ON THE ACTIVE PRINCIPLE OF RENNET, THE SO-CALLED CHYMOSIN.

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The study of the formation of cheese from milk by means of rennet extract has always been a very inviting one. While the cheese manufacturers endeavored to find out how to obtain a very active rennet extract, and for a number of years manufactured it themselves, they were careful at the same time to study the conditions under which such extracts acted. Scientific chemists in their researches, added, as a matter of course, the critical inquiry into the nature of the active principle, its preparation and the manner of its chemical action.

At first sight the chemist is inclined to compare. Glancing at the curdling of milk he finds that a comparatively small quantity of the curdling substance acts upon a relatively enormous quantity of liquid with a given effect. He knows of similar actions more or less unexplained in the vast territory of general chemical reactions, I mean those which in former times were called "catalytic," and which, at present, are generally known as "contact" actions. I recall the formation of ammonium nitrite from ammonia and air in the presence of a glowing platinum spiral, or of formaldehyde from methyl alcohol, under similar conditions. He understands that in these cases the contact substance in a glowing state acts as a conveyer of oxygen. In the general case of etherification the sulphuric acid is no longer considered as a "contact" substance, since we know that the part it plays in these actions is that of taking up the elements of water with which it dilutes itself, from two different chemical compounds containing them. The study of this latter case has contributed much to the present tendency to assume that all the so called contact actions, when sufficiently studied, will prove to be simple chemical reactions under peculiar conditions.

From a chemical standpoint we are not yet clear as to the peculiar action of rennet upon milk ; more than that, we have not yet studied the chemically active principle so as to be familiar with it as we are with sulphuric acid, or even with glowing platinum.

In our case matters are, moreover, complicated by the fact that several substances have the peculiarity of curdling milk, such as certain juices extracted from plants*—that a precipitation of casein, by means of acids, different from the real curdling of milk by rennet may take place and confuse the observations; and finally, that the state in which the casein is actually contained in milk (let us call it the physical state), seems to be a very peculiar one, perhaps similar to glue, when we speak of its colloid nature to absorb water and swell with it, so that the entire fact of the curdling of milk by means of rennet cannot be expected to offer us simple conditions of observation.

Since we do not feel justified in assuming that the active principle in milk coagulation is the same in an extract of the stomach of ruminants and in the juices of certain plants, we are led to suppose that different substances react here in a similar manner upon one and the same body. By means of such conclusions we may succeed in devising correct experiments for the solution of the question before us.

Let us first see then how far we have succeeded in this up to the present time.

We find that the coagulation of milk by means of rennet depends upon the quantity of rennet added, as far as the practically important fact of rapidity of coagulation is concerned. This is not surprising if we consider that a larger quantity of active substance attacks the body upon which it is made to work, over a larger area—

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* A. Mayer proved that the action of the extracts of *Cirsium Arvense Oxalis Acetosella, Rumex Patienta, etc.*, coagulated milk by force of their acidity; while *Cynara Scolymus* (artichoke) and, according to Wittmack, also *Carica papaya* and *Ficus Carica* curdled *per se.*

attacks it at more points at once. If it were possible to regulate the action of sulphuric acid upon alcohol in a corresponding way, we should, doubtless, find within certain limits, a similar relation: also, the temperature at which the rennet acts upon milk is a factor upon which the result depends. The individual character of the milk and the presence of certain foreign substances added to the same, are included in the conditions under which milk will be coagulated by rennet.

Concerning the quantity of rennet with respect to the swiftness of coagulation, Soxhlet's* experiments are of interest. He found that the same rennet and the same milk reacted upon each other in forty minutes, if the proportion was one of rennet to ten thousand milk ; but in two minutes and six seconds, if that proportion was one to five hundred. By means of a number of such experiments it was found that the time which it takes to coagulate milk, at the same temperature and in equal quantities, is inversely proportional to the quantity of rennet used. These experiments give us the means of determining analytically the real value or contents of a given rennet extract in active coagulating power, as we shall see later.

It seems that the manufacturers of cheese, at least in this country, do not attribute much value to these facts ; they find that a certain amount of rennet is necessary to curdle the milk, and they are satisfied with this.

The maximum power of coagulation lies between + 38° and + 40° C. A temperature of + 70° C. entirely destroys the activity of rennet upon milk.

Concerning the individual character of milk and its influene upon coagulation, it has been found that the relative proportion of casein to water in milk is of the greatest influence. Thus A. Mayer found that milk which coagulated in 25 minutes in a jelly-like state, took 30 minutes for precipitation in the same state when five per cent. of water were added, it took 36½ minutes, and the precipitate showed a finely flocculent form, when the dilution with water had been increased to ten per cent., and at twenty per cent.

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* Milch-Zeitung, 1877, page 513.

the time was 73½ minutes and the form of the precipitate was also flocculent.

It is not difficult to understand that if the milk of one animal contains the caseïn and the calcium salts in a different proportion to water than that of another animal, the coagulation will be accordingly influenced. The reason why a certain apparently normal milk will not coagulate, is still unexplained, but seems to be dependent upon foreign substances contained in the same. As far as the reaction of milk is concerned, it has been found that the coagulation is at its height if the milk reacts acid, lowest when alkaline and intermediate when neutral.

The foregoing remarks have outlined the peculiar *conditions* under which rennet and milk react upon one another more or less favorably. We now have to approach this reaction itself somewhat more closely.

What is the difference for example between skimmed milk, before and after successful coagulation? Before coagulation we had a peculiar, uniform liquid containing casein, albumen, peptones, sugar of milk and salts including the important calcium phosphates. After coagulation we have the casein combined (or mixed) with a certain amount of calcium phosphate as a precipitate and the rest of the salts, the albumen, peptone and the sugar of milk constitute the whey. This result was obtained by the addition of a small quantity (one part) of rennet to, as I will show further on, sixty thousand parts of milk. The immensity of this reaction reminds of the sudden crystallization of certain salts in supersaturated solutions upon introduction of a minute fragment of the same salt in the solid state, but we see at once that we cannot continue to draw a parallel here. We introduce into the milk a foreign substance, and only certain constituents of the milk are precipitated from sixty thousand times the quantity of the latter in comparison to the former. That milk contains its two most important substances, the fat and the casein both in a condition from which they are, the former by shock, the latter by rennet, *suddenly* and almost totally precipitated is no doubt an interesting one. The solidification of the milk fat may well be compared to the above mentioned crystallization from supersaturated solutions, particularly since my own experiments have satisfactorily proved to me that *milk* churns quicker if butter is added, a fact which I suppose is known also in dairy establishments, but for the explanation of cheese precipitation we have to look for other facts.

To my knowledge the first one who, though unsuccessfully, attempted to obtain the active principle of rennet extracts (rennet) was Deschamps. (*Dingler's Polytechnisches Journal* 1840, No. 78, P. 445). He thought that he had found the real active principle of rennet, to which he gave the name "chymosin." Deschamps' results were tested and contradicted by Dr. Husemann in Chur who found the chymosin prepared according to Deschamps' method to be nothing but a mixture, of the weakest coagulating power possible, and consisting mostly of calcium phosphate and mucous nitrogenous organic substances, so that it could not be the active principle of rennet.

Liebig, (*Chemische Briefe*, 1865, page 159); Hallier, (*Gährungserscheinungen*, 1867, page 39), and Soxhlet, (*Journal f. praktische Chemie*, vol. 6, page 29 to 38), differed in regard to the peculiarity of the action of rennet.

Liebig, in accordance with his general ideas on fermentation, thought that as soon as a decomposition of the mucous membrane of the stomach had begun, the membrane then had the faculty of transforming the sugar of milk into lactic acid, which then would neutralize the alkali which keeps the casein dissolved, thus precipitating this latter.

Hallier thought that only the organisms contained in calf's stomachs were the cause of coagulation and that microbes of *penicillium* would coagulate milk as quickly as rennet.

In Liebig and Hallier we see also on this subject quite general expressions of the two opposite ideas in regard to the cause of fermentation, characteristic of the period.

Soxhlet's conception was, that the difference between coagulation of milk by means of rennet and spontaneous coagulation was only to be found in the fact that under the influence of rennet it took place more quickly. According to him rennet would transform sugar of milk into lactic acid, by means of which the neutral alkaline phosphate contained in milk would be transformed into the acid salt. If now, consequently *that* definite proportion between neutral and acid phosphate be arrived at, in which the albuminoid (casein) is just kept in solution at low temperatures, and if then the temperature be raised, the albuminoid would be precipitated even if the liquid showed the alkaline reaction. That the acid reaction was also present was, of course, particularly remarked by Soxhlet.

We see that Soxhlet at that time did not admit a difference between coagulated and precipitated casein. We know to-day that in the first place cheese differs mainly from casein by its insolubility in water in which casein is readily soluble, then by its contents of calcic phosphate.

It seems to me, that of the many excellent investigators of this subject "Olof Hammarsten" has pushed the knowledge of milk coagulation by means of rennet farthest, and it was he, who for the first time really prepared the "*chymosin*" or "*lab*," as he calls it in a pure state," particularly free from its companion pepsin. Hammarsten's original report (*Upsala Läkareforenings Förhandlinger*, Vol. 8, 1872, pp. 63-86), was not attainable, but I have read an extract of it in Maly's *Jahresbericht über die Fortschritte der Thierchemie*, 2 Vol., 1872 (ed. 1874), page 118, *et seq.* Hammarsten's researches date sixteen years back. He begins by confirming Soxhlet's observation, that normal, new cow's milk reacts almost invariably amphoteric. He then points out that it is of the greatest importance for the study of the process of coagulation, to work with ferment solutions of great strength, so that the coagulation might take place within a few minutes, thus avoiding a formation of lactic acid which will set in after 1 to 2 hours, and which would lead to a complex result from which no conclusions could be drawn, as he shows further on. He prepares infusions of the mucous membrane of calf's stomach by means of $\frac{1}{10}$ to $\frac{2}{10}$ per cent. hydrochloric acid and states that by 1 c.c. of such an infusion, which may have been previously neutralized or even rendered slightly alkaline, 25 c.c. of new milk will be curdled in two minutes at 36 to 38° C. The reason for preparing a solution by means of hydrochloric acid will be given later.

He then made the following experiments. New cow's milk that had been rendered slightly alkaline by the addition of sodium hydrate was curdled within 4 to 10 minutes by means of an infusion of neutral reaction. The coagulation was complete, so that not a trace of casein could be found in the whey, when tested by means of acids.

The reaction in these experiments was tested from minute to minute, but was not found to have changed either before, during or after coagulation. It was all the time an alkaline one.

Though these experiments speak in favor of the curdling of milk independently of lactic acid formation, Hammarsten furnished the decisive proof of this in another manner. He prepared casein solutions which were entirely free from sugar of milk. This is done by precipitating from milk by means of common salt, applied in a powdered state, the fat and casein and a little albumin, leaving the sugar of milk, most of the albumin and salts in the filtrate. The coagulum is washed with a concentrated sodium chloride solution. Then the curd is taken up with water and the butter is separated for the most part by strongly shaking the mixture. A filtration through linen and a new precipitation by means of common salt follows. The precipitate is pressed thoroughly between bibulous paper so as to free it from most of the sodium chloride, then dissolved in water and filtered through linen or filter paper of rather loose texture. Thus a casein solution is obtained which contains casein and fat but no sugar of milk. This solution remains unchanged in a warm state but upon addition of some of the ferment solution it curdles exactly like ordinary milk. This experiment succeeds not only with the above described impure infusion but also if the perfectly pure ferment, the description of which will follow, is used.

The conclusion to be drawn from these experiments is, that solutions of casein free from sugar of milk coagulate quickly with rennet, exactly as milk does, even with amphoteric or weak alkaline reaction. Therefore the curdling process is a something entirely independent of the formation of lactic acid.

A further proof regarding the non-significance of sugar of milk for the curdling of milk is furnished by Hammarsten by preparing a ferment from the mucous membrane of the calf's stomach which instantaneously coagulates milk or solutions of casein free from sugar of milk, while it is entirely without action upon sugar of milk alone. For this ferment, which is not identical with pepsin, Hammarsten proposes the name *lab.* It *is* evidently the real chymosin, which Deschamps supposed he had found.

The "lab" shows almost all the same reactions as the pepsin, but since it is easier to destroy lab than pepsin, it is more difficult to get pure lab solutions than pure pepsin solutions.

The method according to which Hammarsten prepared lab solutions, free from pepsin, consists in the first place in a fractional precipitation by means of magnesium carbonate or solution of lead acetate. Both ferments, pepsin as well as lab, are in this way mechanically precipitated, but for some reason it is possible, perhaps because the pepsin is more easily precipitated, to free liquids entirely from pepsin, while a goodly amount of lab remains in solution. Hammarsten thus prepared solutions, which at blood heat coagulated milk of neutral reaction within 1 to 3 minutes, while properly acidulated, they did not peptonize a little pouch of boiled fibrin.

In order to prepare the lab in a pure state, fractional precipitation by means of lead acetate was applied. The pepsin having been almost or entirely removed, lead acetate, with the addition of very little ammonium hydrate, is used for precipitation. The precipitate is decomposed by means of very dilute sulphuric acid and the acid solution, containing mere traces of albumin, is mechanically precipitated according to Brücke's method (used by B. for pepsin) with the aid of cholesterin, or by means of a watery solution of palmitin and stearin soap.

The method described yields good results only in case the original infusion was rich in lab. The liquids, diluted with ten times the amount of water, must still be so strong that 1 c. c. will coagulate 5 c. c. of milk at blood temperature in one minute.

The pure lab gives the following reactions according to Hammarsten: it does not show the xanthoprotein reaction; watery solution does *not* coagulate it on boiling, it is *not* precipitated by alcohol, nitric acid, tannin, iodine or normal lead acetate, but is precipitated by basic lead acetate. The impure lab on the contrary *is* precipitated by the same reagents.

Alcohol destroys the lab at neutral reaction only slowly. Increase of time of reaction and augmentation of alcohol also increase the amount of lab destroyed.

Fixed caustic alkalies act strongly to decompose lab, particularly at higher temperatures.

The resistance of lab to heat differs with neutral or acid reaction. A solution rich in lab may be momentarily heated to + 70° C., without losing all its ferment, whilst the same solution, acidulated with $\frac{3}{10}$ per cent. of hydrochloric acid loses all its ferment if momentarily heated to + 63° C. or even after heating it for forty-eight hours to + 37° or 40° C. Pepsin on the contrary is not so easily destroyed in acid solutions; we have therefore a comparatively easy means of preparing pepsin solutions free from lab by heating the acidulated liquid for a certain time up to + 40° C.

The only physiological action of lab, according to Hammarsten, is that upon casein, which it coagulates at neutral, acid, or alkaline reaction. The effect appears latest in alkaline solution, and a small excess of alkali is sufficient to destroy it altogether. The best action is obtained in acid solution, and it was shown by controlling experiments that acid to that amount *alone* would not be the reason of coagulation.

The addition of certain salts (as we have seen above) retards or even stops a coagulation altogether.

Sugar of milk is not changed by lab into lactic acid, nor is albumin digested by it.

Hammarsten puts the question : in which part of the mucous membrane of the stomach is the lab generated? He finds that the pyloric part is much poorer in ferment than the fundus. The stomachs of calves and sheep always showed the presence of lab, while those of the other mammals and birds often failed to contain it. The stomachs of fish hardly ever showed it.*

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* Journal of the Society of Chem. Industry, March, 1887, page 191 : F. Baden Benger states that Sir Wm. Roberts obtained it from the digestive organs of the fowl, and Baden Benger has extracted it from the stomach of the codfish.

But it was found that this variable behavior of the infusions depended upon the more or less strong spontaneous formation of acid, and a continued research taught that the mucous membrane of every animal heretofore studied in this regard, contains a water soluble substance which is not lab, but out of which lab is formed in a short time after addition of an acid. Experiments in this regard were performed with the infusion of a pike's stomach, which, by means of a titred hydrochloric acid was brought to an acidity of 0.1 per cent. After twelve to twenty-four hours the liquid was exactly neutralized, and a liquid was thus obtained which coagulated milk within a few minutes.

The fact that there exists a substance in the mucous membrane of the stomach, which, on addition of an acid yields lab, furnishes an explanation *why* acidulated water produces an infusion richer in ferment than neutral water.

Finally, the following statements are made by Hammarsten in regard to the coagulation of milk at acid reaction. An acid infusion of 0.1 per cent. hydrochorlic acid was divided in two portions. One half was heated for some time, using 70-80° C. in a closed vessel and, after cooling, five times its volume of new cow's milk was added. This mixture remained for hours liquid at the temperature of the blood, while at the same temperature the non-heated infusion, the same milk in the same proportion, coagulated instantaneously.

This shows that the ferments of the calf's stomach at acid reaction act very energetically, but the question to be answered was still this: is this action besides being due to the lab also to be attributed to the pepsin? His experiments permitted him to conclude that pepsin solutions, freed from lab in the above mentioned manner, when neutralized, showed no effect upon milk, while in acid solution there seems to be still a slight action present.

I shall have to show later on that my own experiments have forced me to draw the conclusion that pepsin free from lab, even in acid solution, does *not* coagulate milk.

Since the pure lab is without action upon sugar of milk, or albumin solutions containing sugar of milk, while the impure neutral rennet extract shows this reaction, Hammarsten is justified in concluding that the mucous membrane of the stomach contains a third ferment, which forms lactic acid. And it is possible to prove in a different manner the presence of such a substance. Both pepsin and chymosin (lab) can be destroyed by means of sodium hydrate solution, but the remaining liquid, free from either lab or pepsin, still converts sugar of milk quite energetically into lactic acid. Therefore a third ferment, forming lactic acid *is* contained in the mucous membrane of the stomach, and the frequent references to the fact that milk at coagulation turns acid are easily reconciled with these apparently diverging remarks. Because, if we work with a liquid poor in lab, the spontaneous acidulation of the milk, the action of the ferment which forms lactic acid, comes into play, and the milk may turn sour before coagulation. But if liquids rich in lab are used, which act strongly and rapidly, so the other ferment has no time to begin its action and the milk coagulates at amphoteric or alkaline reaction.

Hammarsten's conclusions are as follows. The mucous membrane of the stomach contains, beside pepsin, two ferments, the one, lab, the other the lactic acid forming one. Of these two, only the first named one has a specific action upon casein, while the latter exclusively attacks the sugar of milk. The reaction of this latter ferment only comes into play exceptionally when cheese is formed and *if* the casein is precipitated by this secondary factor, lactic acid, this is a chemical process non-identical with the formation of cheese.

Within the stomach of the living animal the possibility of a coagulation of milk by means of acid alone, seems only to be actually existing in the newly born animal. Hammarsten found in the stomach of young dogs, one to two days old, neither pepsin nor lab, not even in cases in which the stomachs were filled with coagulated casein and contained a strongly acid liquid.

A considerable length of time after these researches of Hammarsten, a patent was granted to M. Blumenthal in 1886, for the practical and wholesale manufacture of chymosin. The patentee describes his own method of preparation (U. S. Pat. No. 344,-433 as follows):

"I have found that chymosin, the active agent in milk, may be obtained from rennet or the stomachs of ruminants, and more especially that of the calf, which contains comparatively little pepsin, the chymosin preponderating ; in the stomach of the pig or sheep, on the the other hand, I have found that pepsin preponderates. Hence, if the chief product is to be chymosin, it will be found of advantage to employ the stomach or rennet of the calf as a raw material, and if pepsin is to be the chief product it will be of greater advantage to employ the stomach or rennet of the sheep or pig.

The treatment of the raw material being the same whether the stomach of the pig or that of the calf or other ruminant is employed, I will confine this description to the treatment of the stomach of the calf with a view to obtaining chymosin as a principal and pepsin as a by-product. The stomach of the calf is cut into small pieces and macerated or digested for about twenty-four hours in a solution preferably of common cooking salt, containing about 0.5 per cent. of salt, kept at a temperature of 30° C, more or less. The solution is then filtered, and a small amount of mineral acid is—such as hydrochloric, sulphuric, or phosphoric acid —mixed therewith in the proportion of about 0.1 per-cent. The reaction of the acid on the saline solution gives rise to a thick precipitate of mucous matter, which contains but traces of chymosin and no pepsin, the solution during the acidulation being preferably kept at a temperature of about twenty to thirty degrees C., as at that temperature the mucous matter agglomerates more rapidly or readily and may, in this condition, be easily separated from the solution which is effected only with the greatest difficulty otherwise. The filtered solution is again acidulated to the extent of about 0.5 per cent. of acid and pulverized cooking salt is added until a precipitate of the latter is formed.

This supersaturated, acidulated salt solution is now brought to a temperature of 25 to 30° C and kept at this temperature for two or three days under constant agitation and then allowed to rest for a day or so, the temperature being increased to 30 or 35° C. A separation then takes place in the form of a white flocculent substance, which floats on or in the solution, and may be readily collected on a filter, and then dried at a temperature of about 28° C. The substance separated from the solution is the pure zymotic product called 'chymosin.' It is an amorphous, white, gelatinous substance greatly resembling aluminium hydrate, is without taste or smell, and soluble in water, forming a limpid or clear solution. It may be kept for years without deterioration, and is not injured by temperatures reaching as high as 35° C. The remaining saline, supersaturated acid liquor or mother liquor free from chymosin does not cause milk to curdle when mixed therewith, the active agent, chymosin, which alone produces this reaction in milk, having been eliminated. The pepsin held in solution in this mother liquor may now be separated therefrom by neutralizing the solution with an alkali and agitating the same for some time, the pepsin being obtained as a gelatinous precipitate insoluble in the concentrated neutral salt solution but soluble in the acid salt solution.

Pure pepsin may also be obtained from the so-called 'impure pepsin essence,' or 'extracts of rennet of commerce,' by acidulating these extracts or the solution of the dry rennet with one of the mineral acids above referred to in the proportion of about 0.2 per cent. of the acid, whereby the impurities are precipitated. These are removed by filtration, an excess of cooking salt added, as described, to separate the chymosin which is collected, and the remaining solution is neutralized to precipitate therefrom the pepsin. In this case, also, chymosin and pepsin are separately obtained free from any albuminous mucous or other impurities."

Here then is offered an entirely new way of preparing the active principle for milk coagulation, for which the inventor very aptly uses the name chymosin.

Experiments which I performed myself at different times, working exactly according to the directions of this patent convinced me of its value. Working on a small scale and using about five calf's stomachs at a time, the preparation of chymosin was performed successfully. The only difference from the results, as stated in the patent, is a very agreeable one, *i. e.*, "that on so small a scale the chymosin is obtained immediately after supersaturation of the acid solution by common salt (Na Cl). A snow white substance accumulates then, floating upon the liquid. It is even preferable at once to skim off this substance from the liquid and to put it on porous clay or a piece of glass, because it easily might be lost in the three to four days' consecutive standing of the liquid, either because the small amounts of chymosin obtained sinks down to the surface of the salt and is thus rendered inaccessible, or because under such conditions it might decompose." I advise the removal of the white surface at once and then letting the liquid stand to see whether the crop will be augmented after a few days, as the patent indicates.

Comparing the reactions that this substance yields with the reagents which Hammarsten used for characterizing his lab, I found each and every one reaction to be also correct for Blumenthal's chymosin, so that I am inclined to consider the two substances as identical.

Since I referred above to Hammarsten's remark, that it seemed as if pepsin still and *eo ipso* had a slight power of coagulating milk, I made the following assay. Merck's pepsin was taken and freed from chymosin according to the above mentioned patent. The resulting pepsin, which was perfectly odorless and dissolved to an almost absolutely clear liquid, digested albumen actively, while in a very slightly acid solution (of HCl) it did not curdle milk within forty-eight hours and this same milk would always curdle in the shortest time when at the end of the above mentioned period of the experiment chymosin was added.

It seems, therefore, that according to the patent, the separation of chymosin from pepsin is more thoroughly arrived at than in Hammarsten's fractional precipitation and consecutive heating of the substance to a temperature at which it is supposed that chymosin is destroyed. Besides, it would follow, that under such conditions (the pepsin of Hammarsten actually showing the power of curdling), the chymosin is not fully destroyed.

Pure pepsin, free from chymosin, does NOT *curdle milk according to my experiments.*

On the other hand, I have to confirm the statement that the time of curdling milk by chymosin is shortest in acid solution and longest if the solution is purposely made alkaline.

In regard to the systematic analysis of chymosin products as they are offered to the consumer, I intend to present later, if time permits a shortpaper. I only wish here to point out the fact that great care has to be taken in determining the nitrogen, *i. e.* the albuminoids. If the analysis be performed according to the Kjeldahl method, results are obtained which do *not* permit multiplication of the nitrogen found, by 6.25, to indicate albuminoids. It will almost invariably be found, that the quantity of albuminoids, so calculated, is greater than the total quantity of organic substance, the "loss by ignition." If, on the other hand, the soda lime determination is used, the result of multiplication by 6.25 remains within reasonable limits.

But whether the truth is revealed by the soda lime determination, or whether the chymosin is so constituted as not to permit the ordinary calculation of nitrogen found in it to albuminoids by applying the factor 6.25, remains still a mystery to me. The Kjeldahl method being approved and rendering good service in so many cases, may here be misleading, but we certainly know that the soda lime method is not an infallible one and thus we stand before the query: does the chymosin belong to the albuminoids proper?

I intend to approach this question by preparing chymosin entirely free from chloride of sodium etc., which requires time and is by no means an easy matter, and then to perform the nitrogen determination by means of cupric oxide.

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