CX. STUDIES ON THE EFFECT OF HEAT ON MILK.

I. SOME PHYSICO-CHEMICAL CHANGES INDUCED IN MILK BY HEAT.

BY HUGH EDWARD MAGEE AND DOUGLAS HARVEY.

From the Rowett Research Institute, Aberdeen.

(Received May 26th, 1926.)

EXPERIENCE has proved that pasteurisation of low-grade cow's milk makes it safer for human consumption than similar milk in the raw state. The process also helps to preserve its value as a marketable commodity for many hours after being milked; but the question whether its nutritive properties are detrimentally affected cannot yet be taken as settled. Opinions on the matter are very conflicting and further investigation appeared therefore desirable. The problem has engaged the attention of several workers at this Institute for the past 2½ years.

Perhaps no one constituent of milk is of more importance than another for the building up of new tissue in the young animal. There is, however, evidence that the calcium and phosphorus contents occupy an important position in this respect. Thus, Telfer and Crichton [1924] showed that comparatively trifling reductions in the calcium and phosphorus of goat's milk had profound effects on the young goats fed on it. It may be also, as suggested by Bunge [1904] and more recently by Hunt, Winter, *et al.* [1923], that the physical state of the calcium and phosphorus of the diet is of some importance for their proper assimilation by young and adult animals alike.

Since Sydney Ringer [1890] showed that addition of soluble calcium salts to heated milk restored its coagulability by rennin it has been generally accepted that, of all the constituents of milk, calcium was the one most affected by heat. It follows from the work of Van Slyke and Bosworth [1914], György [1923] and others, that calcium is found in two forms in milk, some in soluble or in ionic form and some organically bound with caseinogen in the form of a protein salt. In comparatively recent times several estimations of the relative amounts of soluble and colloidal calcium in fresh milk have been made, but attempts to determine whether the ratio is affected by heat have been very few.

Grosser [1913] found a loss of 5.4 % of soluble CaO in cow's milk (expressed as percentage of the total CaO) after boiling 15 minutes. The amounts of

soluble P_2O_5 and N were only slightly affected. In human milk, however, after boiling 15 minutes the losses were, CaO 21%, P_2O_5 7 % and N 12 % of the respective totals. Bell [1925] found that when cow's milk was kept at 77° for 30 minutes the soluble CaO fell by 4.6 % of the total. His results cannot, however, be accepted as confirmation of Grosser's because the degree and duration of heat were not the same in the two cases.

The experiments recorded in this paper were directed to determine what alterations were brought about in the state of some of the milk constituents by varying amounts of heat. Attention was focused chiefly on the calcium content for reasons which will be obvious from the foregoing. Two other papers in this series will appear later dealing with the relative effects of feeding fresh and heated milk on (a) the metabolism of young animals as determined in balance experiments, and on (b) the rate of growth of groups of young animals.

EXPERIMENTAL.

The research was directed broadly along three lines: the effect of heat on (a) the calcium content, (b) the colloids, and (c) the digestibility of milk.

Determination of soluble calcium. This was determined (1) by precipitation of the proteins with colloidal ferric hydroxide; (2) in rennet whey, and (3) by dialysis of milk for varying lengths of time.

Exp. 1. Precipitation of proteins with colloidal ferric hydroxide. According to Rona and Michaelis [1909] colloidal ferric hydroxide splits up the "casein-calcium compound" of milk and 70–80 % of the total CaO appears in soluble form, most of the P_2O_5 being carried down as an adsorbate. Kay [1925] has confirmed the disappearance of soluble P_2O_5 by this method of precipitation. Very probably this action of colloidal ferric hydroxide is due to the free Cl' its solutions contain [Pauli, 1922].

In four samples of whole fresh milk we found an average of 85.1 % of the total CaO in soluble form and 0.0122 % of P_2O_5 . In four samples of whole milk pasteurised at 65° for 30 minutes, we found 71.3 % of CaO and 0.0117 % of P_2O_5 in soluble form. These results confirm those of Rona and Michaelis and indicate that about 14 % of the total CaO was changed by heat into a form that the colloidal ferric hydroxide could not attack.

Exp. 2. Coagulation by rennin. The three kinds of milk used for all the experiments which follow are defined as under:

- (1) Fresh milk: skimmed cow's milk 3 hours after milking.
- (2) Pasteurised milk: (1) kept at a temperature of 70° for 30 minutes in a flask immersed in a water-bath.
- (3) Boiled milk: (1) kept in a flask immersed in a water-bath at boiling point for an hour.

Reflux condensers were fitted to the flasks to prevent loss of water during the heating processes. The baths of water were quickly brought to the desired temperature and the flasks of milk were kept in the baths while the temperature

was being raised. After heating, the milk was cooled rapidly. There was never any skin formed on the surface of the milk.

The p_H of milk is not altered by rennin coagulation [Milroy, 1915; György, 1923]. Most probably, therefore, splitting up of the colloidal calcium does not occur and all the soluble calcium is present in the whey except what may be carried down by adsorption. Heated milk is very slowly acted on by rennin or not at all. To make it coagulable CaCl_2 in solution was added. No buffer mixture was added to counteract the slight lowering of the p_H caused by the addition of CaCl_2 [Milroy, 1915]. The commercial rennin used, originally of p_H 2.8, was dialysed for 24 hours in running tap water and the p_H became 6.1, the same as that of the tap water. The rennin was still quite active and contained only an insignificant amount of CaO , 0.008 %.

5 cc. of this solution and 5 cc. of a solution of CaCl_2 containing 0.0252 g. CaO were added to 50 cc. each of fresh, pasteurised and boiled milk in 100 cc. centrifuge tubes, mixed, kept at 40° until coagulated and centrifuged for 20 minutes. The CaCl_2 added was the minimal amount necessary to start coagulation in the boiled milk. 40 cc. of whey were pipetted off and ashed, the calcium precipitated as oxalate and titrated with $N/10 \text{ KMnO}_4$. From the data obtained, the amount of soluble CaO in 100 cc. of milk was calculated, assuming that the concentration was the same in the coagulum as in the whey. (The amount of CaO in the CaCl_2 added was, of course, deducted.) For reasons given above, the assumption is probably not quite correct, but we were not aware of any means of correcting for the loss due to adsorption. All experiments were done in duplicate.

Table I.

No.	Total CaO %	cc. $N/10 \text{ KMnO}_4$ for 40 cc. whey		
		Fresh	Pasteurised	Boiled
1	0.1610	11.00	10.00	9.80
2	0.1663	10.30	10.10	9.30
3	0.1743	9.60	9.10	9.15
4	0.1739	11.80	11.37	11.15
5	0.1834	10.69	10.10	8.76
6	0.1736	10.00	9.67	8.99
7	0.1729	9.62	9.47	9.70
8	0.1757	12.36	12.30	11.65
Av.	0.1726	10.67	10.26	9.81
CaO as % of total		22.72	20.73	18.52

The results (Table I) show on the average a loss in pasteurised milk of 2 %, and in boiled milk of 4 %, of soluble CaO expressed as percentage of the total CaO .

Exp. 3. Dialysis of milk. By dialysing milk against running water all the soluble calcium will in time pass out and the amount so lost can be estimated by difference. The CO_2 dissolved in the water, however, complicates the method. Being diffusible it can break up the Ca caseinate, precipitating the protein and bringing the Ca into solution as $\text{Ca}(\text{HCO}_3)_2$. Most probably, however, this

action would not occur to any appreciable degree until the buffer action of the soluble phosphates had ceased on their complete disappearance from the inner fluid. Prior to this the observed rate of loss of soluble calcium should give a fairly accurate picture of the relative concentration of soluble calcium in different kinds of milk since the rate of loss is directly proportional to the concentration.

Fat extraction thimbles 4.3×12.3 cm. were impregnated with collodion meth. (B.P.) as described by Hatschek [1920]. After practice membranes of approximately equal permeability could be prepared. Care was taken that the sacs were of like permeability by testing them on the same sample of milk for a certain time. 20 cc. of fresh, pasteurised and boiled milk were pipetted into each of three sacs suspended in running tap water in a wide-mouthed vessel. The depth of the water was such that, when, owing to the osmotic pressure of the milk, the volume inside the sacs was greatest, the sacs remained floating. Thus, approximately equal areas of membrane surface were in contact with water. Nine sacs at a time—three for each kind of milk—were accommodated in the vessel and care was taken that good circulation of water was maintained round each sac. After dialysis the amount of CaO in the contents of each sac was determined. The addition of a preservative to the milk was considered unnecessary because, as the experiments were carried out in winter, the temperature of the water was never more than a few degrees above 0° . It is to be remembered also that lactose would soon diffuse out. The formation of lactic acid was therefore considered highly improbable.

Fig. 1.

Dialysis was carried out for 8, 16, 24, 32, 40 and 48 hours. The results expressed as loss per cent. of the total CaO are shown graphically in Fig. 1. Each point is the average of nine analyses of dialysates, except at 8 and 24

hours where each is an average of three. Several other experiments were done at these times using sacs made from 4 % acetic collodion which were found more permeable than those made with "collodion meth." The former gave results showing similar relative differences as between the three kinds of milk, and therefore acted as a check on the results obtained with the "collodion meth." sacs; but the losses were too great to warrant their inclusion with the latter for averaging.

Table II is an example of the results obtained.

Table II.

cc. $N/10$ $KMnO_4$ required for 20 cc. milk.

Dialysed milk (16 hours).

	Whole milk	Fresh	Pasteurised	Boiled
1	13.10	10.62	11.15	11.30
2	11.80	10.12	10.30	10.55
3	11.08	9.28	9.48	9.70
Av.	11.99	10.01	10.31	10.54
Loss of CaO as % of total		16.70	14.20	12.30
Loss % per hour		1.04	0.89	0.77

Each $KMnO_4$ reading is the average of three.

Fig. 1 shows that at every interval the percentage loss of CaO from fresh milk was greater than from pasteurised and still greater than from boiled.

The differences in loss per cent. of the total CaO between fresh and heated milks at each interval are given below:

Hours' dialysis ...	8	16	24	32	40	48
Fresh—pasteurised	2.6	2.5	3.3	6.0	2.1	8.4
Fresh—boiled	6.4	4.4	4.5	10.7	4.5	12.5

Fig. 2 shows that the rate of loss of soluble CaO agrees with theory until after 32 hours, when the curves rise up to 40 hours and then fall slightly to 48 hours. The more marked rise and fall in the case of the heated milks suggests the intervention of the solvent action of CO_2 on the colloidal calcium, there being, as will be pointed out later, very probably more colloidal calcium in the heated milks. For example, on two occasions a slight precipitate of caseinogen was noticed in the fresh milk after 48 hours' dialysis, but never in the heated milks. Experience having shown that the method was not accurate enough to demonstrate significant losses after 4 hours' dialysis, no readings were attempted at intervals of 28 and 36 hours where, if they had been possible, they would have had a special significance in view of the commencing action of dissolved CO_2 .

Exp. 4. Δ of milk. The average readings of the Beckmann thermometer for six samples of milk were: fresh 5.502° , pasteurised 5.494° , boiled 5.487° . The readings for pasteurised milk were higher in two cases by 0.001° and

0.002°, than for the corresponding fresh samples. The boiled samples invariably gave smaller depressions than the fresh. The results show that heating progressively reduces the numbers of free ions and of molecules in solution in milk.

Fig. 2.

Exp. 5. Effect of heat on the p_H of milk. Milroy [1915] showed that the p_H of milk is lowered by boiling for an hour. His average figures for six samples were: fresh 6.73, boiled 6.59. We determined the p_H in fresh and boiled milks by a modification of the colorimetric method of Kramer and Greene [1921]. Our average results for six samples were: fresh 6.69, boiled 6.55, *i.e.* as in Milroy's experiments a lowering of the p_H by 0.14. The method was not considered sufficiently accurate to bring out the change in p_H after pasteurisation. Milroy suggested that the increase in H^+ was due to the separation of $Ca_3(PO_4)_2$: thus $3CaHPO_4 \rightarrow Ca_3(PO_4)_2 + H_3PO_4$.

If the $Ca_3(PO_4)_2$ were separated as a precipitate it should be possible to show a loss of calcium in heated milk by filtration or by centrifuging. He showed an average loss of total CaO of 12.8 % after boiling for an hour and then filtering.

Exp. 6. Filtration. Table III shows that almost as much CaO was removed from the fresh as from the heated milks by filtration through paper. The slightly greater losses in the latter could be accounted for by greater volume of colloidal particles; but the possibility of adsorption of ionised proteins by filter paper as shown by Christiansen [1912] is worthy of consideration in this connection. In either case, these results when compared with those of Exps. 2 and 3, are opposed to the idea of the separation of $Ca_3(PO_4)_2$ as an amorphous or crystalline precipitate in heated milk. The results of the next experiment point in the same direction.

Table III.
cc. $N/10$ $KMnO_4$ for 20 cc. milk.

	Unfiltered	Filtered		
		Fresh	Pasteurised	Boiled
1	12.80	12.50	12.60	12.60
2	13.10	12.85	13.10	12.60
3	12.35	12.25	12.20	12.20
4	13.30	13.30	13.20	13.10
5	12.75	12.75	12.50	12.35
6	12.05	12.00	11.90	11.50
7	13.15	13.00	12.95	12.70
8	12.05	12.05	11.95	11.95
9	11.95	11.90	11.85	11.60
Av.	12.61	12.51	12.47	12.29
% CaO	0.1766	0.1752	0.1746	0.1721
Loss as % of total	—	0.8	1.1	2.5

Exp. 7. Centrifuging. 50 cc. of milk were centrifuged for from 5 to 30 minutes and the CaO content of the supernatant fluid determined by analysing 20 cc. The experiments were done in duplicate.

Table IV.
cc. $N/10$ $KMnO_4$ required for 20 cc. milk.

	Time of centrifuging (mins.)	Non-centrifuged	Centrifuged		
			Fresh	Pasteurised	Boiled
1	30	11.85	10.85	10.92	10.95
2	30	12.00	11.12	11.15	11.17
3	30	11.85	11.05	10.90	10.92
4	15	11.35	10.88	10.92	10.77
5	5	12.00	11.80	11.82	11.72
Av.		11.81	11.14	11.14	11.11
CaO %		0.1654	0.1560	0.1560	0.1555
Loss as % of total		—	5.7	5.7	5.9

The results show that the losses of CaO were practically identical in all three cases and thus tend to confirm the opinion expressed above. The deposit had similar microscopic appearances in all cases and consisted in greater part of a gelatinous mass containing some leucocytes, bacteria and epithelial cells. Crystals and anything resembling an amorphous deposit were absent. If, then, amorphous or crystalline $Ca_3(PO_4)_2$ is formed by heating milk, not only would it be detected in the deposit after centrifuging but losses proportionate to the amount formed would be detected in the calcium content of the heated milks after filtration or centrifuging. It therefore looks as if the disappearance of soluble calcium after heating is due to its being changed into colloidal form. This idea suggested investigation of the effect of heat on some of the physical properties of milk for which the colloids are chiefly responsible.

Exp. 8. Viscosity. Table V shows the relative viscosities (η) of ten samples of milk at 26°.

All the samples of boiled milk showed higher viscosities than the corresponding fresh samples. Except for Nos. 1 and 6 all the samples of pasteurised milk showed slightly lower viscosities than the fresh samples. The reason for

this varying effect of heat on the milk was not clear and further experiments bearing on the relation of the caseinogen to the continuous phase were undertaken.

Table V.

	Fresh	Pasteurised	Boiled
1	1.736	1.745	1.958
2	1.747	1.688	1.902
3	1.725	1.721	1.880
4	1.589	1.555	1.692
5	1.552	1.527	1.613
6	1.584	1.601	1.769
7	1.566	1.521	1.634
8	1.563	1.528	1.661
9	1.530	1.512	1.561
10	1.514	1.506	1.662
Av.	1.610	1.590	1.735

Exp. 9. Swelling. 20 cc. each of fresh, pasteurised and boiled milk were clotted as in Exp. 2 and centrifuged for 30 minutes. The curd was then dried on filter paper and its volume determined by displacement.

Table VI.

Volume of coagulum, cc.

	Fresh	Pasteurised	Boiled
1	3.20	3.40	4.20
2	2.20	2.20	3.40
3	2.10	2.30	3.60
4	2.25	1.80	4.20
5	1.90	1.90	3.40
6	2.10	2.10	5.40
7	1.55	2.10	3.00
Av.	2.19	2.26	3.89

The results (Table VI) show that in every sample the volume of curd from boiled milk was much greater than that from the corresponding fresh milk. In sample 4 the volume of curd from the pasteurised milk was less than, but in every other sample either equal to, or greater than, the volume from the corresponding fresh milk. The averages show an increase in swelling, of casein from 20 cc. of milk, of 0.07 cc. after pasteurising and of 1.70 cc. after boiling. The increase of 0.07 cc., however, falls within the limits of accuracy of the measurements, 0.25 cc., and must be regarded therefore as of no significance. We may conclude then that boiling for an hour increases the volume of the casein particles; but that the method employed showed no significant change in volume after pasteurising for 30 minutes. On account of the fall in the viscosity of pasteurised milk one would have expected a corresponding fall in the volume of the casein since the viscosity of protein solutions "is primarily a function of the relative volume occupied by the protein in solutions" [Loeb, 1922].

Hardy [1905] and others have shown that the viscosity of protein salts varies directly as the degree of ionisation and Pauli [1922, p. 29] has shown that, when ionisation is at a maximum, precipitation by alcohol is at a minimum

and *vice versa*. It therefore seemed of interest to make some experiments on the precipitation of milk proteins.

Exp. 10. Precipitation of colloids. A solution of 10 % colloidal ferric hydroxide was run into 5 cc. of milk diluted with 5 cc. of water until a clear fluid was obtained after filtration. The flask was shaken constantly during the addition, which occupied a minute, and for 1 minute after and its contents were then filtered. The limit of accuracy was 0.30 cc. of colloidal ferric hydroxide solution.

Table VII.

cc. of 10 % colloidal ferric hydroxide for 5 cc. milk.

	Fresh	Pasteurised	Boiled
1	17.5	16.0	16.0
2	21.7	19.8	19.8
3	20.5	18.85	18.85
4	22.6	—	19.4
5	26.6	26.6	26.0
6	25.0	25.0	24.5
7	25.0	23.7	23.6
Av.	22.7	21.66	21.16

The amounts required for every sample of fresh milk were greater than for the corresponding boiled milk and for every sample of pasteurised milk except 5 and 6. On the average, fresh milk required 1.04 cc. more than pasteurised and 1.5 cc. more than boiled milk for 5 cc. of each, thus indicating a diminution of stability as a result of dehydration or decrease of charge of the colloid. These results do not fit in with Pauli's views which associate minimum viscosity and maximum of precipitation with the isoelectric point, or the point where the number of neutral particles is a maximum.

Exp. 11. Digestibility of fresh and heated milk. Liquor Trypsin Co. (A. & H.)¹ was brought to a p_H of 8.1 by addition of Na_2CO_3 and activated by incubating for 12 hours. 2 cc. of activated trypsin were added to 20 cc. of milk and a few drops of $CHCl_3$ and the whole incubated for 1 to 20 hours. The proteins were precipitated with colloidal ferric hydroxide or hot alcohol and aliquot portions of the clear filtrate titrated with $N/20$ NaOH for free acids and for amino-groups by Sørensen's method. Many trials of the method were made, but in most cases the differences observed fell within the experimental error of the method as the results show:

Table VIII.

cc. $N/20$ NaOH for 4 cc. digested milk.

Hours' digestion	Fresh		Pasteurised		Boiled	
	Free acid	Amino-acid	Free acid	Amino-acid	Free acid	Amino-acid
20	4.00	7.60	3.70	7.40	—	—
4	3.03	3.96	2.67	3.76	2.07	3.78
4	2.60	4.13	2.66	4.12	2.55	3.92
4	1.87	2.00	2.12	2.20	2.40	2.30

¹ We are indebted to Mr Rimington of Emmanuel College, Cambridge, for advice on the most suitable trypsin preparation and on some particulars regarding its use.

It appears therefore, as found by Ellenberger and Hoffmeister [1890], that previous heating of milk has no appreciable effect on its digestibility by proteolytic enzymes or else that our method was not sufficiently accurate. Measurements of viscosity before and after digestion as used by Christiansen [1912] or of conductivity would in our opinion be much more accurate.

DISCUSSION.

The 14 % loss of soluble CaO found in Exp. 1 after pasteurising is not substantiated in Exps. 2 and 3. The discrepancy is probably to be explained by the chemical properties of colloidal ferric hydroxide, but the results serve to emphasise the change in the state of the soluble CaO by heat.

The amounts of soluble CaO obtained in Exp. 2 are very probably a little low on account of adsorption by the coagulum. We believe the percentage losses in total CaO after 32 hours' dialysis are the nearest approximation to the true amounts of diffusible CaO present in the original milk. These figures are: fresh milk 26.4 %, pasteurised 20.4 %, boiled 15.7 %. Very probably the solvent action of CO₂ became effective between 32 and 40 hours and caused the washing out of colloidal calcium as suggested. It is of interest to compare these results with those of other workers. Rona and Michaelis [1909] found 44 % of the CaO in soluble form in fresh cow's milk; Grosser [1913] 23.4 % and after boiling 15 minutes 18 %. Wha [1923] found 50 % in fresh cow's milk and Bell [1925] 23.8 % and after heating for 30 minutes at 77° 19.2 %. Bosworth and Van Slyke [1916] found 30.3 % in fresh goat's milk. Our figure for fresh milk, 26.4 %, agrees fairly well with those of Grosser and Bell and the loss we found after pasteurisation, 6 %, is similar to theirs, viz. 5.4 % and 4.6 % respectively. The heat treatment was not, however, the same in the three cases. We are not aware of any analogous results with which to compare the loss of soluble CaO of 10.7 % after boiling for an hour.

It has been seen that our results oppose the suggestion that the change in p_H after heating is a consequence of the separation of Ca₃(PO₄)₂ as a precipitate. It looks therefore as if the loss of soluble Ca was due to its partial transformation into colloidal form; whether as Ca₃(PO₄)₂ by itself or as a further combination of Ca with caseinogen, it is difficult to say. Evidence on the effect of heat on the soluble P₂O₅ of cow's milk would help towards a solution. The only data available are those of Grosser and of Bell, which show respectively losses of 0.4 % and 2.8 % of the total P₂O₅ after heating. These losses appear very small when compared with the corresponding losses in soluble CaO, viz. 5.4 % and 4.6 % respectively; but when calculated as per 100 cc. of milk, Bell actually found greater losses of P₂O₅ than of CaO. Thus after heating at 77° for 30 minutes, the losses per 100 cc. of milk were CaO 0.0053 g., P₂O₅ 0.0059 g. It appears very probable therefore that heat changes some of the soluble CaHPO₄ of milk into Ca₃(PO₄)₂ in colloidal form in such a state of subdivision as to be irremovable by filtration or by centrifuging.

It has been abundantly shown that the addition of neutral salts to protein compounds has a depressing effect on the viscosity and swelling of colloidal particles, and that the ion of the salt carrying a charge opposite in sign to the protein ion is responsible. Consequently, the removal of Ca from solution in milk would increase its viscosity and the size of its colloidal particles. The viscosity of milk would also be increased by a decrease in the lyophilic properties of the colloids in the same way as Loeb [1922, p. 252] demonstrated in a solution of egg-albumin after heating. Such an increase in the number of colloidal micellae, or perhaps their appearance for the first time, would lead to the setting up of a Donnan equilibrium whereby water would be attracted into the micellae by the osmotic pressure of the inorganic ions. In this way the relative volume of the colloids would increase and their stability decrease.

Our findings in regard to boiled milk fit in with these suggestions, but not entirely in regard to pasteurised milk, for the viscosity was slightly lowered and the relative volume of the casein scarcely affected by pasteurisation. These discrepancies indicate the need of further study of the effects of heat varying in degree and duration on the physical properties of milk. Possibly other changes and other factors not mentioned would be shown to be of importance.

SUMMARY.

1. When the proteins were precipitated with colloidal ferric hydroxide 85 % of the total CaO was found in solution in the case of whole fresh milk and 71 % in that of whole milk heated for 30 minutes at 65°.

2. About 26 % of the total CaO was found in diffusible form in fresh milk, about 20 % in pasteurised milk and about 15 % in boiled milk. The losses in soluble CaO after heating were believed to be due to the formation of colloidal $\text{Ca}_3(\text{PO}_4)_2$ from the soluble CaHPO_4 , which would account for the increase in acidity observed. Precipitation of amorphous or crystalline $\text{Ca}_3(\text{PO}_4)_2$ by heat was not detected.

3. Heat progressively lessened the stability of the colloids and increased the size of the protein particles. Pasteurisation for 30 minutes caused a slight fall in viscosity, but boiling for an hour a marked increase. The mechanisms by which heat could cause these various effects are discussed.

4. No differences were observed in the rates of tryptic digestion of milk before and after heating.

We desire to express our thanks to Dr J. B. Orr who suggested the research, and to Dr W. Thomas, University of Aberdeen, for advice and criticism.

REFERENCES.

- Bell (1925). *J. Biol. Chem.* **64**, 391.
Bosworth and Van Slyke (1916). *J. Biol. Chem.* **24**, 176.
Bunge (1904). *Z. Biol.* **45**, 532.
Christiansen (1912). *Biochem. Z.* **47**, 226.
Ellenberger and Hoffmeister (1890), cited by Grimmer (1910). *Chemie und Physiologie der Milch*, 310.
Grosser (1913). *Biochem. Z.* **48**, 427.
György (1923). *Biochem. Z.* **142**, 1.
Hardy (1905). *J. Physiol.* **33**, 284.
Hatschek (1920). *Laboratory Manual of Colloid Chemistry*, 24.
Hunt, Winter, Schulz and Miller (1923). *Amer. J. Physiol.* **66**, 349.
Kay (1925). *Biochem. J.* **19**, 433.
Kramer and Greene (1921). *J. Biol. Chem.* **46**, *Proc.* xlii.
Loeb (1922). *Proteins and the Theory of Colloidal Behaviour*, 209.
Milroy (1915). *Biochem. J.* **9**, 215.
Pauli (1922). *Colloid Chemistry of the Proteins*, 14.
Ringer (1890). *J. Physiol.* **11**, 464.
Rona and Michaelis (1909). *Biochem. Z.* **21**, 114.
Van Slyke and Bosworth (1914). *J. Biol. Chem.* **20**, 135.
Telfer and Crichton (1924). *Brit. J. Exp. Path.* **5**, 84.
Wha (1923). *Biochem. Z.* **144**, 278.