THE PROTEIDS OF MILK.

By W. D. HALLIBURTON, M.D., B.Sc., *Professor of Physiology, King's College, London.*

(*From the Physiological Laboratories of University College, and King's College, London.*)

In addition to the principal proteid of milk, known as casein, there have been at various times other proteids described by different observers; these may be enumerated as follows:—an albumin called lact-albumin, a globulin called lacto-globulin, a proteid which has been called lacto-protein, a proteid which appears on the occurrence of the rennet fermentation, and which has been called whey-proteid, and lastly peptone, which term includes both true peptone, and substances of the proteose or albumose class.

It thus appears, that there may be as many as six or seven different proteids in milk if all these statements are to be taken as correct, and it was with a view to testing their accuracy that the present investigation was undertaken.

The only milk I have examined has been cow's milk; it will be convenient to describe my experiments under the head of the various proteids which have just been enumerated.

1. Casein.

This word is differently used by various writers. Most appear to employ it indiscriminately for the proteid in the milk itself which can be made to clot with rennet, and for the curd which is the result of the rennet action. It appears to be most important that a distinction should be drawn between these two substances; the proteid in the milk itself is apparently partly dissolved, and partly in suspension there; the curd is quite insoluble in the liquid medium (whey) from which it separates. Professor Foster alone, so far as I have been able to find, recognizes this distinction by giving different names to these two substances; he reserves the term casein for the proteid which is present in

the milk, and the insoluble casein or curd, produced by the ferment action of rennet he calls tyrein[1]. It has been my custom in my lectures for some years past to distinguish the two proteids by different names; the proteid in the milk I have called caseinogen, and that which composes the curd, casein. Though the actual nomenclature, ultimately adopted, matters but little, I am disposed to think that the terms I have suggested have certain advantages over those proposed by Professor Foster. Both casein and tyrein mean the same thing, one being derived from the Latin *caseus*, the other from the Greek τυρὸς (cheese); the words caseinogen and casein, on the other hand, have different meanings, which convey to the mind the relationship of the two substances to one another, and are moreover framed on the same pattern as the names of other substances like fibrinogen and fibrin, myosinogen and myosin, which bear similar relationships one to the other. In each of these three cases, we have a soluble proteid (-ogen) the precursor of a less soluble one, and the change is in all three instances the result of the action of a ferment.

Casein is obtainable by one process only, namely by the action of rennet on caseinogen in the presence of certain calcium salts (Hammarsten). Caseinogen on the other hand can be obtained from milk by two methods; it can be precipitated either by saturating the milk with neutral salts like magnesium sulphate or sodium chloride, or by adding a certain amount of acid, preferably acetic acid, to the milk. I have found that by a combination of these two methods, it is possible to obtain very easily a preparation of caseinogen which is perfectly free both from fat and from calcium salts.

Milk is saturated by shaking it vigorously with excess of finely powdered magnesium sulphate crystals; the precipitated caseinogen is on standing carried to the surface of the mixture by the entangled fat globules, and is then collected on a filter. It is washed with a saturated solution of magnesium sulphate, until the washings give no evidence of the presence of albumin. Distilled water is then added to the residue on the filter, the caseinogen, in virtue of the magnesium sulphate still adherent to it, dissolves, and the solution of caseinogen which passes through the filter is collected; the fat is left undissolved on the filter. The solution of caseinogen so obtained is then again saturated with magnesium sulphate and the process repeated. The solution that is obtained in this way does not however clot with rennet, on account of the large amount of salt present which exercises an inhibitory influence on this as on many other ferment actions. The

[1] *Text-Book of Physiology*, 5th ed. p. 376.

process of dialysis to get rid of the salt is a very lengthy one, and the following will be found better. Excess of acetic acid is added to the solution; the precipitate of caseinogen so formed is collected, washed with dilute acetic acid, and lastly with distilled water; it is then dissolved in a weak alkali, preferably lime water, and once more precipitated by acetic acid, washed and redissolved as before.

The solution so obtained is an opalescent one, and on the addition of rennet no coagulation occurs; the addition of a few drops of 0·5 per cent. phosphoric acid however leads to the formation of the necessary amount of calcium phosphate, and the subsequent addition of rennet produces a firm curd, which soon, especially at 40° C. contracts, squeezing out a clear whey.

Dr Ringer has among other points taken up the question of the influence of calcium and other salts on the coagulation of milk and other fluids, and has already published two preliminary communications on the subject[1]. Until the full papers have appeared I do not feel at liberty to offer any further remarks on this portion of the subject, except to say that the similarity of caseinogen and casein to myosinogen and myosin appears to me to be exceedingly close.

Another question, which appears to me to be important to settle, is the place that caseinogen and casein should occupy in the classification of proteids.

It is easy to give casein its proper place; it should be classed with fibrin, myosin, and gluten as proteids more or less insoluble, which are produced by ferment actions from other proteids of more soluble nature.

It is by no means so easy to assign caseinogen its proper position. The precursors of fibrin, myosin and of gluten are globulins. Is caseinogen also a globulin? It is generally classed as an alkali-albuminate and to me it seems with insufficient reason; for alkali-albumin is not coagulated by rennet, and is easily soluble in dilute acids. Still caseinogen is not a globulin, for its solutions are not coagulated by heat. A perfectly neutral solution of caseinogen in dilute magnesium sulphate or sodium chloride solution when heated to 50° C. becomes opalescent. This opalescence disappears on cooling. If the solution is heated to a higher temperature, the opalescence increases up to 80° C. Beyond this temperature it does not increase, but flocculi of coagulated proteid are never deposited. If the solution is heated only just up to 80° the opalescence can be made to disappear by cooling the solution; but if

[1] *Proceedings of the Physiol. Soc.* 1890, pp. i. and iii.

the solution is kept at 80° C. for any length of time, or if it is heated, even momentarily, above 80° the cloudiness is rendered permanent, and does not disappear on cooling[1]. If instead of a neutral, a very faintly acid solution is employed, (the small amount of caseinogen precipitated by the acid being filtered off), the same series of phenomena is observed; opalescence however begins at a somewhat lower temperature (about 40° C.) and becomes permanent also at a somewhat lower temperature (70°—75° C.) than is the case with a neutral solution.

Caseinogen has doubtless certain resemblances to alkali-albumin; it has also certain resemblances to the globulins, for instance in the way it may be precipitated by neutral salts. It however differs from both, and therefore should not be classified with either. It appears to me to be a proteid with such marked characteristics as to constitute a class by itself, and this class will be one intermediate between the albuminates and the globulins.

2. Lact-albumin.

The albumin of milk (lact-albumin) is in its general characters similar to serum-albumin. It is coagulated at about the same temperature, but its specific rotatory power is different. Sebelien[2] gives its specific rotatory power as $(a)_D = -36°$ to $-37°$, and concludes from this that lact-albumin is not identical with serum-albumin, which has a specific rotatory power of $-56°$.

My own observations on lact-albumin have shown that there are other slight differences between the two albumins, and therefore support Sebelien's view of their non-identity. These observations have been in two directions, viz:—(*a*) heat-coagulation; and (*b*) precipitability by certain salts.

(*a*) Heat-coagulation. The lact-albumin was obtained in the following way; milk was saturated with magnesium sulphate, and the precipitate so produced, consisting of caseinogen and entangled fat, was removed by filtration. The filtrate contained the albumin, the coagulation temperature of which was determined without further treatment. In some specimens, however, the excess of salt was first removed by dialysis, and in others the proteid was precipitated by the addition of sodium sulphate in excess; the latter precipitate was washed and redissolved by the addition of distilled water; in all three cases,

[1] I find this fact has been also noted by Dogiel, *Zeitsch. physiol. Chem.* IX. p. 591.

[2] *Maly's Jahresbericht*, Vol. XV. p. 184. Original paper in Swedish.

however, the average results of heat-coagulation determinations were practically identical. It was found that when examined in a faintly acid solution with the precautions I have previously described[1], that opalescence occurs about 70°, and the separation of distinct flocculi appears at 77° C. The greater quantity of the serum-albumin obtained in a similar way from serum separates out at a somewhat lower temperature (73°), but I do not consider this difference sufficient in itself to show that the two proteids are not identical. It is rather in the manner of the precipitation, than in the temperature at which it occurs, that a very striking distinction between serum-albumin and lact-albumin is noticed.

If a solution of serum-albumin is heated gradually, opalescence sets in rather below 70°, and a flocculent precipitate occurs at 73°, the precipitate being immediately dense, and if the temperature is maintained at 73°, it increases somewhat in amount for the succeeding few minutes. If this is filtered off, and the filtrate again heated to 73°, a small amount of precipitate may again occur; if however the temperature of 73° is maintained for ten minutes, no further precipitation occurs at that temperature, however long it is kept up: if the temperature is then raised four or five degrees, a fresh precipitation occurs, and a third one above 80° C. On these grounds I formerly concluded that serum-albumin may be by fractional heat-coagulation differentiated into three proteids, which I called α, β, and γ serum-albumin[2].

The phenomena obtained with a solution of lact-albumin are however different. If the solution is kept at a temperature below 77°, no separation of flocculi occurs however long the heat be applied. At 77°, the separation of flocculi commences; there is however no sudden appearance of a dense precipitate at 77°, but the density of the precipitate increases gradually and very slowly; after half an hour the precipitate however does not apparently increase; if it is filtered off, and the filtrate heated to 77° momentarily, no fresh precipitate occurs, and if the temperature is rapidly raised, a second precipitate occurs above 80°. I therefore at first thought that fractional heat-coagulation demonstrated in lact-albumin, as in serum-albumin, the existence of more than one proteid. This I soon found was a mistake; for if the filtrate is kept at 77° for a second half hour, a precipitate forms slowly at that temperature, and after this lapse of time, does not

[1] This *Journal*, Vol. v. p. 155. [2] *Ibid.* p. 158.

apparently increase in amount; if however the precipitate is filtered off, and the filtrate heated for a third time to 77°, a fresh precipitate forms in exactly the same way, and this may be repeated several times more. Ultimately, however, with patience, the whole of the lact-albumin may be separated out at this temperature, and the final filtrate gives no further precipitate on heating even to boiling; a small amount of proteid is however still present in it, as it gives the xanthoproteic reaction.

The albumin of milk is therefore so far as the method of heat-coagulation will decide the question, a single proteid, coagulating with very remarkable slowness at the temperature of 77° C. At a higher temperature the precipitation occurs more quickly, but even at 100° C. it is half an hour or more before a filtrate is obtained free from coagulable albumin.

I may here take this opportunity of making a reply to Dr Haycraft[1], who has recently, in a very courteous manner, made certain criticisms regarding my work on serum, and regarding certain somewhat similar observations made by Mm. Corin and Berard[2] on the proteids of white of egg.

Haycraft's criticisms relate to the method of fractional heat-coagulation, and the sum and substance of them may be put concisely as follows:—The coagulation point of a proteid is considerably raised by diluting its solution, and a very dilute solution may not coagulate, even on boiling. Without doubting the possibility of fractionating some proteids, this factor in determining the temperature of coagulation has been neglected by Corin and Berard and by Halliburton, and therefore a doubt is cast upon the results they have obtained by fractional heat-coagulation. In order that the proteids separated may be considered distinct from one another, it is necessary that other differences besides that of mere heat-coagulation should be demonstrated to exist. It is thus possible that serum-albumin and egg-albumin may be single proteids, and the fact that various precipitates at different temperatures are obtainable, can be explained in one of two ways: either that the heat when applied for a long time alters the character of the proteid in solution so that its temperature of coagulation is heightened, or that the result is simply the effect of dilution; a solution of serum-albumin, for instance, is raised to 73° C., and the precipitate which occurs is filtered off; that left in solution is now more diluted, hence its coagulation temperature is higher.

These criticisms are exceedingly valuable, and considerably shake my former opinion that serum-albumin consists of more than one proteid. They show that in determining the temperature of coagulation of a proteid, two

[1] J. B. Haycraft and C. W. Duggan, *Brit. Med. Journal*, Vol. I. p. 167, 1890.

[2] Corin and Berard, *Arch. de Biol.* Vol. IX. p. 1.

chief factors have to be taken into account in addition to the degree of heat applied, namely the amount of acidity, and the concentration of the solution. The first of these I recognised, the second I missed. Then I must again freely admit that no differences other than those of coagulation temperature can be demonstrated to exist between the three varieties of serum-albumin; it is therefore unsafe at present to affirm that these are distinct from one another, seeing what a variable factor is the temperature of heat-coagulation. Since the publication of my paper on the serum-proteids, Kauder[1] has arrived at much the same conclusion concerning serum-albumin as I did, only by a different method, namely that of elementary analysis; he finds that the discrepancies between different analyses are too great to come within the limits of experimental errors, and considers that they can only be accounted for by the presence of more than one proteid in varying admixtures.

There is thus a certain amount of evidence that serum-albumin is not a single substance, and evidence that it is a single substance is at present wanting. Granting, however, for the sake of argument, and in view of Haycraft's experiments that it is a single substance, the question arises, what explanation can be given of the fact that various precipitates are obtainable at different temperatures.

Haycraft has suggested three possible explanations, viz:—that the coagulation point rises in virtue of:—

i. The solution becoming continually more dilute;
ii. Its becoming less acid;
iii. Changes which are being produced in the proteid itself, by the action of the high temperature to which it is subjected.

Haycraft appears to lay most stress on the first of these three; I am inclined to regard the third as the most important; the second may at once be discarded as care should be taken, as I have before pointed out, to keep the degree of acidity the same throughout the course of an experiment. Neumeister[2] has shown that the action of acidified hot water will in a very short space of time convert albumin partially into primary albumoses; it is therefore exceedingly probable that less profound changes resulting in the raising of the coagulation point will be produced in a similar way. I cannot say that my own experiments on the effect of dilution entirely confirm Haycraft's; care must of course be taken that the acidity is kept constant; if this is done, the amount of proteid in solution makes very little difference in its coagulation temperature; I may here refer to the paper I published on the serum proteids of certain lower vertebrates[3]; the amount of serum-albumin is there quite insignificant, it is not differentiable into several

[1] *Arch. f. experim. Path. und Pharmakol.* Vol. xx. p. 411.
[2] *Zeitschr. f. Biol.* Vol. xxiv. p. 272.
[3] This *Journal*, Vol. vii. p. 319.

proteids by heat coagulation, and its temperature of coagulation is 73°, the same as that of the α-albumin of mammalian serum; whereas if Haycraft's suppositions be correct, one would have expected that the temperature of coagulation in these dilute specimens would be nearer to that of the γ-albumin. I may also refer Dr Haycraft to some experiments with rabbit's and sheep's serum recorded in this *Journal* (vol. v. pp. 179—180) in which the diminution in the amount of proteid present did not alter the coagulation temperature. Since the appearance of Haycraft's paper I have made similar experiments with the same result.

How is it that Haycraft and myself have arrived at opposite conclusions on this point? Simply I imagine because Haycraft has not taken the precaution to keep the grade of acidity in his solutions constant; in fact he expressly states that his solutions of egg-albumin were always alkaline in reaction; his solutions of serum-globulin appear to have been neutral, and in this case dilution had very little influence in raising the coagulation temperature. With regard to acid solutions he speaks as follows:—"We have not made so systematic an investigation upon the effect, on its coagulating point, of diluting acid solutions of albumin, but we have assured ourselves that the more dilute solutions coagulate at a higher temperature"[1], one or two illustrative experiments with egg-albumin are then given. If however Dr Haycraft makes a systematic investigation as I have done on this most important point, I feel assured that his conclusion will be the same as mine; namely that although dilution of alkaline solutions of proteids raises their coagulation point, yet if those solutions be neutral, or better still, have a faint *and constant* degree of acidity, the disturbing influence of dilution is reduced to a minimum.

I still believe that fractional heat-coagulation is a most valuable method of indicating the presence of more than one proteid in solution. Without confirmatory evidence of other differences between the proteids, it is, however, not in itself an absolute means of distinction between them. It is least liable to give trustworthy results when the temperatures at which the individual proteids coagulate are, as in the case of serum-albumin, high, or close together. It is most valuable when the temperatures of coagulation are low, and far apart; for instance in the separation of fibrinogen from serum-globulin, in the separation of the proteids of the muscle plasma, of lymph cells, and of the proteids which can be dissolved out of kidney cells, liver cells, and nervous tissues. In all these cases (some of which are not yet published) I have obtained other evidence of differences between the individual proteids, quite apart from their temperatures of heat-coagulation.

[1] *Proc. Roy. Soc. of Edinburgh*, p. 381, 1888—9.

I am even inclined to think that there is still something to be said for the view I formerly expressed that serum-albumin is not a single proteid; and I would especially mention the following points that bear out this view:—

i. The remarkable constancy of the coagulation temperatures.

ii. The fact that other proteids coagulating above 70° (such as serum-globulin and lact-albumin) cannot be similarly differentiated, as they would be if all the facts of fractional heat-coagulation were explicable by Haycraft's hypotheses.

iii. The confirmatory experiments on elementary analysis made by Kauder.

(*b*) Precipitation by salts. It was until quite recently believed that one of the distinctions between globulins and albumins was that the former are precipitated by saturation with neutral salts, and the latter are not. We now know that this is only true in reference to certain neutral salts like magnesium sulphate and sodium chloride, but with regard to certain other salts like ammonium sulphate[1], sodio-magnesium sulphate[2], and potassium acetate[3], the difference is only one of degree. These three last mentioned salts precipitate both globulins and albumins; they however precipitate the globulins more readily than the albumins, complete saturation not being necessary to precipitate the former.

In the paper already referred to[4] I showed that saturation with certain pairs of salts will precipitate serum-albumin from its solutions. In the case of magnesium sulphate and sodium sulphate, this is due to the formation of the double sulphate of magnesium and sodium ($MgSO_4 . Na_2SO_4 . 6H_2O$), and in other cases there is also probably a formation of similar double salts.

I have now found that although lact-albumin is in the main similar to serum-albumin in this particular, it differs from it slightly in being only incompletely precipitated from its solutions by sodio-magnesium sulphate, or by double saturation with magnesium sulphate and sodium chloride.

The principal salts now most generally employed for the separation of proteids act in the following way on lact-albumin:—

i. Magnesium sulphate. This does not precipitate lact-albumin even

[1] J. Wenz, *Zeit. f. Biol.* XXII. p. 1.
[2] This *Journal*, Vol. v. p. 181.
[3] *Ibid.* p. 191. [4] *Ibid.* p. 177.

when added to milk to complete saturation; this is known because a solution of the precipitate produced by this means does not coagulate on boiling.

ii. Sodium chloride. This salt has also no precipitating action on lact-albumin.

iii. Sodium sulphate. This salt has little or no power of precipitating caseinogen, much less that of precipitating lact-albumin.

iv. Ammonium sulphate. On adding this salt to saturation to milk, and filtering, the filtrate is absolutely proteid-free; saturation with ammonium sulphate therefore, completely precipitates lact-albumin.

So far, serum-albumin and lact-albumin are alike.

v. Sodio-magnesium sulphate. On saturating milk with this salt and filtering, the filtrate always contains a small quantity of an albumin, coagulating at 75°—80° C. In other words, saturation with this salt only incompletely precipitates lact-albumin.

vi. Double saturation with sodium sulphate and magnesium sulphate. The result is the same as in v. If milk is saturated with magnesium sulphate and filtered, and the filtrate containing the lact-albumin is then saturated with sodium sulphate, an abundant precipitate of lact-albumin is produced. On filtering this off, the filtrate is found to contain a small quantity of lact-albumin; and it is not possible to precipitate this by prolonged shaking of the filtrate with excess of the two salts.

vii. Double saturation with sodium chloride and magnesium sulphate. What has been said with regard to sodium sulphate and magnesium sulphate, is true *mutatis mutandis* for sodium chloride and magnesium sulphate. It is necessary however to notice in view of what has to be said in reference to lacto-globulin (see next section) that the result is the same, no matter what the order is, in which the salts are used. If the milk is saturated with magnesium sulphate first, caseinogen is precipitated, and filtered off; on saturating the filtrate with sodium chloride, the lact-albumin is incompletely precipitated. If the milk is saturated first with sodium chloride, caseinogen is as before precipitated, and filtered off; on saturating the filtrate with magnesium sulphate, lact-albumin is precipitated incompletely.

We thus see from v, vi, and vii that lact-albumin differs somewhat in its solubilities from serum-albumin, which is completely precipitated from its solutions by the salts in question.

3. Lacto-globulin.

This proteid is described as occurring in milk by John Sebelien[1]. He states that casein (caseinogen, as I should call it) is completely

[1] *Maly's Jahresb.* Vol. xv. p. 184 (abstracted by O. Hammarsten). Original paper in Swedish.

precipitated by saturating milk with sodium chloride. This is filtered off. The filtrate is then warmed to 35° C., and a very small precipitate of a proteid which is thus produced, but which was not further investigated, is then filtered off. The filtrate from this is, lastly, completely saturated with magnesium sulphate, and a flocculent precipitate of a proteid then occurs. This proteid appears to be identical in its properties with serum-globulin; it is coagulated at 74°—76° C. and the name lacto-globulin is given to it.

As already stated, my own experiments have shown the truth of the main fact here described. If one saturates milk with sodium chloride, filters off the caseinogen so precipitated, and then saturates the filtrate with magnesium sulphate, a precipitate of a proteid is obtained. My interpretation of this fact is however different from that of Sebelien. Sebelien has overlooked the fact that saturation with the two salts is capable of precipitating albumin as well as globulin. The proteid which Sebelien calls lacto-globulin is in reality lact-albumin. If it be collected on a filter, washed with water saturated with the two salts, and then dissolved in water, and subjected to dialysis, it remains in solution after the removal of the salts, whereas a globulin would be precipitated under these circumstances. In order to obtain the precipitate described by Sebelien, it is immaterial which salt is used first. The salt with which the milk is saturated first, precipitates the caseinogen; the salt with which the filtrate from this is saturated, precipitates the albumin. The precipitation of the albumin is however incomplete, and so differs from what occurs in the case of serum-albumin.

I have attempted to solve the question, whether a globulin exists in milk, in another way. Milk is saturated with magnesium sulphate; if a globulin were present this would be carried down with the caseinogen; but I have never found that such a globulin is present, as the precipitate when redissolved and heated does not coagulate on heating.

My conclusion is, therefore, that milk contains no globulin. This, however, does not cast any doubt on Sebelien's statement[1], that the imperfectly formed milk secreted during the first few days of lactation, and which we call colostrum, contains abundance of globulin. I have not myself had the opportunity of examining colostrum, but Sebelien's analyses appear quite conclusive. He ascertains the quantity of proteid precipitable from colostrum by acetic acid (caseinogen); and in another portion the quantity precipitated by saturation with magnesium sul-

[1] *Zeit. physiol. Chem.* Vol. XIII. p. 135.

phate (caseinogen + globulin); the difference between the two, which is often considerable, gives the amount of globulin.

4. Lacto-protein, proteoses, and peptone.

Many observers have stated that milk contains a small quantity of a proteid which is either a peptone, or a peptone-like substance. The name lacto-protein has been given to it. Most of these observations were made some years ago, before the properties of the proteoses and peptones were carefully distinguished from one another, and before ammonium sulphate was known as a reagent for the separation of true peptones from other proteids.

The principal of these earlier researches are the following:—

Schmidt-Mulheim[1] quotes earlier papers by Syubotin, Hofmeister, Arnold, and Kirschner, and confirms their statement that milk contains peptone, which increases when milk is allowed to stand. The casein is first removed and filtered off; the albumin is then precipitated from the filtrate by adding a small quantity of acetic acid and boiling; this is filtered off. A third proteid is found in the final filtrate which is not coagulated by boiling, and which gives the biuret reaction; this is regarded as peptone, and the amount present averages 0·13 per cent.

E. Pfeiffer[2] precipitates casein by acetic or hydrochloric acid, the albumin by boiling the filtrate, and a third proteid (averaging in amount 0·7 per cent.) is left in solution and is precipitable by tannin.

J. Schmidt[3] states that peptone is present in milk in the merest traces, and that the substance which has been mistaken for peptone is hemi-albumose. He precipitates casein by acetic acid, and albumin by boiling; the third proteid, of which there is a considerable amount, being left in solution.

F. Hofmeister[4] precipitates the proteids from milk by acidifying and boiling, then by adding lead hydrate to the filtrate; no peptone is found in the second filtrate.

E. Duclaux[5] states that milk contains three varieties of proteid:— (1) casein in the solid form; (2) casein in the colloidal condition and removable by filtration through porcelain; (3) lacto-protein under which term he includes the soluble proteids (albumin *plus* peptone).

A. Dogiel[6] like Hofmeister denies that any true peptone exists in

[1] *Pflüger's Archiv*, Vol. xxviii. p. 287; Vol. xxx. p. 602.
[2] *Zeitschr. f. anal. Chemie*, Vol. xxii. p. 14.
[3] *Maly's Jahresb.* Vol. xiv. p. 175.
[4] *Zeit. physiol. Chem.* Vol. ii. p. 288.
[5] *Compt. rend.* Vol. xcviii. p. 373, 438, 526.
[6] *Zeit. physiol. Chem.* Vol. ix. p. 591.

milk; he admits the existence of a third proteid of the nature of hemi-albumose, but states that it does not occur in such large quantities as described by J. Schmidt[1].

These observers thus all found a third proteid which although it gave the biuret reaction was later recognised to be a proteose[2], and not a peptone.

Neumeister[3] was the first to show the fallacy of the method adopted by these investigators, not only with regard to milk, but also in connection with blood, lymph and other fluids. The substance called peptone by some, and hemi-albumose by others, is really a primary proteose (i.e. proto- or hetero-proteose) formed artificially during the manipulations by the hydrating action of the acidified hot liquid. The only method by which peptone may be separated with certainty from other proteids is to saturate the fluid with ammonium sulphate, and filter. The only proteid not precipitable by this means is peptone, which is therefore found in the filtrate. Using this method he found that peptone was absent, and Sebelien[4] has confirmed this statement. I had also previous to the appearance of these two papers arrived at the same conclusion from examining a large number of specimens of cow's milk. I also searched for peptone in stale (acid) milk, and in whey, obtained after the action of rennet on milk, but in all cases peptone was absent. On filtering off the precipitate produced by saturation with ammonium sulphate, the filtrate was examined by the following tests:—

i. Xantho-proteic reaction; negative result.
ii. Biuret reaction; negative result.
iii. Addition of tannin; no precipitate.

I then proceeded to examine milk for proteoses by a method, which is also that given by Neumeister.

To the milk, ten or twelve times its volume of absolute alcohol is added. This precipitates all the proteids. The precipitate is collected, and allowed to stand under absolute alcohol for some weeks; (in my experiments for ten weeks). The prolonged action of the alcohol is to render all proteids, except albumoses and peptones, insoluble in water.

[1] *Loc. cit.*

[2] This term is better than albumose, which should be restricted to the products of digestion of albumin. The term proteose includes albumoses, globuloses, vitelloses, etc.

[3] *Zeit. Biol.* Vol. xxiv. p. 272.

[4] *Zeit. physiol. Chem.* Vol. xiii. p. 135.

The precipitate is again collected, dried in an exsiccator over sulphuric acid, extracted with water, and the watery extract examined. The following different specimens were examined in this way, and the results of the examination of the aqueous extracts are as follows:—

Variety of fluid examined.	Result of examining the aqueous extract prepared as just described.
1. Fresh milk.	Extract contains no proteid at all.
2. Whey prepared by the action of rennet on fresh milk.	Extract contains no proteid at all.
3. Sour milk.	Extract does not coagulate on boiling; it gives a brilliant biuret reaction; an abundant precipitate with nitric acid which disappears on heating, reappearing on cooling. The proteid which gives these reactions is wholly precipitable by saturating with sodium chloride. These are the reactions of a primary proteose; and the fact that there is no precipitate on heating shows that the proteose present is chiefly proto-proteose.
4. Whey prepared by the action of rennet on sour milk.	The extract gives exactly the same result as just described under 3.

Several specimens of each of the above, all gave the same result, and the conclusion to be drawn from them is that fresh milk contains no peptone, and no substance of the nature of a proteose, and moreover that no formation of such a substance occurs under the influence of the rennet ferment. Sour milk (and the same is true for sour whey) contains abundance of a primary proteose. It thus appears that during the fermentation process called the souring of milk, there is not only a change in the carbohydrate leading to the formation of lactic acid, but also a change in the proteids leading to the formation of a peptone-like substance, or proteose.

This latter observation confirms the statements of several investigators who have described peptones (? proteoses, or proteoses *plus* peptones) in koumiss, kephir, and the preparation called 'long milk' in Upper Scandinavia[1].

[1] See Sebelien's paper in *Zeit. physiol. Chem.* Vol. XIII. p. 135.

5. Whey-proteid.

It was Hammarsten who first described the appearance of this proteid, upon the occurrence of the rennet fermentation. Caseinogen when acted on by rennet becomes converted into casein, and this second proteid which remains in solution simultaneously makes its appearance. As it is not coagulated by heat, some have surmised that it is of the nature of a peptone or proteose, and some have used the name lacto-protein for this substance as well as for the peptone-like substance which they consider to exist in the unaltered milk.

I have prepared this substance in two ways; (1) caseinogen obtained as already described, is dissolved in lime water; a few drops of rennet extract, and of 0·5 per cent. phosphoric acid added, and the resulting clot of casein filtered off. The whey-proteid remains in solution, and may be precipitated by saturating with magnesium sulphate. (2) I have obtained a larger supply of this substance by adding rennet to milk, filtering off the curd so formed, and saturating the whey with magnesium sulphate. The precipitate so produced is washed with a saturated solution of magnesium sulphate, and dissolved by the addition of water.

The reactions of the substance obtained by either method are the same. Like caseinogen it is not coagulated by heat, but its solutions become opalescent when heated, the opalescence disappearing when the solution is cooled[1]. Unlike caseinogen it is not precipitable by acetic acid, nor has rennet any coagulating action on it. It is not a peptone as it is precipitable by saturating its solutions with ammonium sulphate. It is not a proteose as it is precipitated and rendered insoluble by the prolonged action of alcohol; it gives with copper sulphate and caustic potash a violet, not a pink (biuret) reaction; it is precipitable by nitric acid, and though this precipitate dissolves to a very slight extent on heating, it is not increased again on cooling. It is not a globulin as it is not coagulated on heating. Though it is difficult to assign to this proteid its proper place in the classification of proteids, I should be inclined to put it into the special class of proteids nearly related to the globulins, which I have already suggested should be made for the admission of caseinogen.

[1] After prolonged boiling after acidification a small formation of flocculi was sometimes observed.

6. General Conclusions.

The principal points, to which I have endeavoured to draw attention in this paper are the following:—

(1) The principal proteid in milk called caseinogen is precipitable by certain neutral salts, or by acetic acid, and may be most satisfactorily prepared free from impurities by a combination of these two methods.

(2) The term casein should be restricted to the curd formed from caseinogen by the action of rennet.

(3) In the classification of proteids, casein should be grouped with other insoluble proteids like fibrin and gluten formed by ferment activity from preexisting more soluble proteids.

(4) Caseinogen should be classified in a new group made to include it, and whey-proteid. These proteids are very similar to the globulins; the chief difference being that their solutions are not coagulated by heat like the globulins, but only rendered opalescent. This opalescence if the heating has not been continued too long, disappears on cooling.

(5) Lact-albumin is very similar in its properties to serum-albumin. Not only does it differ however from serum-albumin in its specific rotatory power as has previously been shown, but in its behaviour on heat-coagulation, and in precipitability by certain neutral salts.

(6) Caseinogen and lact-albumin are the only proteids contained in milk.

(7) The proteid described as lacto-globulin does not exist; it is owing to the error of not recognising that the two salts sodium chloride and magnesium sulphate when both present to saturation precipitate albumin, that this proteid has been supposed to exist.

(8) The proteids variously called lacto-protein, peptone and hemi-albumose do not exist in milk. This mistake has also arisen from faulty methods of analysis.

(9) When milk turns sour owing to the lactic acid fermentation, primary proteoses, chiefly proto-proteose, are developed.

(10) The proteid called whey-proteid, which is formed during the rennet fermentation, is not of the peptone or proteose class, but should be included with caseinogen in a new class of proteids allied to the globulins.

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