **51**: 294. 1907.
[2] Euler, H., Zeits. physiol. Chem. **51**: 213. 1907.
[3] Kober, P. A., and Haw, A. B., J. Amer. Chem. Soc. **38**: 457. 1916.
[4] Kober, P. A., and Sugiura, K., J. Biol. Chem. **16**: 539. 1914.
[5] Smith, C. R., J. Amer. Chem. Soc. **41**: 135. 1919.

SYNTHESIS OF THE PROTEINS AND THE NATURE OF BIOCOLLOIDS

The theory as to the nature of the proteins deduced from the analytical chemical methods described is that they consist of very complex molecules, themselves composed of amino-acids, or rather of condensation products of these. This theory then amounts to the conclusion that protein "molecules" exist as physical units corresponding to definite, if very complex, chemical compounds, from which the so-called "Bausteine" or elemental amino-acid units are derived by relatively simple hydrolytic fissions.

Even if the complete constitutional formula of a protein could be written with any degree of probability, as satisfying the majority of its decomposition reactions, there appears no evidence that it would predict in any way those very essential and specific properties of a protein which are condensed in the term biocolloid. The term biocolloid refers usually to those colloidal bodies or organized materials obtained from plants and animals, and either forming actual constituents of the cells and tissues, or derived from these by relatively simple operations. Hence, they correspond fairly closely to the "matières organisées" as distinguished by Dumas in 1835[1] from definite organic chemical substances. It remains true, now as then, that none of the biocolloids proper has been synthesized, although considerable progress has been made in the case of caoutchouc, or crude rubber, which is obtained from the latex by a relatively simple operation.

There is, however, a counter-theory to the view that we can consider proteins (and other biocolloids) as "composed of multiples of (identical) molecules, the latter having a definite elemental composition and constitution, representable by graphic, or at least stereometric, formulas." This counter-thesis has been stated most specifically by the well-known cellulose chemists, Cross and Bevan. The following quotation from their work illustrates this view:

"Crystalline form, which is proximate and visible, is ultimately related to the form and motions of constituent atoms and molecules; organized matter is in its proximate form essentially amorphous, in fact the inconceivably high order of plasticity postulated in the materialization of vital effects implies a neutralization of motion or stress of the individual component groups which otherwise determine crystallization. —This is characteristic of the colloidal state, is common to a

[1] Dumas, J. P. A., Traité de chimie appliquée aux arts. Vol. V.

diversified range of compounds as they exist in the natural order, and probably a general characteristic of all forms of matter under certain conditions of action of force or superforce.—The current conception of 'starch' as a chemical individual is that of a molecule of large dimensions, and this has been applied by analogy to cellulose. It is an integral expression of the 'constants' obtained for the series of hydrolytic transformation products.

"The alternative view of both starch and cellulose[1] is that the state of matter in these complexes is conditioned by a differentiation affecting the ultimate constituent groups, which are not free molecules of the configuration of dextrose (or maltose), but units which have a pecular mutual relation causing association into complexes; and *complexes are reacting units in the sense that any change impressed upon any constituent group affects the whole complex.* No dimensions can be assigned to these complexes, as there are no physical terms of measurement which can be applied to them. There is evidence that the reactions both in starch and cellolose are continuous virtually."[2]

A similar scepticism of the chemical polymolecule theory is expressed by H. R. Ling,[3] who regards the molecule of starch and of all biocolloids as mobile and as a physiological rather than a chemical entity.

The view held by Cross and Bevan, then, which, being in process of development, cannot in the nature of things be entirely explicit, may be tentatively defined as envisaging cellulose (and, as we have stated, this will apply, *mutatis mutandis*, to any biocolloid), as an ionized solution aggregate, the "ions" being not necessarily those of a very complex supermolecule, but probably of certain "ultimate constituent groups, which are *not free molecules* of the configuration of dextrose (or amino-acid), but units which have a peculiar mutual relation causing association into complexes."

In summoning back organic chemistry to what he terms "the biocentric standpoint" Meldola points out "that there is at present but little reason for believing that our laboratory methods have much analogy with the processes which go on in the living organism." "The ordinary chemical equation representing the genetic relationship of one (vital) compound to another is apt to delude those who are not expert in chemistry into the belief that it is all-sufficient and that it 'explains' the

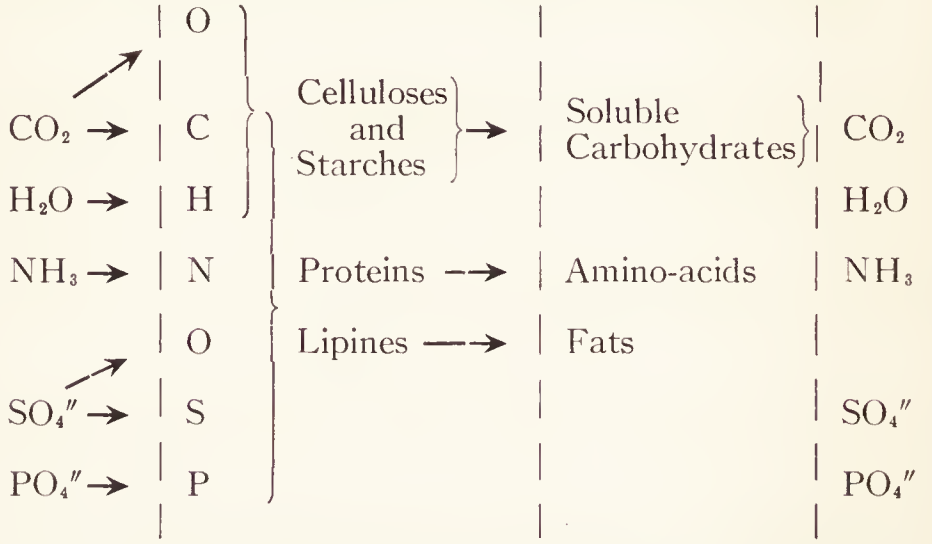
[1] Similarly for proteins.

[2] Cross, C. F., and Bevan, E. J., Researches on cellulose, pp. 5 et seq.

[3] Ling, H. R. A short review of our knowledge of starch. Proc. 7th Internat. Congress of Applied Chemistry, London, 1907. Section VI B. Fermentation.

bio-chemical process. As a matter of fact *the sign connecting the two sides of the equation stands for the whole unexplored region of bio-chemical transmutation.*"

We may outline this in a more concrete form. The vital synthesis on which all industry and life depend may be expressed as the following general cycle, carrying the simple molecular constituents of the earth's atmosphere and soil through the stages of relatively free elements and complexly united proteins, and back to the simple molecular stage.[1]



The essential link is photochemical synthesis, to which it may be suspected all biochemical (enzyme) reactions can be reduced in principle,—i. e., actual reactions, to distinguish from ordinary thermochemical reactions which should be properly termed black radiation reactions. (Perrin). The other aspect is the occurrence of the synthesis by colloids in colloids, and persisting in colloids. Now, just as laboratory organic chemistry has not, for excellent reasons, accomplished its synthesis from the biocentric standpoint, by biochemical methods, so laboratory colloid synthesis has, for the most part, drifted from a chemical to a mechanical conception of colloids. In particular, the chemist has, with reason, regarded them as interfering with the progress of chemical reactions, while in vital synthesis the maintenance of colloid condition is both the means and the end. Hence, just as Cross and Bevan complain that organic chemists see nothing more in cellulose, etc. (biocolloids), than a polyanhydride of glucose, of proteins than a polyanhydride of amino-acids, so colloid chemists are coming to regard no colloids as more than mechanical sub-divisions of phase.

[1] Cf. Mann, G., l. c. Introduction, pp. 1–4.

These purely chemical and purely mechanical approximations are no doubt of much use, and correspondingly true. There is, however, a gap which is of the greatest importance in the case of biocolloids. That is, *how*, in biocolloid synthesis and catalysis, *is the essential coördination of chemical condensation and mechanical dispersion regulated and maintained?*

Naturally, this subject is too large to be completely discussed in the present monograph. It appears desirable, however, that it be opened up somewhat, in view of the natural tendency to accept partial explanations. Therefore there are added to this general critique:—

First, an outline of certain integral reactions of gelatin, in which its behavior is similar to that of other colloids which differ widely in chemical composition; secondly, a comparison of a possible purely chemical structure theory of colloids with the existing purely mechanical theory; lastly, a suggestion which may lead toward harmonizing the chemical and physical theories of the emulsoid colloids.

INTEGRAL REACTIONS OF GELATIN

The following reactions are of interest as showing the protein complex functioning chemically without hydrolytic decomposition, and as indicating the analogy between proteins and celluloses.

Proteins can be combined with halogen acids without hydrolysis by using concentrated acids and dry proteins at relatively high temperatures,—i. e., 105° C.[1]

Then again, proteins form copper complexes direct, the copper combining only with the nitrogen of the R.CO.NH.R groups.[2]

(The adsorption reactions of gelatin and proteins, which are also of an integral nature, will be treated in Volume II.)

Just as celluloses can be alkylated, so Skraup and Böttcher[3] find that gelatin can be methylated, by using methyl iodide. They consider that there is both introduction of methoxy-groups and fixation of methyl to the nitrogen of the amino-radicle. On hydrolysis the methyl derivative yields no lysine, and only small proportions of histidine and arginine are found; glycocoll is present in larger amounts than in the original; alanine, pyrolidine-carboxylic acid, and phenylalanine are also present.

[1] Long, J. H., and Hull, M., J. Amer. Chem. Soc., **37**: 1593. 1915.

[2] Osborne, T. B., and Leavenworth, C. S., Protein copper compounds, l. c.

[3] Skraup, Z. H., and Böttcher, B., J. Soc. Chem. Ind. **30**: 39. 1911; Monatsh. Chem. **31**: 1035. 1910.

The reactions of methyl-gelatin are similar to those of gelatin, the reactions with α-naphthol and with thymol and the biuret reaction being identical; but in the xanthoproteic test methyl-gelatin gives a brighter yellow color and a sharper change to red with ammonia than gelatin.

The pre-existence of methyl groups in proteins has been investigated by Burn,[1] who used Herzig and Meyer's reaction, —i. e., heating with hydriodic acid, so that methyl iodide is formed, as in Zeisel's methoxy determinations. Decomposition of methyl-amino groups requires a temperature of 230° C., whereas methoxy-groups decompose at 130° C. Commercial gelatins, *inter alia*, give positive results, but although a methyl-amino group may exist in proteins, the test is not decisive.

Engeland[2] tried methylation of amino-acids as a method of quantitatively following protein hydrolysis, and found that the amino-acids are converted into their betaines.

ORGANIC CHEMICAL VIEW OF PROTEINS

If the organic chemistry of the carbohydrates and the proteins be contrasted with that of dyes, one is struck with the fact that the chemistry of dyes has been dominated and guided by the effort to relate chemical structure to physical color. But no effort has been made to correlate the chemical constitution of the carbohydrate and protein colloids with their distinguishing physical property—colligation or agglutination. Only Sadikoff, who has shown that with carbon bisulphide and alkali proteins undergo a colloid-chemical reaction essentially similar to that of celluloses, has put forward this possibility.[3] As elsewhere noted, caustic alkali at 50° C., even in small concentrations, rapidly destroys the setting and other properties of the "agglutinative complex" (klebende Komplex). In the presence of carbon bisulphide peculiar sulphur derivatives of glutin (gelatin) or thio-glutins are formed, characterized by increased capacity of condensation.[4]

On cooling, the solutions of thio-glutin form jellies, the consistency of which depends largely on the pretreatment and history of the gelatin—(hysteresis factor).

The "colligative property" or cementing capacity (klebende Komplex) of the thio-glutins can be enhanced still further by combination with lead salts and tannin.[5] Sadikoff points out

[1] Burn, J. H., Biochem. J. **8**: 154. 1914.
[2] Engeland, R., Analyst **39**: 316. 1914.
[3] Sadikoff, W. S., J. Soc. Chem. Ind. **26**: 105. 1907; Koll. Zeits. **1**: 193. 1907.
[4] The similarity to the case of cellulose is evident. (Viscose reaction).
[5] Compare similar actions by mordanting and lakeing with dyes.

that with either extreme of the opposite processes of condensation and hydrolysis the colligative property is lost. It is therefore associated with a certain equilibrium of these opposite processes.

All in all, it appears that it is a purely arbitrary judgment which has decreed that the basis for color and selective absorption of light of organic compounds should be sought for in characteristic groups (chromogens, chromophores and auxochromes) and constitutional arrangements of atoms—in structural chemical bases,— while the grounds for colligation (tenacity) and selective adsorption (adhesion) of matter in other organic compounds should not be sought for in characteristic groups (collogens, collophores and auxocolls), but brushed aside as purely physical or mechanical in origin. Actually, they are measured, no doubt, as physical or mechanical quantities,—as jelly strength (tenacity, etc.,) and surface tension. But precisely the same is true of color and selective absorption.

Now, inversely as modifications of the static chemical theories of color by considerations of physical electronic theories and dynamic isomerism are being forced on the chemist, so modifications of the physical or mechanical theories of cohesion and capillarity are being injected by chemists into the haphazard domain of molecular physics.[1]

This new departure, remarkable because experimentally founded and quantitatively expressed, gives aid to the view that in the colloidal condition there is not only mechanical subdivision but at the same time enhancement of the residual affinities of every atom and potential atom group, and it is precisely this far-reaching and deep-seated dissolution of molecular bondage which allows both the physico-chemical activity and the structural chemical inertia of colloids. The true antithesis is not between crystalloids and colloids, which are indeed continuous, but between crystals and organisms, and the cycle which an atom can undergo could or should be represented in its main features by a closed hysteresis cycle, in which crystallization would be equivalent to disorganization. Actually, laboratory colloids and biocolloids tend continually, through all changes, to increase of entropy (reduction of motivity) and crystallization, or, at least, segregation of definite chemical compounds and physical phases. This has been inverted to the point of considering colloids as essentially dispersed systems of ultramicroscopic droplets or crystallites.[2] Yet this extreme view is not entirely incom-

[1] Langmuir, I., J. Amer. Chem. Soc. **38**: 2221. 1916; *ibid.* **39**: 1848. 1917.
[2] von Weimarn, l. c.

patible with the counter-theory just discussed, for it is expressly argued by the supporters of the dispersion theory that "the surface layer of crystals consists of relatively uncoördinated molecules and atoms with greater free energy." Now, as the crystal diminishes to ultramicroscopic dimensions, its surface increases relatively to its volume, so that for the postulated super-ultramicroscopic crystals of emulsoid colloids this "true crystalline" part would be an approximately negligible amount—particularly *in vivo*, though tending to increase *in vitro*.

THE EMULSOID CONDITION

In what direction are we to look for a chemical constitutional influence and control of cohesion, elasticity, and agglutination? It appears likely that the answer is to be found along the lines indicated by Hardy[1] and followed up with great success by Langmuir[2] and Harkins,[3] namely, in consideration of molecular orientation due to structure, in surface and interfacial planes, as determining surface tension, cohesion, and agglutination.

In the following we shall briefly indicate that existing theories of the chemical constitution of the proteins are, if certain assumptions as to molecular orientation be allowed, compatible with both the solution-aggregate theory of Cross and Bevan, and with the general physical properties of the proteins.

The chemical equilibrium of the elements in a protein is expressed by its constitution. The possible types of constitution are:[4]

1. Linkage by C-atoms, as in $-\overset{|}{\underset{|}{C}}\ -\overset{|}{\underset{|}{C}}-$, as in carbohydrates. This is improbable because the decomposition of proteins by enzymes into larger and smaller complexes is difficult to understand.

2. Linkage by O-atoms, as in $-\overset{|}{\underset{|}{C}}\ -\ \overset{|}{\underset{|}{O}}\ -\ \overset{|}{\underset{|}{C}}\ -$, is improbable because of the small number of oxygen-atoms.

[1] Hardy, W. B., Proc. Roy. Soc. **86** A: 610. 1912.

[2] Langmuir, I., l. c.

[3] Harkins, W. D., Brown, F. E., and Davies, E. C. H., J. Amer. Chem. Soc. **39**: 354. 1917, *ibid.* p. 541.

[4] Plimmer, R. H. A., l. c., p. 1.

3. Linkage by N-atoms. Here three sub-types are possible:
(a) $-CH_2-NH-CH_2$; found in proline, but free COOH group would give an acid character;

(b) $-CH_2-NH-C(NH)-$ occurs in guanidine and arginine, but not elsewhere in decomposition products;

(c) $-CH_2-NH-CO-$. This is the most feasible mode, according to Plimmer, because:

(i) A small portion of the total nitrogen is found as ammonia on hydrolysis, corresponding to acid amide $-CO-NH_2$;

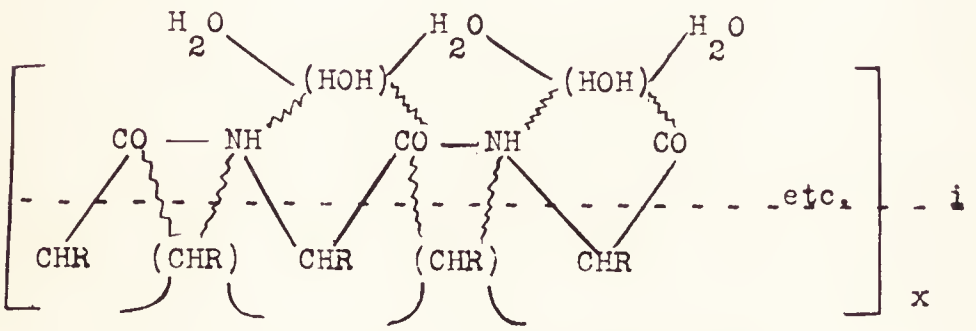
(ii) The greater part (90 per cent) of the nitrogen is obtained as amino-acids. These are not present as such in proteins, because so little nitrogen is given by nitrous acid; hence probably present as $-CH_2-NH-CO$;

(iii) The biuret reaction makes presence of $-\underset{|}{CH}-\underset{|}{NH}-$ / $\underset{|}{CO}-\underset{|}{NH}-$ very probable.

This formulation is probable for the polypeptides, and, accepting polypeptides as equivalent to polysaccharides, we have generally for proteins groupings of the type:

$$\begin{array}{l} NH_2.\ CHR.\ CO-(NH.\ CHR.CO)_n.NH.CHR \\ \quad\vdots\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad\vdots \\ \quad\vdots\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad COOH \end{array}$$

Beside the probability of chemical ring formation (as by salt formation of the free carboxyl group from a dibasic acid with the terminal NH_2 group), or of a diketopiperazine or anhydride ring by final union of the terminal $-NH$ and $CO-$ groups, a wider potentiality exists in the nature of this formu a-tion. Let us write the inner chain in the following manner:



— = Valence ~~~ = Residual affinity

There is indicated here first, an imaginary plane or intramolecular interface *i* separating the hydrophile groups

$$-\mathrm{CO}-\mathrm{NH}-,$$

which are consolute with water from the hydrophobe or hydrocarbon groupings CHR. We may suppose that, in the admittedly large protein complex there is, in the presence of water, a mutual attraction of the water-soluble and of the water-insoluble groups, without actual cleavage. For convenience we may call these groups saloid and lipoid respectively. Further, it may be supposed that tautomeric oscillation between enol-keto positions is concomitant with displacement of hydration-dehydration of the saloid portion on the one hand, and with condensation-dispersion of the lipoid portion on the other.

The mechanism thus provided would affect both the physical and chemical properties, since we should have constantly nascent (or renascent) atom-groups, limited electrolytic ionization, and inter-group mobility.[1] Activation of the mechanism is provided for by the fact that thermal and variation conditions tend to maintain inequality of diffusion potentials of hydrogen ions, the importance of which in the maintenance of a zonal or strati-chemical tension (regarded as a generalized field of force in colloids) will be discussed more fully in the next volume. It is noted here merely as an attempt to harmonize the antagonistic theories advanced.[2] The conception of partial polymerization and fractional molecular stability offers a *modus vivendi* between (a) the synthetic organic chemical view of proteins, as polymolecules of amino-acid anhydrides giving specific hydrolytic products, and (b) the technological and colloid-chemical view of biocolloids, as solution-aggregates giving certain generalized reactions, in which the reaction is virtually continuous through stages varying continuously in nature and products.

[1] The nature of the primary particles provided (neutral and ionic micelles), and their aggregation in secondary granules, filaments, sheets, etc., requires further consideration.

[2] This suggestion of the writer's, endeavoring to reconcile the requirements of the organic structure theory with the colloid chemical peculiarities of proteins and similar colloids will be found in *Nature* (**107**: 73. 1921) together with a criticism by J. W. McBain. Further advance on the chemical side would seem to be likely by application of the coördination theory of valency.

CHAPTER IV

Technical Testing of the Physical Properties of Gelatin

In technology, the most important properties of gelatin are physical ones, or, more properly, chemico-physical or colloidal. The tests usually made are for the determination of:[1]

1 Capacity for absorption of water;
2 Viscosity of solution;
3 Melting and setting points of jellies;
4 Mechanical strength of jellies;
5 Foam test;
6 Specific gravity of solutions; and
7 Clarity of solutions.

These properties will be considered at greater length in Volume II. Here we shall consider only approximate determinations or typical "tests" as used in industrial and routine laboratories for grading and controlling gelatins and glues.

(1) Capacity for Absorption of Water

Abney[2] states that a good photographic gelatin should absorb five to ten times its own weight of water. Actually, the absorption depends upon many secondary factors, such as acidity, temperature, etc., and on the conditions under which the gelatin was dried and prepared—e. g., as leaf, flake, fibre, etc.

In order to standardize conditions for this test it is well to powder the gelatin, but quite coarsely.[3] A known weight of the air-dry gelatin or glue is immersed in water at room temperature (18 to 20° C.) for twenty-four hours, and weighed. It is stated that in this time high-grade glues absorb from ten to fifteen times their weight of water, poorer grades three to five times. Rideal recommends soaking 10 gms. of granulated gelatin in water at 15° C. for 48 hours. He summarizes his results as follows:

Grade	*Weight water absorbed*	*Character of jelly*
Good	5 to 9 times its own weight	Firm
Poor	Low or uncertain	Slimy to liquid

The relation of water-absorption to acidity, to the presence of electrolytes and non-electrolytes, and to the form and history of the gelatin, will be dealt with in a later section.

[1] Cf. Alexander, J., in Allen's Commercial Organic Analysis, 4th edition, Vol. VIII, p. 611.
[2] Abney, W. de W., Instruction in photography, 10th edition, 1900, p. 113.
[3] Rideal in Allen's Commercial Organic Analysis, Vol. VIII, p. 611.

(2) Viscosity of Solution

The viscosity of glue and gelatin sols is one of the easiest properties to measure to any desired precision, one of the most sensitive to changes of character, and therefore one most likely to mislead if the conditions determining its variations are not understood and controlled. In practical testing and routine work, solutions varying in strength from one to ten per cent are used, the majority of tests favoring the higher concentrations. Determinations are made either relative to water or as purely arbitrary numbers for the time of flow through a selected pipette or burette.

Thus Lambert[1] recommends taking as a standard the length of time required for a one per cent solution to flow through a burette as compared with water taking twenty-five seconds to run through the same burette. The one per cent solution of gelatin or glue is cooled to 18° C., and the viscosity determined by the number of seconds required for 50 cc. to run through the burette. According to this, the higher the strength of the glue (adhesiveness or jelly-strength), the greater the viscosity. Lambert gives the times of flow as:

Strong glue.....32 to 34 seconds;
Medium glue.....28 to 30 seconds;
Weak glue.....26 to 27 seconds;

Actually, it is incorrect to term the time of flow viscosity, since the dimensions of viscosity are dynes/sq. cm. (see Vol. II). It would be better to take the ratio of time of flow to that of water, thus having an approximation to specific or relative viscosity. This, however, will be far from accurate with a time of flow as short as twenty-five seconds for 50 cc. of water.

The use of Engler's viscosimeter[2] with a fifteen per cent solution at 30° C. gives somewhat better results. Fels gives the following table for glues:

No.	Sample	Per cent moisture	Time of efflux	Viscosity
1	Light yellow plates	16.3	149	1.65
2	Brown transparent	14.0	125	1.36
3	Cherry-colored	15.4	171	1.91
4	Light yellow plates	18.2	150	1.60
5	Turbid glue	15.2	199	2.21

[1] Lambert, T., Glue, gelatin, and their allied products, p. 117.

[2] Engler, C., and Künkler, A., J. Soc. Chem. Ind. 9: 654. 1890.

These results were obtained with the improved type of Engler viscosimeter, shown in Fig. 31.

It is to be noted that the Engler values are not accurate measures of relative viscosity, owing to the systematic errors of short-tube viscosimeters. Rideal[1] prefers to use a one per cent gelatin solution at 18° C., with a modified Slotte viscosimeter. This consists of three bulbs in line (see Fig. 32) kept

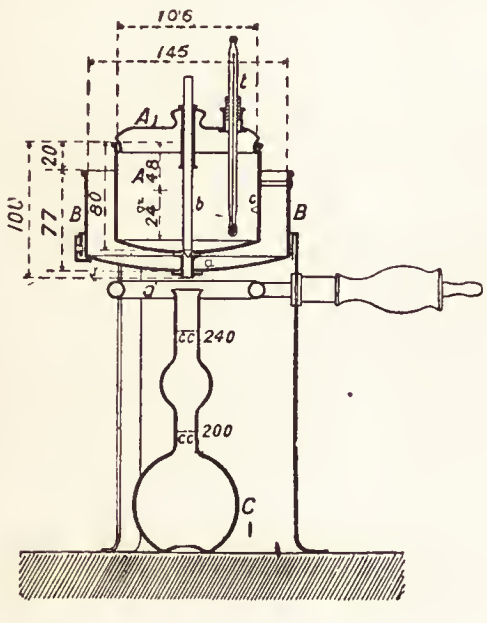
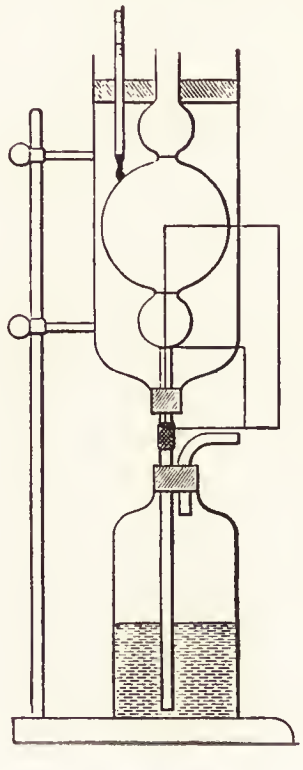


FIG. 31
Engler viscosimeter



at the required temperature by a water-jacket and terminating in a short capillary tube. The time required for the middle bulb to empty through the capillary, either at atmospheric pressure, or under suction with a gauge in to note the difference in pressure, is measured. Formulae and corrections are given in an article by Rideal and Youle.[2] The values obtained, in terms of the viscosity of water, vary from 1.19 to 1.6.

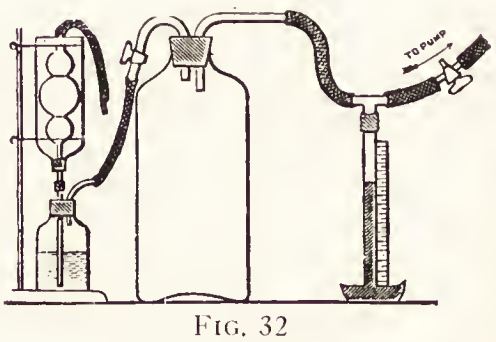


FIG. 32
Slotte viscosimeter

[1] Rideal, S., Glue and glue-testing, p. 129.
[2] Rideal, S., and Youle, W. E., J. Soc. Chem. Ind. **10**: 610. 1891.

Alexander[1] uses a pipette of 45 cc. capacity which permits the efflux of hot water from the glue bath in exactly fifteen seconds. The pipette adopted as standard had the following dimensions:

Capacity—45 cc. water at 80° C.	
Internal diameter effluent tube	6 mm.
External diameter effluent tube	9 mm.
Length of effluent tube	7 cm.
Smallest diameter of outlet	1.5 mm.
Outside diameter of bulb	3 cm.
Length of bulb	9.5 cm.
Length of upper tube	22 cm.

The efflux hole was made by cutting the effluent tube squarely across and holding it vertically in a Bunsen flame until it contracted to the desired size. A constant-temperature jacket surrounds the pipette. The viscosity may then be expressed in terms of water as unity by dividing the number of seconds for the glue or gelatin solution by 15. However, with such a rapid efflux, and with no correction for kinetic energy, the values obtained can not represent true viscosity coefficients (cf. Volume II).

Fernbach[2] recommends the use of a jacketted pipette, having a water value of fifteen seconds at 180° F.

In routine testing it is generally desirable to take observations of viscosity at two temperatures, high and low—e. g., 100 and 150° F.

As has been stated, the viscosity value, however determined, is subject to a great number of variations which may be due, not to intrinsic differences in different samples of gelatin, but to differences in the preparation and treatment of the solutions. As these will be dealt with fully in the next volume, they will be only briefly noted here. We have to consider:

(i) Thermal hysteresis. Thus if a ten per cent solution is prepared at 70–80° C., and rapidly cooled to 30 or 35° C., it will give a considerably lower viscosity than if it is kept at the lower temperature for 24 or 48 hours. This increase of viscosity on standing may, however, be changed to decrease if alcohol is present in the solution. Conversely, a jelly of ten per cent congealed at, say, from 0–10° C., and raised to 30 or 35° C., will give higher viscosity values at first (i. e.,at the lower temperature).

[1] Alexander, J., J. Soc. Chem. Ind. **25**: 158. 1906.

[2] Fernbach, R. L., Glues and gelatins, p. 38.

(ii) HYDROLYSIS. Gelatin solutions maintained at temperatures much above 70° C., or jellies repeatedly melted at or above such temperatures, lose their setting power or jelly-forming capacity and thereby a large part of their viscosity. Further, according to the degree of hydrolysis, the above-mentioned thermal hysteresis is altered, so that the actual viscosity becomes correspondingly indeterminate. (See p. 194.)

(iii) RATE OF SHEAR. Colloidal solutions give different values of the viscosity coefficient according to the rate of shear. This will be discussed in Volume II. A commercial viscosimeter operating by concentric shearing of the gelatin solution is shown in Fig. 33, while Fig. 34 shows an instrument designed by the writer for concentric shearing between two cylinders.

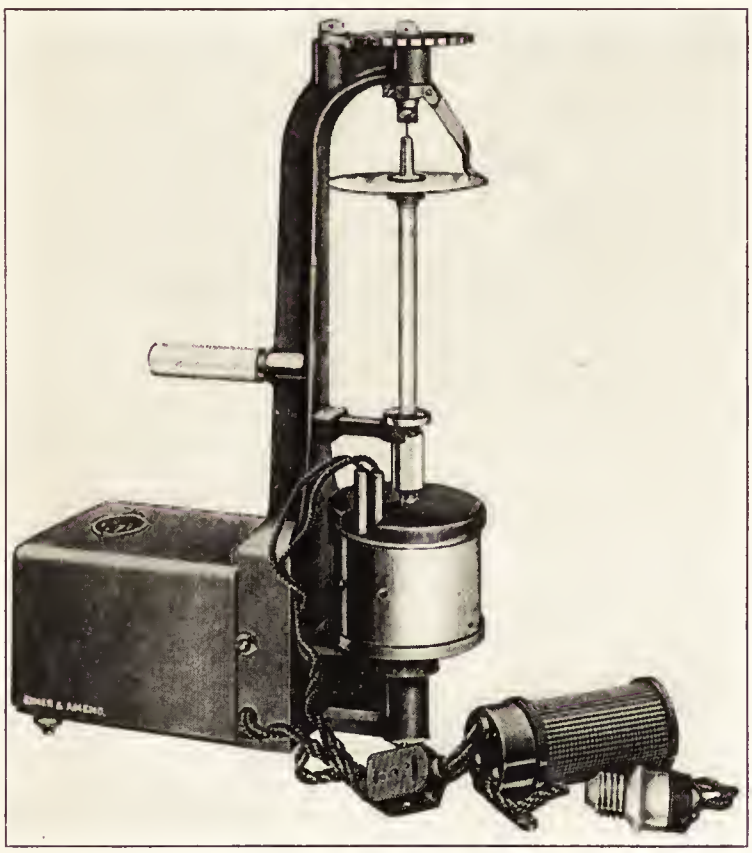


FIG. 33
MacMichael viscosimeter[1]

To sum up, the determination of viscosity of gelatin solutions, even for routine testing requires specification of conditions. These conditions have only recently been determined in the light of physico-chemical investigations (Volume II).

[1] Courtesy of Eimer and Amend Company of New York City.

Provisionally it may be stated that the viscosity should be compared for much the same P_H, that the solution should be prepared on a definite schedule, without any period of heating above 70° C. and "aged" for twelve hours in a thermostat,[1] and that the viscosimeter should give readings in specific viscosity—centipoises. It has been shown by R. H. Bogue[2]

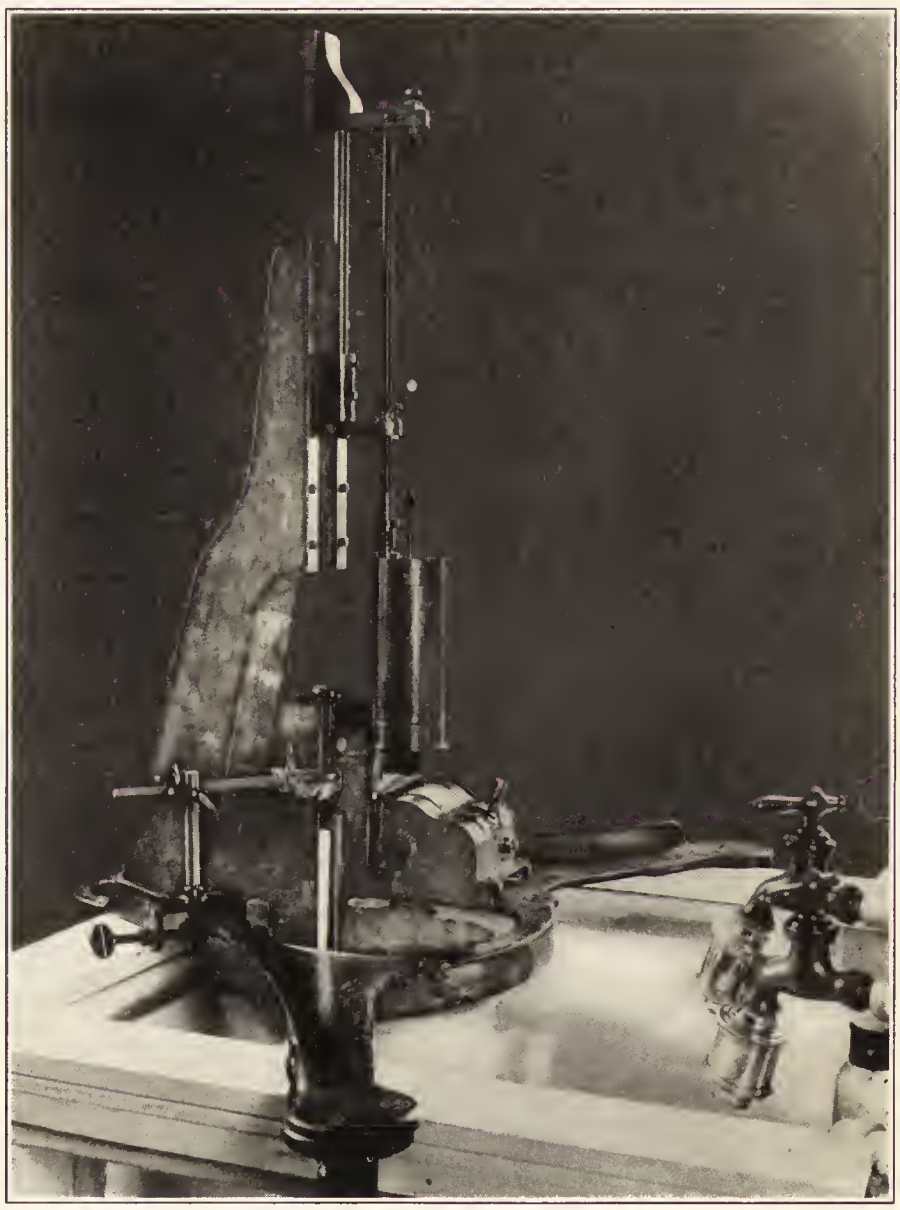


FIG. 34
Couette-Sheppard viscosimeter

[1] Davis, C. E., Oakes, E. T., and Browne, H. H., J. Amer. Chem. Soc. **43**: 1526. 1921.
[2] See p. 207.

whose work will be noted later, that using the MacMichael instrument (new form) under specified conditions, viscosity may be made the basis of a scientific system of grading glues, and, as far as mechanical properties, gelatins. In a paper, read before the New York Section of the American Chemical Society[1] Bogue emphasized the desirability of the adoption of such an instrument for standardizing tests and developed a system of *primary* evaluation based chiefly upon viscosity measurements.

(3) The Melting and Setting Points of Gelatin Jellies

These so-called "points" are not in any case to be classed with the definite points of fusion and solidification of pure crystalline solids. The transition from the solid to the liquid state in the latter case is usually abrupt or discontinuous in a high degree, so that very characteristic temperature points are obtainable for the transitions. On the other hand, with colloid gels (jellies) the transition from jelly-solid to liquid is virtually continuous. The points obtainable are relatively arbitrary ones, being temperatures at which the elastic resistance becomes very much less than the small applied load, so that a rapid shearing takes place—the melting point—or, conversely, a temperature at which the growth of elastic resistance overcomes some small steady shearing stress—the setting point. It follows that in the determination of the melting points and setting points of gelatin jellies, the method is everything, the values in any case being only comparative.

Melting Point of Jellies. The capillary tube method used in organic chemistry for melting point determinations of crystalline solids is not altogether satisfactory, although Gamble[2] has used it, taking the temperature at which the concave meniscus at the surface disappears. Working with a concentration of 5 gms. gelatin per 100 cc. water, he recommends that the mean of six readings be taken. Herold[3] uses an apparatus very similar to Ubelohde's tester for fats and greases.

A glass vessel is closed at its lower end with a short piece of rubber tubing containing a glass bead. Mercury is poured in to form a layer 2 mm. thick and the gelatin solution introduced to a definite height. When the gelatin has set, the vessel is immersed for thirty minutes in a water bath at 19° C. Then the cap is removed, the mercury shaken out, and the

[1] Bogue, R. H., J. Ind. Eng. Chem. **14:** 32. 1922

[2] Gamble, C. W., J. Soc. Chem. Ind. **30:** 1324. 1911.

[3] Herold, J., Chem. Ztg. **35:** 93. 1911.

vessel and thermometer placed in a test-tube surrounded by water at 40° C. The temperature at which the vessel begins to slip off is taken as the melting point of the jelly.

The "fusiometer" of Cambon[1] is a device of the same character. Here also there is a "test piece" consisting of an ordinary umbrella or walking stick ferrule weighing about 7 gms. In this is "set," by the glue or gelatin solution to be tested, a piece of wood of specified dimensions. The complete tester is suspended in a bath under specified conditions, and the temperature at which the ferrule slips off is taken as the melting point.

In the method devised by Chercheffsky[2] small cubes of jelly are cast in molds from solutions of specified concentrations of air-dried gelatin, as 10, 15, or 20 per cent. These small cubes are suspended on the horizontal arm of an L-shaped wire in a 250 cc. beaker filled with clear paraffin oil. The oil is gradually warmed up, and the temperature at which the cubes lose their rectangular form is taken as the melting point. For greater accuracy the oil-bath may be heated indirectly through a water-jacket.

A somewhat similar method for testing the melting points of gelatin jellies containing alum and other hardening agents has been described by Bayley.[3] His apparatus consists of a water bath so arranged that the products of combustion of the flame used to heat it do not reach the largest side. (See Fig. 35.) Along this side a straight line is ruled one inch from the top, and one or more thermometers are set in the bath so that their bulbs are as close as possible to this line The bath is laid on its side and discs of the jellies to be tested, cast from little paper tubes, are allowed to solidify with the

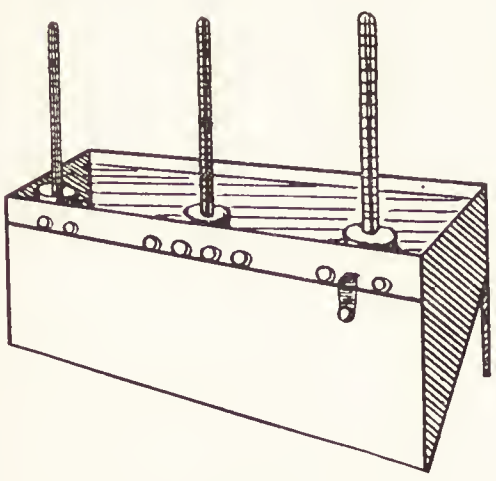


FIG. 35
Bayley's melting point apparatus

[1] Küttner, S., and Ulrich, C., J. Soc. Chem. Ind. **26**: 703. 1907; Chem. Centrbl. V. **11** 1703. 1907.

[2] Chercheffsky, N., J. Soc. Chem. Ind. **20**: 731. 1901.

[3] Bayley, R. Child, Phot. J. **20**: 224. 1895–96. Abney, W. de W., Instruction in photography, 10th ed., p. 113.

lower edge of each disc upon the line on the face of the tank. When the jelly has set, the paper molds are removed and the tank set upright and filled with water. The temperature is then raised gradually, and the point observed at which the discs commence to slide down the side of the bath. It is recommended to use discs one-half inch in diameter and one-quarter inch in thickness. With discs of this size, and gentle heating of the bath, results checking to 0.5° C. can be obtained. The values obtained by this method are, however, uniformly 2° C. higher than those obtained by immersing a thermometer in a jelly, and then raising the temperature until the melting point is reached, as for example, in the methods described below.

Pauli and Rona[1] have used an apparatus similar to that arranged by Beckmann for the determination of depression of freezing point. They took as the melting point the temperature at which the layer immediately contiguous to the thermometer began to flow.

Bechhold and Ziegler[2] employed an air-bath in which is placed a small test-tube containing the jelly and a thermometer, which may be imbedded in the jelly. The jelly is loaded with 5 gms. of mercury, and the temperature at which the mercury breaks through the jelly is taken as the melting point.

The writer has had fairly satisfactory results with Bechhold's method, slightly modified. The thermometer was placed axially in the gelatin solution and set in this position, the mercury load being poured around it. The test-tube was placed in a larger one which acted as an air-bath and which was immersed in a beaker of water heated evenly and slowly. Readings of the thermometer were taken at definite intervals and plotted against times as abscissae. A break in the curve is obtained at the yield region (due to the disturbance of the conduction by sudden convection), the lower limit of which is taken as the yield point, the upper as the melting point.

Another modification tried in the Eastman Research Laboratory consists in using a small annular test-piece of metal surrounding the thermometer and resting on the jelly by three equidistant wedge-shaped feet. The yield-point is taken as the temperature at which the test-piece just commences to sink into the jelly, the melting point as the temperature when the piece has sunk until its base is level with the jelly. (See Fig. 36.)

[1] Pauli, W., and Rona, P., Pflüger's Archive. **7**: 333. 1898, and cited by H. Bechhold, Die Kolloide in Biologie und Medizin.

[2] Bechhold and Ziegler: see H. Bechhold, Die Kolloide in Biologie und Medizin.

Data on the relation of melting points of jellies to the hardening of gelatin will be dealt with in Volume II.

The determination of the comparative melting points of glues as a guide to their jelly strength has been advocated by Sammet.[1] The melting point in this case is taken as the temperature of flow of small heaps of ground glue particles swollen for equal periods in water. It is evident that jelly particles of different concentration will be compared here, the concentrations depending upon the initial swelling rates of the respective powdered glue samples. Sammet claims, however, that the results are strictly comparable with jelly strengths determined by other (unspecified) methods.

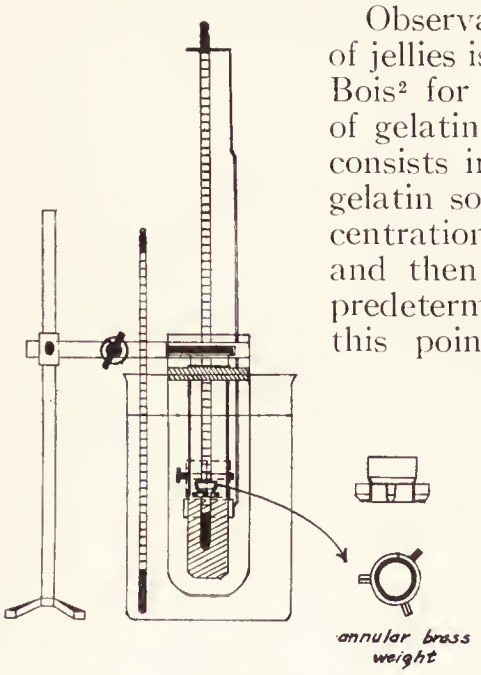


FIG. 36
Melting point apparatus

Observation of the melting phenomena of jellies is proposed by Clarke and DuBois[2] for the assay of the "jelly value" of gelatins and glues. Their procedure consists in making up a series of glue or gelatin solutions of different known concentrations, cooling them until well set, and then slowly warming them to a predetermined fixed temperature. At this point observations are made by tilting the test tubes containing the jellies to determine which concentrations are solid and which are not. A temperature of 10° C. was found most generally satisfactory.

SETTING POINTS. Determination of the "setting" or "solidification point," that is, the temperature at which jelly formation takes place, is beset with difficulties similar to those arising in defining and determining the melting point. Here again the transition from the liquid to the solid state is practically continuous. Therefore it is again necessary to establish definite experimental conditions and to adhere to certain arbitrarily fixed conventions, though those adopted by different workers have varied considerably.

Valenta[3] recommends taking 50 gms. of ten per cent jelly in a beaker, placing it in cold water, and stirring with a ther-

[1] Sammet, C. F., J. Ind. Eng. Chem. **10**: 595. 1918.

[2] Clarke, A. W., and DuBois, L., J. Ind. Eng. Chem. **10**: 707. 1918; J. Soc. Chem. Ind. **37**: 665. 1918.

[3] Valenta, E., Jahrb. Phot. **23**: 179. 1909.

mometer until solidification occurs. No precise definition of solidification is given, however.

Winkelblech[1] points out that a warm glue or gelatin solution sets at different temperatures, according as it is cooled rapidly or slowly. Thus a ten per cent solution cooled at room temperature set at 18° C., but, when cooled in a cooling bath, set at 15° C. This investigator found that the most satisfactory results, as regards abruptness of setting and reproducibility, are obtained by steadily cooling a solution kept in continuous swirling and oscillatory motion, so that the cooling is uniform throughout the mass. The originally readily mobile liquid becomes markedly more viscous as the setting point is approached, and entrapped air bubbles escape very slowly when the motion is stopped. On further cooling these no longer move, the whole mass rapidly loses mobility, and the thermometer remains stationary. When thin jellies are used—e. g., those of two per cent gelatin content, which easily break on shaking—observations must be taken rapidly, as they are liable to undercooling.

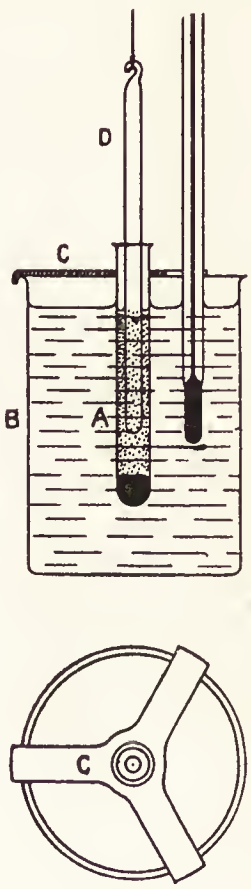


FIG. 37
Apparatus for comparing melting and setting points

The following methods are given by Hatschek in his new manual of colloid chemistry:[2]

"The melting and setting points are, of course, not strictly defined, and can be determined and compared only by conventional methods. An apparatus suitable for this purpose is illustrated in Fig. 37. A test tube A is suspended in the center of a 300 or 400 cc. beaker B, which serves as a water bath, by means of the guide C, through which it must slide freely. A tube 5/8" diameter x 6" long is suitable; it is weighted with 15 to 20 gms. of mercury. It is essential that the tube should be perpendicular when it is resting on C; if the rim is not sufficiently regular to ensure this, a square collar, say of rubber, should be used and permanently attached to the tube. A glass rod D, about 3/8" diameter for a 5/8" tube, is

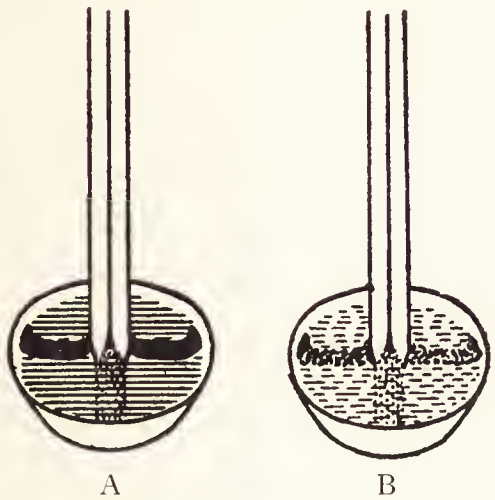
[1] Winkelblech, K., Zeits. angew. Chem. **19**: 1260. 1906.
[2] Hatschek, E., Laboratory manual of elementary colloid chemistry.

suspended exactly in the axis of the test tube. (If the apparatus is to be used frequently, it is advisable to mount it permanently to ensure correct alignment.)

"To determine the melting point the test tube is filled with a definite quantity of the gelatin sol under examination, the beaker filled with water at a definite temperature, say 15° C., and the sol allowed to set for a definite time. The rod, with the test tube hanging to it, is now raised a definite height (which stage is shown in the illustration), and the temperature of the bath slowly raised, with constant stirring, until the test tube slides off the gel cylinder surrounding the rod and comes to rest on C. The temperature at this moment is noted as the 'melting point.' If the 'setting point' is also to be determined, the rod is lowered to its original position, the flame extinguished, and the bath allowed to cool. The rod is raised very slightly from time to time, until it just lifts the test tube with it, the temperature at this point being noted as the "setting point." It must be remembered that there is considerable hysteresis and that the setting point of harder brands may be as much as 7° or 8° C. lower than the melting point of about 10 per cent gels.

"A more delicate method of determining, with very simple means, the setting point is based on the well-known fact that the exposed surface of a gelatin gel which has been allowed to set quietly is *not smooth like that of a liquid, but shows a network of wrinkles.* The formation of these wrinkles is not due to drying, but occurs actually during the last stage of setting. The alteration in the appearance of the surface is very striking if it is observed under an acute angle in reflected light, and it may be used for determining the setting point in the following manner: A small porcelain crucible is filled with about 10 cc. of sol and the bulb of the thermometer completely immersed in the latter. The reflection of the window in the surface is then observed, attention being fixed on some dark object in the light field, such as the window-frame or the like. The reflection of such an object is, of course, distorted by the menisci formed by the sol at the wall of the crucible and the stem of the thermometer, but is a smooth and unbroken curve. As soon as wrinkling commences, the image is broken up into fringes (see Fig. 38, A and B); the fall of temperature between the time when this alteration in appearance becomes barely perceptible and when it is quite unmistakable rarely amounts to more than 0.1°, which is a more than sufficient accuracy."

A method which requires no special apparatus beyond test-tubes and a thermometer is as follows: Equal amounts of the gelatin solutions to be tested are placed in similar test-tubes of one inch inside diameter. These are then cooled in ice-water, being examined at definite intervals until the approach of solidification (jelling) is shown by the fact that the solution is hardly disturbed when the tube is tilted. After



A B

FIG. 38
Apparatus for determining setting points

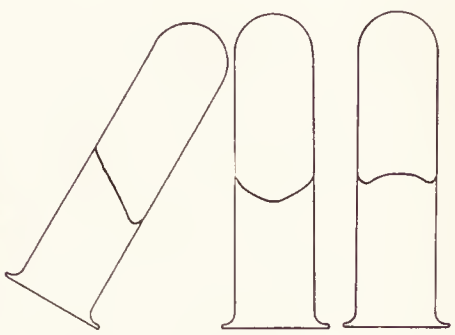


FIG. 39
Setting point apparatus

this stage is reached, the tubes are removed at frequent short intervals and inverted. When the meniscus (see Fig. 39) no longer sags, a thermometer is thrust into the jelly, and the stationary temperature taken as the setting point. This method is not capable of very great precision, particularly since the cooling is not uniform when the solution is not stirred. It is, however, the same for different specimens, and the temperature is read in the region last setting (centre of the meniscus), and is capable of considerable practical service.[1]

Considerable data on effects of hardeners, etc., on the setting point are given by Cobenzl[2] and will be referred to again. The method of observation used by Cobenzl is as follows: A thermometer divided in $^1/_5°$ C. is set in a 250 cc. graduated cylinder by means of a tight-fitting cork stopper, in such a way that the scale divisions in question are below the liquid. In each test 12.5 gms. of gelatin were placed in the cylinder, covered with cold water, left to swell one hour, and made up to 250 cc. The gelatin was then dissolved on a water-bath of temperature not above 50° C. The solution was then allowed

[1] Used for glue testing in the Eastman Research Laboratory.
[2] Cobenzl, A., Phot. Ind. 317. 1919.

to cool in a place free from draughts until the temperature was two or three degrees above the first expected observation point, after which the cylinder was kept in motion by inclining it on its axis and rotating, etc., to induce gradual and uniform cooling. The originally mobile liquid became viscous. As soon as the flow, particularly off the walls of the cylinder became "wavy" or rilled, with simultaneously a momentary cessation of the fall of temperature (sometimes even a rise of 0.1–0.2° C.), the temperature was read as Point I (of the setting points). At this stage bubbles of about 1 mm. easily followed the motion of the liquid. As soon as bubbles of 10 mm. magnitude only slowly followed the flow, temperature Point II was taken. When the air within or without the fluid could no longer follow the motion, indicating complete solidification, temperature Point III was taken. It is admitted that much personal variation is probable in estimating Point II, but Points I and III are regarded as definitive.

Greater precision in the determination of the setting points, and also ready adaptation to determination of the time of setting can be obtained by arranging for the intermittent passage of air bubbles under constant pressure through the solution. The method used in the Eastman Research Laboratory is as follows:

Solutions of gelatin in water at various concentrations (1, 3, 5, 10, 15, and 20 per cent air-dry basis) were prepared under standardized conditions—i. e., definite period of swelling in cold water, definite time of heating and stirring at 70° C., definite short heat at 100° C. Then 20 or 30 cc. were placed in one-inch test-tubes which were clamped in fixed position in constantly stirred ice-water, this assuring a constant rate of cooling. Air-bells were passed through the hydrosol at intervals of fifteen seconds, using a modification of Flemming's apparatus for determining the rate of coagulation of silicic acid hydrosols, which is described below. The precautions necessary are: (a) fifteen-second intervals between passage of air-bells; (b) slow flow of bells; (c) exit at definite depth, 1 or 1.5 inches below the surface; (d) water in compensator tube at same level throughout the experiment. (See Fig. 40.)

The thermometer is immersed with its bulb next to the air-passage, and the temperature at which bubbles cease to pass is taken as the setting-point. Inversely, the jelly thus formed may, after suitable chilling, be surrounded by water at a definite higher temperature, and the melting-point taken as the temperature at which bubbles again pass through.

Setting Power. The transition from the sol or liquid state to the jelly-solid is, as has been stated, practically continuous.[1] Therefore, the viscosity-temperature curve with falling temperature shows a continuously progressive increase in viscosity, the refractive index shows no break between the solution and jelly states, and the vapor-pressure also changes continuously. The transition would be most naturally defined in terms of the appearance of an elastic coefficient which is in a measure what is attempted in the previously described methods. The potential of this elasticity (or the setting power), may be measured either by the time of setting or the rate of setting, analogously to the similar determinations for the setting of cements.

The setting time for a given temperature fall may be determined by using Flemming's apparatus for the study of the rate of coagulation of colloidal silicic acids mentioned above.[2] The arrangement is as follows: Compressed air passes a manostat B (see Fig. 40), or Marriotte bottle B through a mercury valve D (to be described later), to the distributor. Here the air-current is split, part going through the hydrosol in K, part through a T-shaped tube to a pneumatic release, which operates an alarm clock (not shown in Fig. 40).

As long as air passes K the pressure is insufficient to disturb the pneumatic release, but once the passage of air through K is stopped the pressure rises in the other arm and expands the release, making this stop the timer electrically.

The aforementioned mercury valve D is necessary to make the passage of the air through K intermittent, as a continuous air-current maintains a channel through the jelly. The valve is constructed as follows: The U-tube, which contains mercury, has an iron core floating in one limb. If this is attracted by the electromagnet E the level of the mercury sinks and allows air to pass in the other limb of the U-tube, which is otherwise closed by the mercury. The electromagnet is operated by making the second-hand of a clock C effect contact every thirty seconds with the battery energizing the magnet. (See Fig. 40). The apparatus can be used for a variety of relatively slow setting phenomena of colloids. Instead of the time of setting, the rate of setting may be determined, defined as $\left(\frac{\Delta y}{\Delta t}\right)_{\Delta T}$, the rate of increase of viscosity for a given drop in temperature. A discussion of this will be given in Volume II.

[1] Recent work suggesting a definite transition point will be treated in Volume II.
[2] Flemming, W., Zeits. physik. Chem. **41**: 427. 1902.

MELTING POINT DEFINED BY RELATION TO VISCOSITY. In view of the difficulty of defining a melting point of gelatin : water systems, because of the apparent continuity of the gel⇌sol transition, Bogue[1] has proposed a method for the measurement of relative melting points of glues and gelatin based on the relation which exists between this value and the jelly strength and viscosity. Bogue states: "If one may assume as a meas-

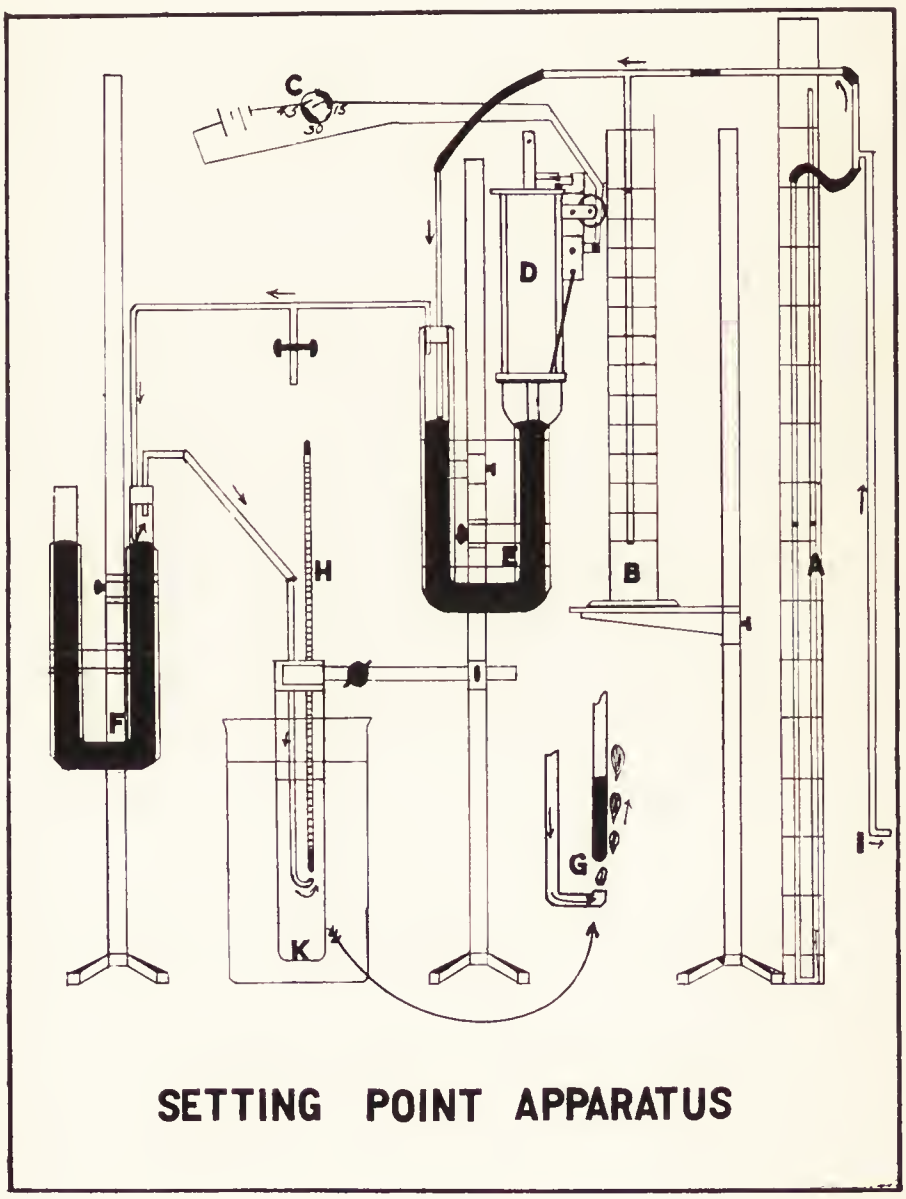


FIG. 40

[1] Bogue, R. H., l. c.

ure of melting point that degree of viscidity at which a glue will no longer flow through the narrow orifice of a viscosimeter tube, then it becomes an easy matter by taking a series of viscosity readings at decreasing temperatures to plot the point at which the rate of flow would be zero. For comparative purposes, however, it should suffice only to plot the values actually obtained and note the relative rates at which the curves tend to approach the vertical (time of flow = infinity). Thus, if the curve of one glue tends to approach the vertical more rapidly than another, as both are reduced through equal intervals of temperature, then the former has the higher melting point". This conclusion is supported by Bogue's data. (See Fig. 41.)

In a paper[1] dealing with the setting and melting point apparatus herein described, the writer has discussed this criterion of Bogue's in relation to the converse process—change from solid to liquid state, with diminution of elasticity. Bogue's convergence point (for time of flow = ∞) might more correctly be regarded as the setting point; however, thermodynamically, melting points and setting points should be identical. It is suggested that at this point not the viscosity but the time of relaxation (of Maxwell's elastic theory) becomes infinite, this point or zone being one in which the substance is plastic.

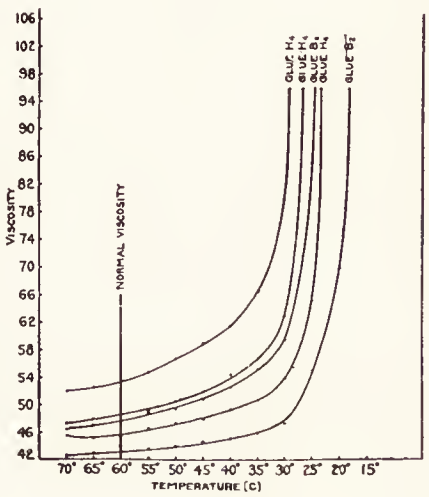


FIG. 41
Viscosity-jelly strength curve

It has been pointed out that the (apparent) viscosity of a gelatin sol is a function of the shearing stress. It appears from the recent investigations of Bingham[2] that the transition from sol to gel is likely to be capable of more precise definition by determining the flow under varying shear at each temperature, and ascertaining the point of zero fluidity. As this very important conception of plastic flow in relation to colloids and particularly to gelatin systems has not yet been specifically adapted to gelatin technology, it will be discussed at greater length in the second volume.

[1] Sheppard, S. E., and Sweet, S. S., J. Ind. Eng. Chem. **12**: 167. 1920.
[2] Bingham, E. C., Science **54**: 17. 1921.

(4) Jelly Strength

For some purposes the mechanical strength of a jelly is regarded as the most important of all tests.[1] This is particularly the case in rating glues, but the test is also of great importance for photographic and paper gelatins. Analogously to the "wet strength" of papers, the jelly strength of a gelatin is obviously of first importance for its use in photographic operations, and more so than ever with the increasing use of flexible film support in place of the rigid glass base.[2]

The method most highly recommended for practical commercial purposes is that of measuring, or rather comparing, the resistance of the jelly by means of the finger. The fourth finger of the left hand is used, being considered the most sensitive. Reference should be made to the work cited for a description of the procedure.

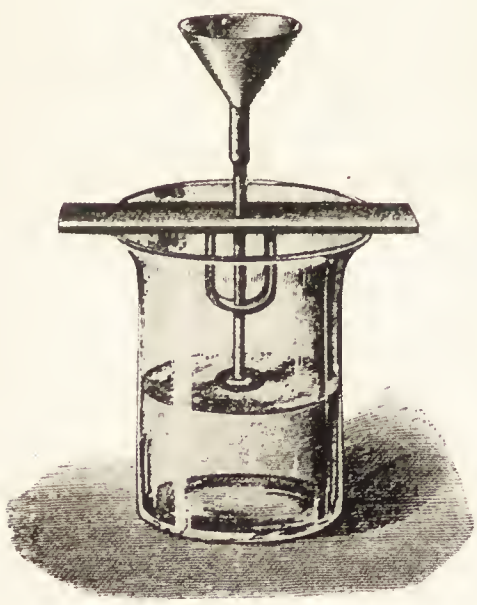


Fig. 42
Lipowitz jelly strength apparatus

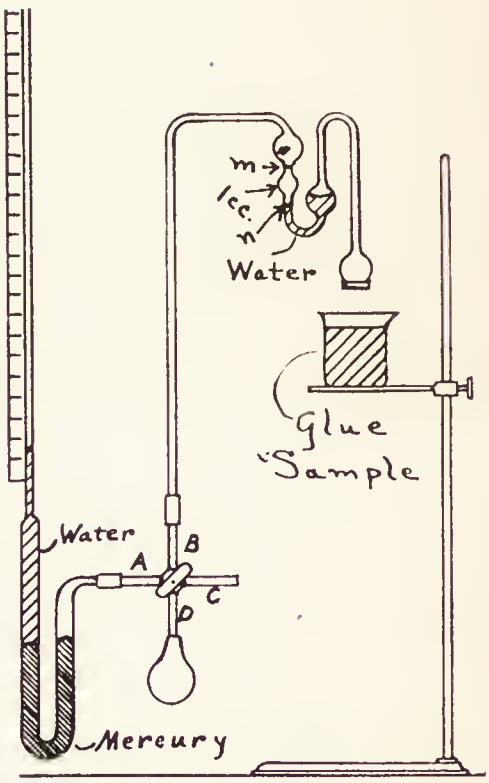


Fig. 43
Hulbert's modification of Smith's jelly strength tester

[1] Fernbach, R. L., l. c.

[2] The wet strength of photographic papers is indeed chiefly determined by the jelly strength of hardened gelatin.

Although no doubt satisfactory for many commercial purposes, the purely comparative and very subjective nature of this test obviously makes it desirable to have a method giving values independent of comparison with an arbitrary standard, and objective in character. Of the various mechanical tests developed the following are worthy of note:

(a) The Plunger Test (Lipowitz). This test is to determine the weight necessary to force a loaded plunger through the surface of a jelly on which it rests. The apparatus in its simplest form is shown in Fig. 42. Shot is poured into the funnel connected to the plunger until it just breaks through the jelly. A high grade glue (ten per cent solution) carries 60 grams, a poor one 8 grams.[1] A modification due to Valenta[2] is as follows: To reduce the friction of the plunger in its bearings Valenta used a square shaft made to run in roller bearings. He also substituted mercury for the lead shot used for loading the plunger. With this apparatus, using ten per cent gelatin jellies, values from 800 to 1500 grams were obtained for the jelly strengths.

Scott's glue tester is based on a similar principle.

A jelly-strength tester patented by Smith[3] consists of a pressure chamber, one side of which is provided with a flexible elastic membrane (e. g., rubber), which is brought into contact with the surface of the jelly to be tested. Means for generating pressure in the chamber are provided, thereby expanding the membrane and deforming the jelly surface. There is also a device for measuring the amount of displacement and the pressure in the chamber. A modification of this type of tester has been described by Hulbert.[4] (Fig. 43).

As pointed out by Alexander,[5] all methods which depend upon the breaking or compression of the jelly in glasses or open molds are subject to error due to the formation of a skin of greater or lesser thickness and to variations of diameter, giving a surface of variable area. To avoid these troubles, Alexander[6] devised a tester for determining the resiliency of jelly blocks free from containing walls, with the surface skin layer at the bottom. This tester consists of a cup-like brass vessel supported, like a gas tank, by four vertical rods, against which it slides on friction-reducing roller bearings. This

[1] See Lambert, T., l. c.

[2] Valenta, E., l. c., p. 181.

[3] Smith, E. S., U. S. Patent No. 911, 277. Feb. 2, 1909.

[4] Hulbert, E. C., J. Ind. Eng. Chem. **5**: 235. 1913.

[5] Alexander, J., in Allen's Commercial Organic Analysis, l. c., p. 607.

[6] Alexander, J., U. S. Patent 882, 731. (March 24, 1908.) J. Soc. Chem. Ind. **27**: 459. 1908.

brass cup is allowed to rest on a truncated cone of jelly of definite size and composition, and at a definite temperature. Then shot are gradually poured into the cup until a definite compression of the jelly is observed. Beneath the cup are two vertical adjustable brass uprights, 3.5 cms. high, connected with an electrical indicator circuit. When the cup reaches the level of these uprights, corresponding to a definite displacement, it completes the circuit. The weight of the cup plus the weight of shot added gives a figure for the jelly-strength.

In preparing the truncated cones, the jellies are cast in cone-shaped brass cups 6 cms. high, 5.5 cms. in diameter at the open top, and 5 cms. at the bottom, which is closed by a tightly fitting cap. If the jellies do not come out readily on removing this cap the molds may be momentarily heated in hot water. The height of the cones should be exactly 4.5 cms.

FIG. 44
Forest Products Laboratory jelly strength tester

A series of standards, to compare to some extent with the Cooper grades, is described in the article cited.

A jelly-strength tester recently described by the U.S. Forest Products Laboratory[1] is based on the finger-test principle, but gives numerical results. Its essential parts are: a light cylindrical frame or float, which rests upon the surface of the jelly, and a heavy graduated plunger which moves vertically in the frame. The depth of its depression in the jelly is a measure of the jelly-strength. The nose of the plunger is hollow so that it may be filled with shot to bring the weight to the most sensitive point. By means of a set-screw at the top of the plunger, the zero-mark on the scale may be set opposite the zero-

[1] Technical Notes from the Forest Products Laboratory, No. F-32.

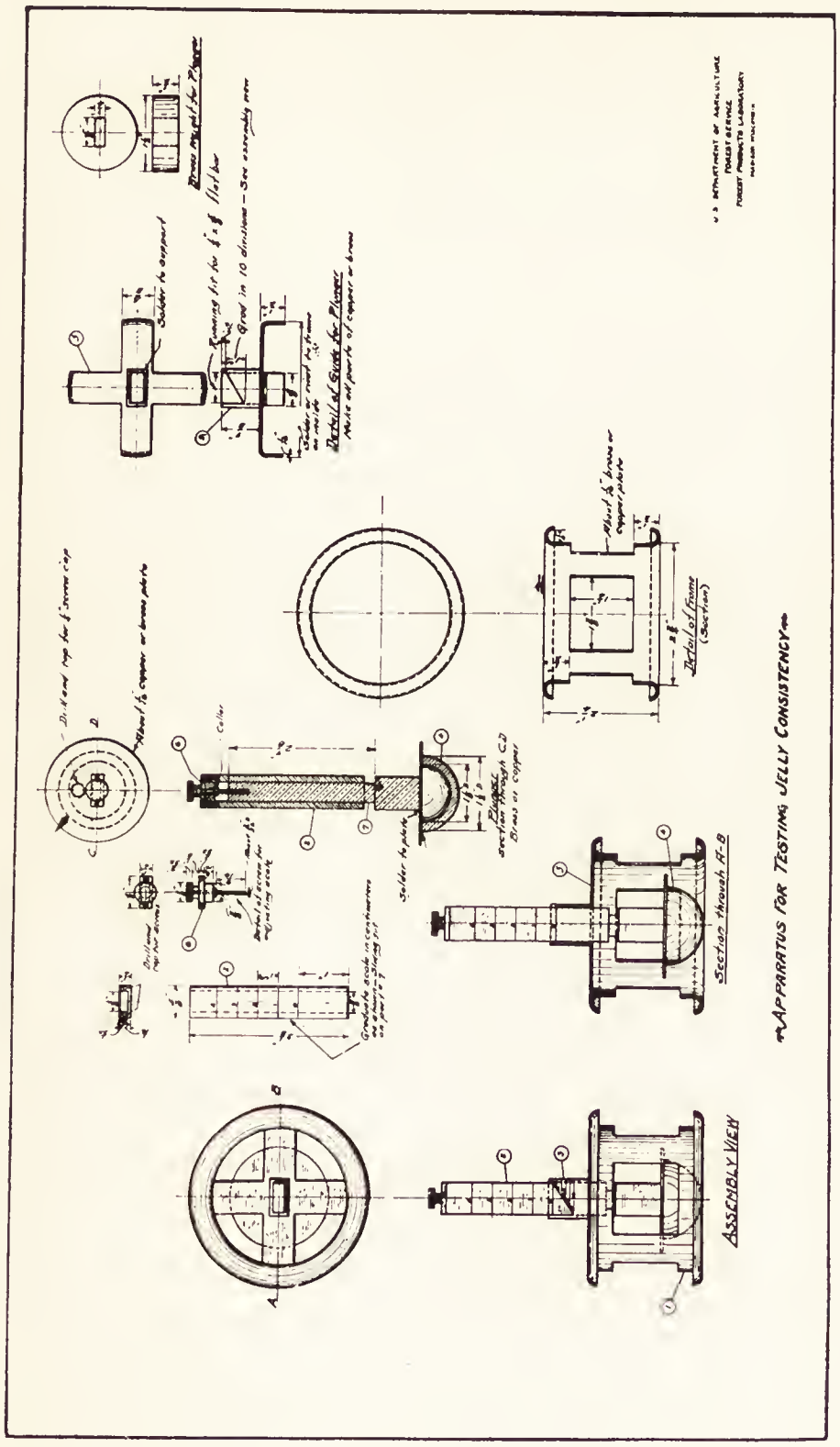

Detail of Stock for Plunger
Detail of Frame (Section)
Section through A-B
ASSEMBLY VIEW
*APPARATUS FOR TESTING JELLY CONSISTENCY

FIG. 45

mark of a scale on the frame. The article cited gives details for preparing the jellies to be tested.

It is stated that the depth of depression varies inversely as the consistency (concentration?) of the jelly, though not in exact proportion. The general disposition of the apparatus will be clear from Figs. 44[1] and 45.

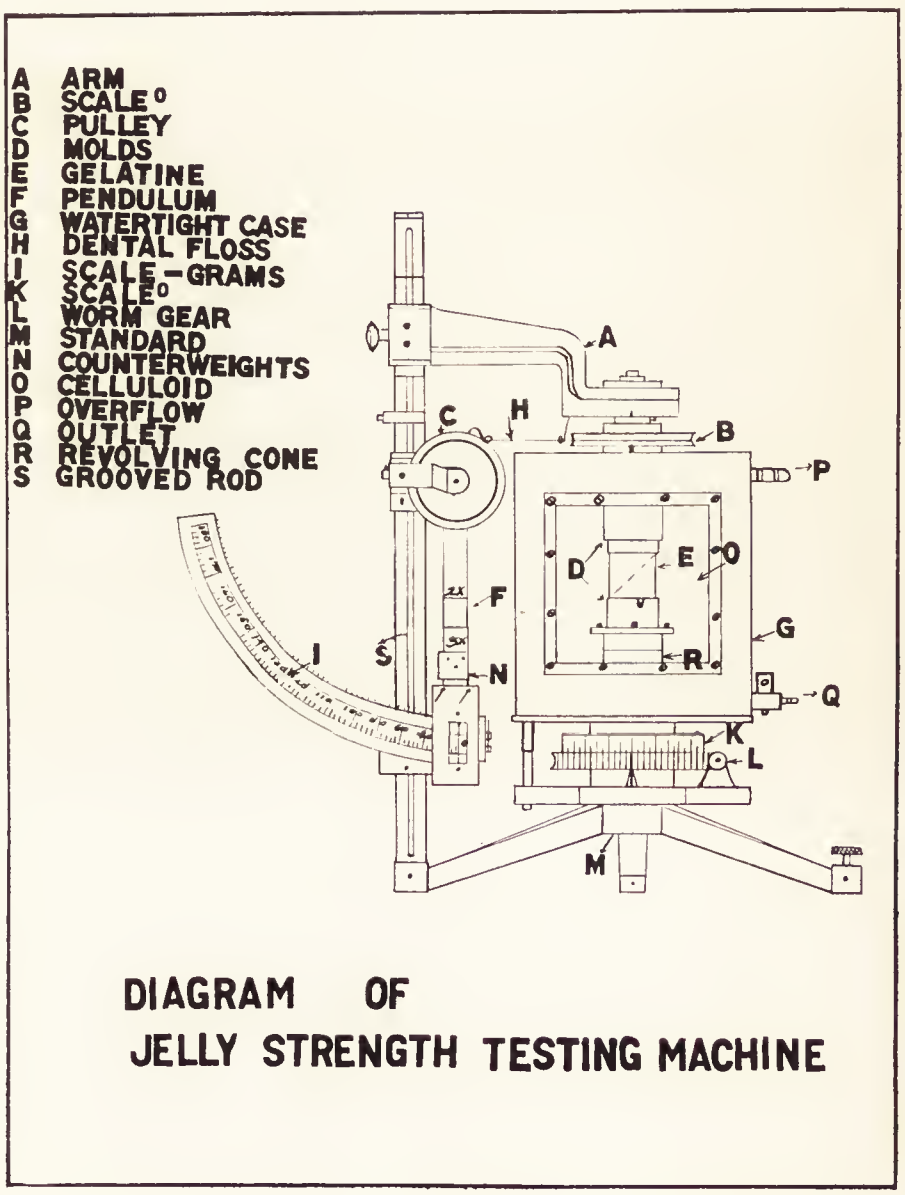


FIG. 46

[1] Furnished by the Forest Products Laboratory.

(b) Torsional Elasticity. For research on the properties of jellies and for more exact testing a jelly-strength testing machine operating by torsion has been devised by the author. To obtain the closest correlation between viscosity coefficients and elasticity coefficients or moduli, it is desirable that elastic values, e. g., the tenacity or breaking strength, should be obtained for pure shear. This is most easily secured by subjecting cylinders of the material to be tested to torsional stress.[1] The general construction and mechanism of the instrument are shown in Figs. 46 and 47.[2]

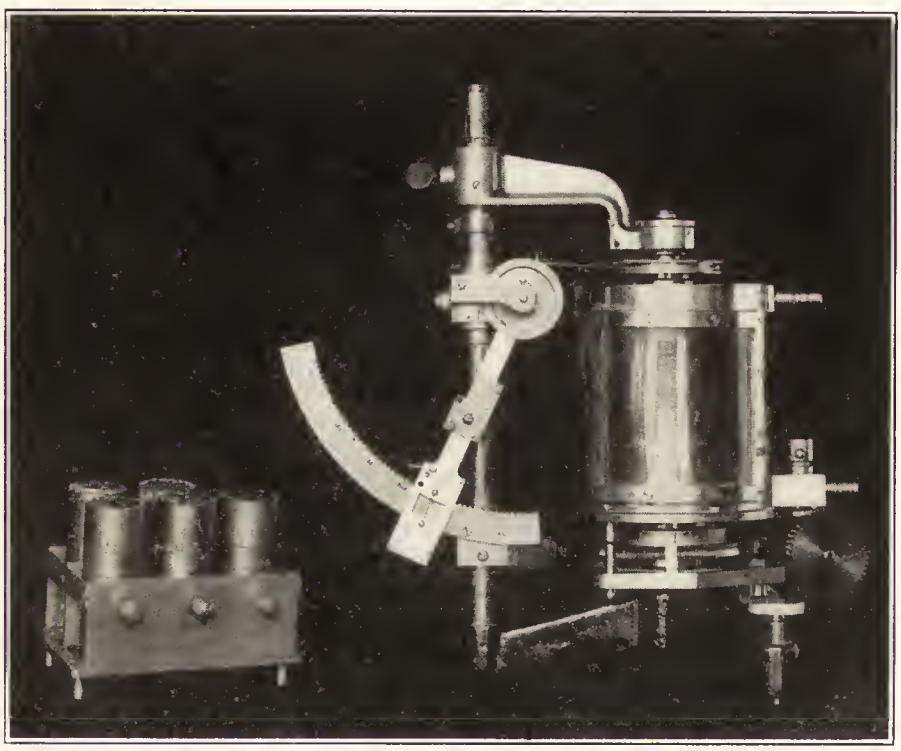


Fig. 47
Jelly strength testing machine

The jelly is cast in a cylindrical mold having a split jacket which may be removed after the test cylinder has been fixed in position in the instrument. In operation the jelly cylinder is twisted at a uniform rate by rotating the base at constant (angular) speed. This is effected by a worm drive, operated either by hand, or, where greater accuracy at low speeds is required, by a constant speed electric motor. The circular base is graduated in degrees—scale K—and the upper head of the "test cylinder" also carries a circular scale in degrees—

[1] Cf. any text-book on strength of materials.

[2] See Sheppard, S. E., and Sweet, S. S., J. Ind. Eng. Chem. **13:** 423. 1921.

scale B. One of the feet of the tripod base carries as a standard a steel rod grooved on opposite sides throughout its entire length. A short section at the top is free to rotate. This grooved standard carries an arm which is free to move up and down except as fixed by an adjustable setting collar, but which can not rotate except when in position on the top section of the standard. This arm carries the upper "grip" for the jelly cylinders, which is rigidly connected with a pulley revolving freely on a ball race. The pulley (about 2½ inches in diameter), carries scale B. The grooves in the standard bring the pulley and the upper grip into perpendicular and centered alignment with the lower grip, the pulley wheel connecting, as shown, by a silk or fine chain cable with a lever, moving along a calibrated scale I. The lever or pendulum carries a set of pawls running lightly over the ratchet on the scale and keeping it from dropping back from any position to which it has been raised. The load of this counter-poise can be altered by placing jockey weight on the bob, so that multiplying factors of two times and three times the scale readings are obtainable. The actual range with the first instrument constructed is from 10 to 550 grams. The "breaking load" W is obtained from the limit position reached on the scale C, the "twist" as the difference K′—B′ between the readings of the scales K and B at the moment of break.

It is desirable in any test of the strength of materials that the dimensions of the test piece be as definite as possible, and that the portions gripped do not affect the result. It has been found that this may be secured very simply, with glue and gelatin, by filling the grips with wood, the surface of which has small transverse grooves cut on it.[1] The grooving not only increases the surface available for adhesion, but also facilitates molding free from air-bells.

In filling the molds it is necessary to avoid formation of air bubbles, particularly at the interface of the wood and the jelly. After the side pieces are fitted on the bottom grip of the mold, and this placed in the clamp shown in Fig. 47 (which insures a tight fit and no leakage), the gelatin solution, at about 40° C., is poured in until it forms a meniscus at the top of the side plates. The upper grip is now carefully pushed across in the direction of the grooves, thus allowing air to be displaced. It is then pushed down into the mold, the excess gelatin solution and residual air being displaced through vent holes in the wooden core.

[1] For information on this, I have to thank Major Taylor, R. A. F., who has found in experiments with gelatin models of shafts that gripping by adhesion gives the least "end" error.

Experiments in chilling the test cylinders showed that after three to six hours at 0° C. no further increase in strength occurred.

In making the test, the entire mold is inserted in the lower clamp of the instrument, and the rotatable arm carrying the upper grip or clamp is swung around and fastened to the top of the mold by set screws. The scales A, B, and C are set at zero. The sides of the mold are then split off and the twisting started. This is stopped immediately on completion of a "break," and the angular difference K′—B′ read off, as also the breaking load on I. It is desirable also to note the character of the break. This should be a helical cleavage, of an angle of 45°, extending from the base to the upper end of the jelly column, with no sign of imperfect adhesion. (See Fig. 48.)

FIG. 48

Imperfect adhesion, which seldom occurs if proper precautions in filling and casting are taken, should lead to rejection of the sample and repetition of the test. There are six molds in the set, but usually two or three check values are sufficient, allowing two or three different gelatins to be tested simultaneously. Apart from imperfect adhesion, however, a jelly may break off "short," corresponding to a low angle of twist and a brittle or crumbly structure.

Effect of Temperature. Experiment showed that, after forming a jelly at 0° C. for three hours, then bringing it to a higher temperature and testing it at that temperature, the strength did not alter materially until a temperature above 10° C. was reached. Above this point the jelly-strength rapidly diminished. See Fig. 49. For experimental control

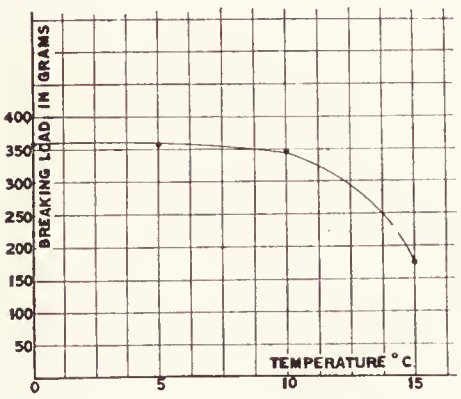


Fig. 49
Temperature-jelly strength curve

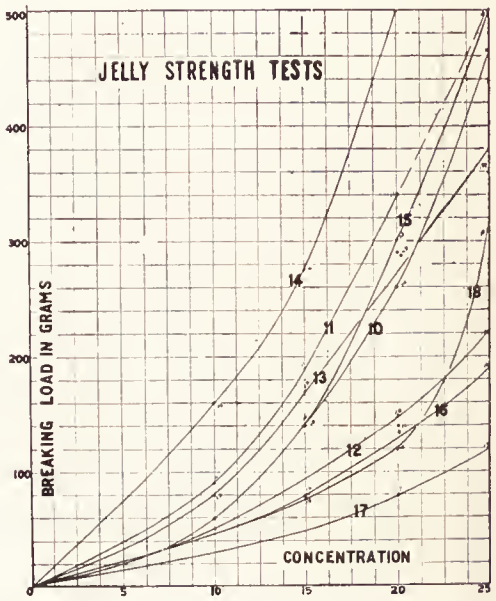


Fig. 50
Concentration-jelly strength curve

of the temperature a water-tight jacket, revolving with the base and having transparent windows to facilitate observation, is provided. (See Fig. 47.)

Effect of Concentration. The general elastic properties of gelatin jellies in relation to various factors will be discussed more fully in Volume II. This relation of jelly strength to concentration is important in making comparisons between different gelatins and glues. Although the jelly-strength in any case increases more or less rapidly with the concentration, different gelatins differ considerably in this respect, as is shown in Fig. 50, where the curves of "breaking load" plotted against "concentration" frequently cut. Here concentration is based on air-dry gelatin. Consequently, according as comparisons were made below, at, or above the concentration where the curves cut, the one gelatin would be regarded as having lower, equal or greater jelly-strength than the other. For complete comparison it would be necessary to compare

the areas enclosed by the breaking-load concentration curves from 0 to 100 per cent (based on dry gelatin). As this is not practicable, more or less arbitrary methods of comparison must be adopted. For some purposes we have taken the jelly strength value of a photographic gelatin or paper gelatin as the slope $\triangle W/\triangle C$ between concentrations of ten per cent and twenty-five per cent. As the actual expression for jelly strength, instead of the breaking load W, it appears better to use the value

$$T = \tfrac{1}{2} \text{ Breaking load} \times \text{twist},$$

which is proportional to "proof resilience."

A method of determination of "jellying power" of gelatins and glues by polarimetry has been developed by C. R. Smith.[1] In a previous investigation on the mutarotation of gelatin[2] he found that gelatins of the highest jelly strength approached a definite maximum value of mutarotation measured between 35 and 15° C., and that the jellying power decreased in weaker gelatins parallel with reduction of the mutarotation. Smith's conclusion is that the existence of the sol $\rightleftharpoons$ gel equilibrium is connected with definite gelatin molecules responsible also for the mutarotation.

For testing jellying power the powdered air-dried samples are weighed, soaked in cold water for 30 minutes, and dissolved, in graduated flasks, between 50 and 60° C., and made up to the mark at 35° C. The rotations at 35° C. are measured in 2 dm. tubes, and again after cooling to 15° C. over night (12 to 18 hours). The rotations finally reach constant values and represent the maximum change in rotation and jellying power at that temperature. The samples showing the greatest mutarotation in the 3 gms. per 100 cc. concentration required the smallest amounts to produce the standard jelly. As standard procedure for grading, Smith recommends polarizing 3 gms. per 100 cc. at 35 to 36° C. in a 2 dm. tube; then cooling a portion rapidly to 10 or 15° C. and transferring the sample before it has jellied to a cold 1 dm. tube. If the samples need clarification, digest with 5 cc. light powdered magnesium carbonate at 30 to 40° C. for one hour or more, and filter until clear, avoiding evaporation. Considering a sample which polarizes −20.5° at 35° C. and −40.0° at 15° C. for a concentration of 3 gms. per 100 cc., it is suggested that the strength be expressed as 19.5 points at 15° C., the increment of rotation in Ventzke degrees.

[1] Smith, C. R., J. Ind. Eng. Chem. **12**: 878. 1920.

[2] Smith, C. R., J. Amer. Chem. Soc. **41**: 153. 1919.

(5) Foam Test

Excessive tendency to foaming in a glue or gelatin solution is not only objectionable for several technical applications—e. g., veneering glues, paper-box making and sizing—but also indicates the presence of impurities or decomposition products, such as soaps, mucins or peptones. Foam tests are usually comparative, no absolute standard of experimental conditions having been adopted or even proposed. One of the first systematic investigations is due to Trotman and Hackford,[1] who state that "the literature on the subject may be summed up in the statement that a ten per cent solution of glue is employed in testing foaming capacity, and that glues give from one-half to eight inches of foam." The method used in testing is of course immaterial where only comparative results are required, but those at present employed are so varied that there is little chance of obtaining comparable values.

Since the amount of foam given by a solution of glue depends upon (a) the height of liquid in the tube, (b) the diameter of the tube, (c) the temperature of the solution, and, to a less extent, the time and method of test, Trotman and Hackford proposed and used the following apparatus for testing foam: A graduated tube, about 70 cms. in length and of such diameter that each division is 1 cm. in length for 1 cc. capacity, is half filled with a ten per cent solution of the glue or gelatin to be tested. It is then placed in a water-jacket, the temperature of which may be raised by passing steam into it. Even distribution of the steam is effected by means of a ring distributer at the end of the delivery tube, and an overflow is provided. The temperature of the bath is maintained at 60° C. After allowing the glue solution to reach this temperature the tube is withdrawn from the bath and its level adjusted by a tap till it stands at the zero mark, set for exactly 25 cc. The tube is now corked and shaken vigorously for about one minute, replaced in the bath, and the height of the foam read off. The top of the foam is read, since this is found to be constant with constant temperature. The line of demarcation between the foam and the liquid is usually too indistinct to allow of the lower reading being taken accurately. Since the foam produced varies greatly with temperature, it is very important to arrange for a constant temperature bath. Prolonged delay in carrying out the test leads to hydrolysis and increased values. The conclusion of these authors as to the causes of foaming will be discussed later. It may be noted here that an average pure gelatin "containing less than

[1] Trotman, S. R., and Hackford, J. E., l. c.

one per cent peptone" and purified by precipitation with zinc sulphate as already described,[1] gave, on redissolving, a foam figure of 10.5 which, on the apparatus described, may be considered a relative minimum for pure gelatin. The fact that some glues give lower figures than this shows that they contain foam-inhibiting substances. Again, the foaming is a maximum for the same gelatin at the iso-electric point, i. e., P_H about 4.8.

In discussing the above paper, Watson[2] described the following method of testing, which does not differ in principle and requires no special apparatus. The glue to be examined is broken up in an iron mortar (covered to prevent the glue from flying about), and 5 gms. of the powder are weighed out and put in a beaker of 100 to 150 cc. capacity. This is covered with 40 cc. of water and allowed to stand for several hours, after which the glue is melted at about 130° F. (53.3° C.) When completely melted the solution is poured into a stoppered graduated cylinder of 100 cc. capacity and about 2½ cms. in diameter. The volume is made up to 50 cc. with the hot water used for washing out the beaker. The temperature now being about 120° F. (48.8° C.), the stopper is replaced and the cylinder shaken vigorously for a few seconds, or until no more foam is produced. The cylinder is then allowed to stand for a few minutes, during which the foam collects at the top of the solution. As soon as the height of the solution has reached 45 cc., the reading at the top of the foam is taken, the difference between this and 45 cc. giving the amount of foam. Watson considered this better than reading the height immediately after shaking and subtracting 50 cc., first, because it gives an idea as to the permanency of the froth, and, secondly, because the foam reading is practically the same, as shrinkage of the foam is compensated in taking the reading this way.

(6) Specific Gravity

Specific gravity or density measurements are used in control of manufacture of glue and gelatin, a hydrometer being employed. Use of immersion hydrometers or the Westphal balance, is rendered inaccurate, in some cases with quite dilute solutions, in any case with more concentrated ones, first, by turbidity or opacity of the sols, second, by viscosity and surface tension. To allow for the first, Winkelblech[3] takes the reading where the surface of the solution cuts the spindle and then adds a correc-

[1] See Ch. III, p. 132.
[2] Watson, H. J., J. Soc. Chem. Ind. **25**: 108. 1906.
[3] Winkelblech, K., l. c.

tion (estimated at three scale divisions on the 15° C. hydrometer, specific gravity scale). Winkelblech compares results by pycnometer (weighing) and hydrometer, from which he concludes that hydrometer methods are sufficiently accurate and unaffected by viscosity even for very strong solutions.

Winkelblech's results are as follows:

Grams in one liter	One liter = grams by weighing	One liter = grams by hydrometer
300	1072	1071
200	1046	1045
150	1033	1030
100	1018	1017
75	1013	1011

Further differences in the specific gravities of solutions of the same concentration are hardly noticeable in gelatins and glues of normal type, but obviously peculiar kinds give deviations. In addition to this it may be remarked that deviations will occur for loaded materials, or for those with abnormally high mineral content. In plotting specific gravity against concentration the latter should be expressed as "gelatin or dry matter at 100-105° C.," as air-dry gelatins have a variable moisture content.

(7) The Clarity of Solution

The clarity of solution may be measured by means of a turbidimeter devised by the author, and described below:

Turbidimeters as used in water analysis, based on the vanishing of a line or filament for a given depth of liquid, are well known, the turbidities being expressed generally on an arbitrary scale. For this method there is generally assumed a more or less unlimited amount of the liquid.

In many cases, however, especially in research and development work with nonaqueous systems, only a limited amount of the material may be available—for example, in development of a new lacquer or varnish. For such work an instrument operating on a small constant thickness becomes desirable. While this could be attained photometrically, measuring either the relative transmitted light (transparency) or the relative side scattered light (turbidity), yet in some respects the method of judging the distinctness of some standard object seems simplest for practical purposes. What is required now for the case of a limited thickness of fluid is an object the visibility of which can be varied in some continuous and easily measurable way. For this purpose advantage was taken of the well-known properties of crossed gratings, on lines similar to those employed by H. E. Ives[1] in his test object for visual

[1] Ives, H. E., J. Opt. Soc. Amer. **1**: 100. 1917.

acuity. The essential part of the apparatus consists of two superposed opaque line gratings arranged to rotate relatively to each other about an axis perpendicular to their plane. Viewed by transmitted light, at such a distance that the grating lines are below the limit of resolution, parallel dark bands are seen. The separation of these alters quite continuously as the gratings are rotated, so that there is a continuous change from extreme visibility to invisibility—when the bands can no longer be resolved. The average brightness of the field remains constant, and also the variation of brightness from the center of a bright band to that of the adjacent dark band is the same for all sizes of bands.

The following diagrams, taken from Ives' paper, illustrate the important relationship between the width of the irradiation bands and the angle of relative rotation of the gratings.

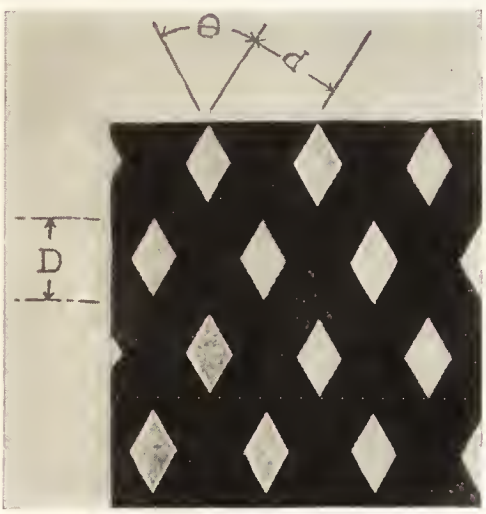


FIG. 51
Grating lines

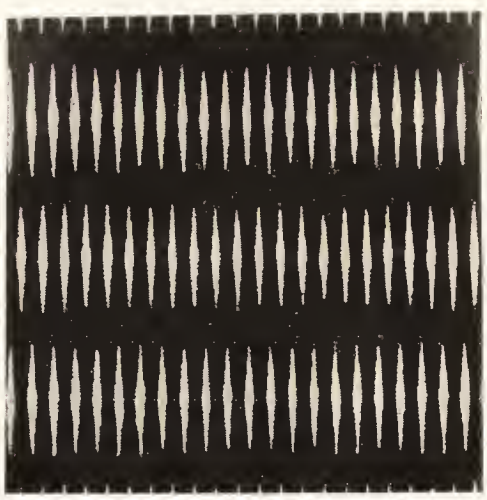


FIG. 52
Photomicrographs of crossed gratings

In Fig. 51, *d* is the distance between centers of the grating lines, A the angle of relative rotation, and D the distance between those regions where continuous straight lines can be drawn across without meeting a clear space. These latter lines form the centers of the dark bands visible on viewing the crossed gratings.

The distance between them is given by the formula

$$D = \frac{d}{2 \sin A/2}$$

In Fig. 52 is shown a photomicrograph of a pair of crossed gratings, turned through a small angle. At a sufficient dis-

tance—several yards—the component lines vanish, only the interference (or irradiation) bands showing. At the ultimate resolving distance the variation of intensity over the width of the bands is lost and they appear as sharp, black lines. For details as to their use in forming visual acuity tests, reference should be made to Ives' paper. Their application to turbidimetry depends on an inversion of the same principles. If a scattering (turbid) medium be placed in front of the crossed gratings, the visibility limit $1/D = 2 \sin A/2$ will be lowered, according to the turbidity. For relatively small angles, secured by a proper choice of d, the expression $1/D = 2 \sin A/2$ reduces to $1/D = \sin A = A$. From this the visibility limit—for one and the same observer, *i. e.*, for the same visual acuity—can be measured by the angle through which the gratings must be turned to make the bands just disappear.

If $A_{max.}$ be the angle for the visibility limit for a given thickness of some standard medium,—e. g., distilled water, lacquer solvent—and A the angle for the same thickness of a turbid solution, then putting $A_{max.} / A = 100/C$, gives C,

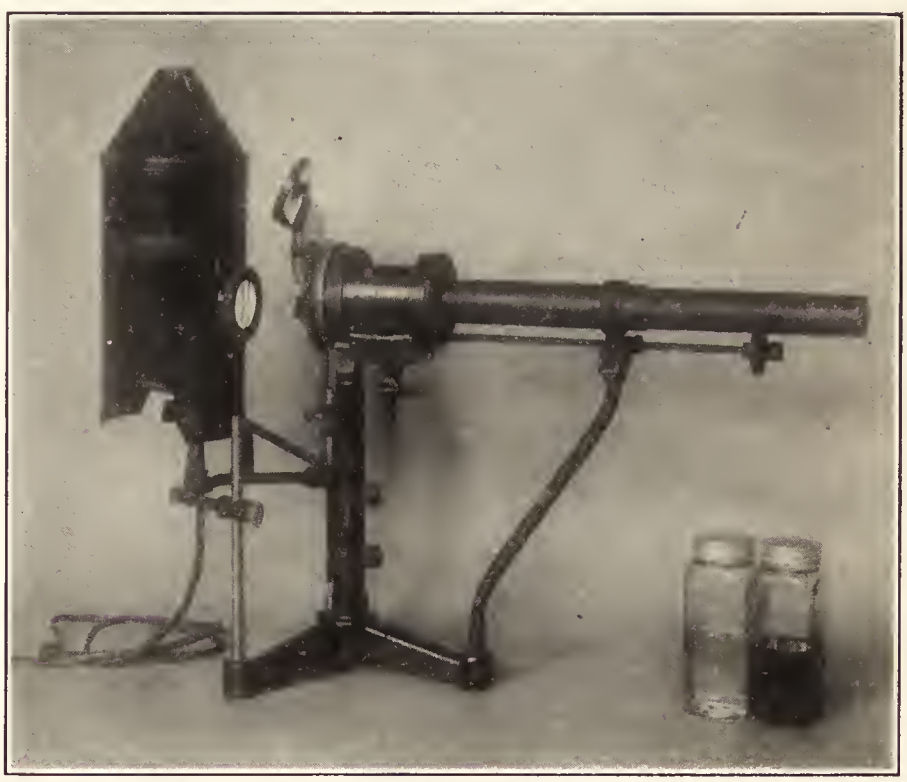


FIG. 53
Instrument for measuring clarity

the per cent clarity, from $C = \frac{100\ A}{A_{max.}}$.

The instrument constructed for measuring clarities in this way is shown in Fig. 53. The solutions to be tested are conveniently put up in 8-oz. square jars, with screw-on caps. These are obtainable commercially and are much more convenient for work with varnishes, lacquers and other sticky substances than corked or stoppered bottles. Since the sides are not perfectly plane parallel, distortions of the grating image will occur on placing a jar in position in front of the gratings. To eliminate this refraction error, the test jars are placed in a cell with glass walls parallel to the gratings, and immersed in a liquid of refractive index equal to the glass, monochlornaphthalene proving very satisfactory and eliminating all distortion. The readings of A are made on a circle and vernier reflected in the concave mirror above, readings being possible to 2′ of arc. Error in zero can, if necessary, be eliminated by alternate readings on each side. Readings can be taken so rapidly that it is easy to secure a mean of 5 or 6 for each observation. As with methods depending upon visibility of interference fringes, the possible precision is much higher than that practically obtainable, owing to the fatigue factor in making such readings. But this error, for a normal observer, does not appear to be at all prohibitive, as the following results indicate. They were made without special precautions, such as dark room, resting the eye, or head rest, and the gratings were not of first-class quality.

Sample	A	P. E.	C	P. E.
Solvent	41°24′	±16′	100	....
Solution 1	2°30′	± 2′	6.0	±0.2
Solution 2	3°38′	± 9′	8.7	±0.4
Solution 3	10°15′	±17′	24.7	±0.5
Solution 4	22° 0′	± 8′	53.1	±0.5
Solution 5	30°28′	±21′	73.5	±0.95
Solution 6	35°10′	±23′	84.9	±1.00

The probable error for A is for the mean of five readings, hence the probable error for a single observation would be larger, amounting for the largest value to 10 per cent on the reading. The probable error on the per cent clarity C is calculated from the value

$$P.\ E. = \pm \frac{\sqrt{\left(\frac{Aa}{A_{max.}}\right)^2 + b^2}}{A_{max.}} \times 100$$

where $A_{max.}$ = reading for solvent; A = reading for sample; b = P. E. for setting of A; a = P. E. for setting of $A_{max.}$

The probable error of a single setting increases on the whole with the angle of setting, i. e., with the clarity, but the per cent probable error remains much the same. The readings are made as rapidly as with the polarimeter or refractometer.

In the instrument constructed two photographic line gratings were used, having about 50 lines per in., and Amax. was about 40° for normal visual acuity. The error using the simplified formula

$$C = A/A_{max.} \times 100 \text{ instead of } \frac{\sin A/2}{\sin A_{max.}/2} \times 100 \text{ is not,}$$

however, serious for the work required. Thus for A = 5°, Amax. = 40°, we have

Approximate	Accurate
C = 12.5 per cent	12.7 per cent

There appear to be several advantages in replacing such expressions as "water-clear" by definite per cent clarity and definite colorimetric values, where color is a factor.[1]

Test Forms

The suggested test sheets following do not cover the applicability of gelatin for photographic use, but pass on their physical and chemical qualifications prior to photographic adaptations.

[1] Reprinted from J. Ind. Eng. Chem. **12**: 167. 1920.

TYPICAL PROTOCOL OF PHYSICAL TESTS, FOLLOWING BOILING SCHEDULE

No. 132

Serial	Lot	Kind of Stock	Run	Gravity of draw	PH	10% Viscosity		10% Jelly Strength	10%		Water Absorption per gm. /24 hrs.	Foam	Clarity and Color
						100° F.	150° F.		M. P.	S. P.			
532	1	1 Br. 1	1										

TYPICAL PROTOCOL OF CHEMICAL ANALYSIS

No. 132

Serial	No.	Lot	Kind of Stock	Gravity of draw	On dry gelatin, Batch No.							
					Gelatin Content	Acidity in N/100 per 1 gm.	Ph	Moisture	Ash	Amide Nitrogen	Wash out Nitrogen	Inorganic Constituents, etc.
												Copper, per million. Lead, per million. Iron, per million. Sulphurous acid, per million

General Remarks Appearance (whether flake, sheet, etc.)

Odor (sweet, sour, offensive, etc.)

Tested by .....

Approved by .....

Chemist

Abbreviations adopted in citations of serial publications.

Amer. J. Botany	American Journal of Botany
Amer. J. Physiol.	The American Journal of Physiology
Amer. J. Sci.	American Journal of Science
Analyst	The Analyst
Ann. Chem.	Annalen der Chemie (Liebig's)
Ann. Physik	Annalen der Physik
Arch. Anat. Physiol. (Physiol. Abt.)	Archiv für Anatomie und Physiologie. Physiologische Abtheilung
Arch. fisiol.	Archivio di fisiologia
Arch. Hyg.	Archiv für Hygiene
Ber. chem. Gesell.	Berichte der deutschen chemischen Gesellschaft
Ber. Verh. k. sachs. Gesell. Leipzig.	Berichte über die Verhandlungen der k. sachsischen Gesellschaft der Wissenschaften zu Leipzig
Beitr. chem. Physiol.	Beiträge zur chemischen Physiologie und Pathologie
Biochem. J.	The Biochemical Journal
Biochem. Zeits.	Biochemische Zeitschrift
Brit. J. Phot. Almanac	The British Journal of Photography Almanac
Bull. soc. chim.	Bulletin de la société chimique de France
Bull. soc. phot.	Bulletin de la société française de photographie
Chem. Abst.	Chemical Abstracts
Chem. Centrbl.	Chemisches Centralblatt
Chem. Met. Eng.	Chemical and Metallurgical Engineering
Chem. News	Chemical News and Journal of Physical Science
Chem. Ztg.	Chemiker-Zeitung
Collegium	Collegium
Compt. rend.	Comptes rendus hebdomadaires des séances de l'academie des sciences
Compt. rend. soc. biol.	Comptes rendus hebdomadaires des séances de la société de biologie
Gaz. chim. ital.	Gazzetta chimica italiana
Helvetica Chim. Acta.	Helvetica Chimica Acta
J. Agr. Res.	Journal of Agricultural Research, U. S. Department of Agriculture
J. Amer. Chem. Soc.	Journal of the American Chemical Society
J. Amer. Med. Assoc.	Journal of the American Medical Association
J. Amer. Leather Chem. Assoc.	Journal of the American Leather Chemists' Association
J. Biol. Chem.	Journal of Biological Chemistry
J. Chem. Soc. (Abst.)	Journal of the Chemical Society (Abstracts)
J. Chem. Soc. (Trans.)	Journal of the Chemical Society (Transactions)
J. Exp. Med.	Journal of Experimental Medicine
J. Frankl. Inst.	Journal of the Franklin Institute

J. General Physiol.	Journal of General Physiology
J. Ind. Eng. Chem.	Journal of Industrial and Engineering Chemistry
J. pharm. chim.	Journal de pharmacie et de chimie
J. Physic. Chem.	Journal of Physical Chemistry
J. Physiol.	The Journal of Physiology (London)
J. prakt. chem.	Journal für praktische Chemie
J. Russ. Phys. Chem. Soc.	Journal of the Russian Physical-Chemical Society
J. Soc. Chem. Ind.	Journal of the Society of Chemical Industry
Jahrb. Phot.	Jahrbuch für Photographie und Reproductionstechnik (Eder's)
Koll.-Zeits.	Kolloid-Zeitschrift
Monatsh. Chem.	Monatshefte für Chemie und verwandte Teile andere Wissenschaften
Phil. Mag.	Philosophical Magazine and Journal of Science
Phot. Ind.	Die photographische Industrie
Phot. J.	The Photographic Journal
Physic. Soc. Lond.	Physical Society of London
Polytech. J.	Dingler's polytechnisches Journal
Proc. Roy. Soc. Lond.	Proceedings of the Royal Society (London)
Proc. 7th Internat. Congress Appl. Chem.	Proceedings of the 7th International Congress of Applied Chemistry, London, 1909
Science	Science
Zeits. anal. Chem.	Zeitschrift für analytische Chemie
Zeits. angew. Chem.	Zeitschrift für angewandte Chemie
Zeits. anorg. Chem.	Zeitschrift für anorganische und allgemeine Chemie
Zeits. Biol.	Zeitschrift für Biologie
Zeits. klin. Med.	Zeitschrift für klinische Medizin
Zeits. physik. Chem.	Zeitschrift für physikalische Chemie, Stöchiometrie und Verwandtschaftslehre
Zeits. physiol. Chem.	Zeitschrift für physiologische Chemie (Hoppe-Seyler's)
Zentrbl. Physiol.	Zentralblatt für Physiologie

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