XCIV. THE DIALYSIS OF MILK. THE DISTRIBUTION OF PHOSPHORUS.

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NOTWITHSTANDING the vast amount of work which has been carried out in attempts to define the constituent compounds of normal cow's milk, no very clear or even comprehensive statement can yet be made. A gross division has been effected, or rather perhaps a number of compounds have been separated from milk, but in the majority of cases chemical methods have been employed which, in themselves, must have brought about interaction between the reagents used and the constituents of the milk. The problem is, of course, complicated by the fact that milk is not a true solution, but a mixture of substances in true solution with others dispersed through the medium as colloids: this introduces the question of adsorption on surfaces and also the possibility of change, according to conditions, of some substances from true solution to colloidal dispersion, or *vice versa*.

It is suggested that until some method be devised whereby the constituents of milk can be separated by physical means, or without the use of chemicals which themselves react with the milk, no definite hypothesis concerning the composition of milk or even of any of its constituent parts such as caseinogen, can be held to be free from serious criticism. This fact has been realised by some workers, and Lenstrup [1926] has stated that in the separation of the acid-soluble phosphorus compounds the difficulty is overcome by the use of picric acid. The constituents of milk are, however, so numerous, so little understood and some of them certainly so easily changed in composition that it appears that dialysis and ultrafiltration are methods least open to criticism from the point of view of outside interference. It was with the object of using such physical methods that the present investigation was undertaken and the continuous dialysis apparatus developed.

It is obvious that the progress of the dialysis of milk can be followed by tracing the changes in the distribution of any of the numerous dialysable constituents. In this particular study, phosphorus (as inorganic and organic) was chosen, but when it was desired to compare the results obtained with those of other workers, it was found that there was no general consensus of opinion regarding the distribution of this element; Table I demonstrates this point. It appears that there are two main reasons for this lack of conformity, namely, variation in the milk and variation in the apparatus used.

(a) *Milk*. It is self-evident that if there is any variation in the natural phosphorus content of milk, it will be almost impossible to express in general terms the amount—both inorganic and organic—dialysable under any series of conditions. Moreover, in order to make comparison possible, all results should be referred to a common basis, such as the non-fatty solids content of the milk. The majority of recorded analyses have not been so calculated nor has the

Table I. *The phosphorus distribution in milk reported by other workers.*

Reference	Method of separation	Form of phosphorus	Amount of phosphorus found in milk %	Amount of phosphorus found in serum %
Van Slyke and Bosworth (1915)	Pasteur-Chamberland filter Separated milk. CHCl_3 extracted. (Chloroform as preservative)	Inorg. Org.	0.096, 0.087 0.029 (2 samples)	0.067, 0.056 Nil (54 % of total)
Van Slyke and Bosworth (1915)	Pasteur-Chamberland filter	Total	0.087-0.131 (13 samples)	0.029-0.082 (27-64 % of total)
Van Slyke and Bosworth (1916)	Pasteur-Chamberland filter Separated milk. (No preservative)	Inorg. Org.	0.083 0.021 (1 sample)	0.043 —
Bell (1925)	Pasteur-Chamberland filter Separated milk. (No preservative)	Total —	— —	0.028 —
György (1923)	Ultra-filtration through de Haen filter membranes	Inorg.	0.030-0.036	0.013-0.019 (40-50 % of total inorg. P)
Wardlaw (1914)	Dialysis (collodion membranes) 25 cc. water in 1000 cc. separated milk	Total	0.093-0.115 (7 samples)	0.033-0.067 (35-55 % of total)
Mattick and Hallett (1929)	Dialysis of milk 25 cc. (parchment thimbles) in 150 cc. of 3 % NaCl for 18 hours	Total	0.099-0.114 (11 samples)	0.030-0.038 (29-38 % of total)

composition of the milk used been quoted. A further complication is introduced by the fact that a number of investigators have used milk from individual cows in order to evaluate the possible effects of factors incidental to the animal itself, such, for example, as the lactation period. The effects of these complicating factors must be obviated before conclusions, deduced from any phosphorus distribution determinations, can be regarded as reliable; this end could be realised either by averaging a large number of individual determinations or by carrying out experiments on what may be called "average" milk. In order to achieve this object, separated bulked milk (or spray process powder made therefrom) has been used in the work now described. The idiosyncrasy of the cow has thereby been nullified, but the possible effect of seasonal variation has not yet been considered; it appears that this is not, however, of great importance. The difficulty with all milk experiments is the progressive change taking place on keeping, and therefore milk in the form of powder would be of great value, as experiments could be carried out and repeated on the same bulk without fear of alteration having occurred; but it would be first necessary to show that dialyses of a milk and of a solution of a milk powder made therefrom, would yield identical results. This has not yet been done, but for convenience of the comparison of the various methods of serum production, milk powder has been used in the majority of experiments now described.

(b) *Apparatus used.* In the past the variations in the means of obtaining sera have added still more to the difficulty of comparing the results. Consequently in the present study three methods of separating a serum have been compared: (i) a continuous complete dialysis method (involving ultrafiltration); (ii) dialysis with minimum equilibrium disturbance (called herein "static" dialysis) and (iii) ultrafiltration. In all cases collodion membranes were used. These membranes were hand-made, and it is realised that criticism of this fact might be raised, but tests have shown that the errors introduced by slight variations in the structure of the membrane, made according to the technique described, are not significant to the present argument.

EXPERIMENTAL.

(a) *Method of continuous dialysis.*

The apparatus used is shown in Figs. 1 and 2. It will be realised that certain particular requirements are satisfied: (a) The speed of dialysis is increased by

Fig. 1.

Fig. 2.

Fig. 2a.

continuous stirring and by applying a pressure¹ which induces ultrafiltration of the milk through the collodion sac. (b) The milk is maintained at a low

¹ Obtained by a reduced pressure outside the sac equivalent to 70 cm. of mercury.

temperature (0° to 5°). (c) The volume of water used is reduced to a minimum, thus enabling distilled water to be used, and in the process the dialysate is concentrated to convenient volume.

A point of major importance is that the speed and low temperature employed enable dialyses of milk to be conducted without the use of any preservative, as, at the temperature in question, very little change, induced by micro-organisms likely to be present, occurs. In all cases dialysis was continued until equilibrium had been attained (usually 40–48 hours).

The variation in the rates of dialysis using two different membranes is shown in Graph 1. Curve a_1 and curve b_1 represent experiments using sodium

Graph 1.

chloride solution (0.07 %), and curves a_2 and b_2 using a solution of separated milk powder¹ in which the reducing power² of the dialysate was used as the index of dialysis. In the experiments represented by curves a_1 and a_2 the same sac was used and similarly in the case of curves b_1 and b_2 . The graph demonstrates that there is a satisfactory similarity between the two collodion thimbles used. Incidentally, it also shows that the dialysis of milk is very much slower than that of salt solution.

The general procedure of a dialysis by this method is as follows.

The liquid (approx. 20 cc. of milk) to be dialysed is poured down the stirrer (a glass tube, see Figs. 1 and 2) thus enabling the air in the sac to escape into the cut-down pipette. Distilled water is added to bring the level of the liquid approximately to the position shown in the figure. The collodion sac is surrounded by distilled water in the vessel shown, and between it and the walls of this vessel are two capillary tubes to ensure free passage of water. This water is in continuous circulation, being delivered to the dialysis chamber by the condenser, and returned to

¹ In all cases the milk powder "solutions" have a concentration of about 10 % total solids, the actual concentration being checked by analysis. The milk powder used in this work was made by the "Milkal" spray process.

² In all cases a measure of the lactose has been obtained by using the ferricyanide reduction method of Hanes [1929] and when the reduction was small the method of Hagedorn and Jensen [1923]. The protein precipitant was trichloroacetic acid (2 %) which was neutralised in the filtrate prior to sugar determination.

the flask by means of the siphon. Since the distillation is carried out under reduced pressure, the temperature of the dialysate concentrating in the flask is between 45° and 50°. Moreover, the reduced pressure induces filtration of the milk through the membrane. The volume of milk is maintained by the addition of distilled water through the hole in the bulb of the pipette. Brine of suitable temperature is circulated through the condenser and the jacket of the dialysing unit during dialysis in order that the milk may be maintained below 5°.

At the termination of an experiment the dialysate in the flask may be recovered quantitatively (with careful washing of the bung and return pipe from the dialysing cell); the milk residue may be obtained likewise quantitatively by removing part of the dialysing unit Fig. 2 *a*, inverting so that the milk flows along the stem of the cut-down pipette and out through the hole in the side; the sac may be washed by the addition of water down the stirrer, the washings being recovered as described for milk.

In connection with the method of cooling, certain precautions should be taken, *e.g.* the brine must not cause blockage in the condenser due to freezing of the condensate, and, more important still, the temperature of the liquid surrounding the collodion sac must not be allowed to drop below about 2°, otherwise freezing may occur, with consequent puncturing of the sac.

The efficiency of the method using milk is illustrated by Table II.

Table II.

	Lactose (as % of the milk) removed in dialysis	Efficiency of dialysis calculated from lactose removed %
Dialysed 12 hrs.	4.20	90.0
Dialysed 24 hrs.	4.62	99.0
Dialysed 40 hrs.	4.65	99.6

The original milk contained 4.67 % lactose.

(b) *Method of static dialysis.*

The method was developed by Wardlaw [1914] who based it on the work of Rona and Michaelis [1909]; see also Moore and Bigland [1911].

The procedure by which the results now presented were obtained is as follows (see Fig. 3).

A collodion sac containing 25 cc. of distilled water is suspended in 900 cc. of milk contained in a 1-litre conical flask. The sac is supported by attaching it to the lower end of a piece of wide glass tubing and fixing the tubing in the neck of the flask so that the level of the water inside the sac is the same as that of the milk outside.

When equilibrium has been attained (after 48 hours at 0–5°) the milk, with regard to the dialysable portion, will have been diluted to the extent of the water in the thimble, that is, the effective volume becomes 925 cc. instead of the original 900 cc. Analysis of the liquid in the sac, when corrected for this dilution gives, therefore, the composition of the dialysable portion of the original milk. It may be noted that the liquid in the sac decreases in volume from 25 cc. to approximately 15 cc.

The statement of Wardlaw was confirmed that equilibrium is attained by this method in 16 hours but, since the continuous dialysis method requires 40–48 hours to come to completion, the static dialysis was continued for the same period in order that the figures obtained might be strictly comparable.

(c) *Method of ultrafiltration.*

The apparatus is shown in Fig. 4 and is a slight modification of the dialysis chamber used in the continuous method. Under these conditions approximately 5 cc. of filtrate were obtainable in 1–2 hours.

(d) Method of determination of phosphorus.

Inorganic phosphorus. A minimum amount of trichloroacetic acid, usually less than 0.5 % of the milk, is added to precipitate the organic matter, and the filtrate is used for the determination; the colorimetric method of Kuttner and Lichtenstein [1930] was adopted.

Fig. 3.

Fig. 4.

Total phosphorus. This involves the destruction of organic matter with fuming nitric acid followed by "ashing." It is well to describe this in some detail, as modifications of technique are liable to result in erratic figures.

A suitable weight or volume of solution is evaporated to dryness in a platinum dish on a water-bath. Fuming nitric acid (5 cc.) and approximately 0.01 g. of calcium carbonate are added and the mixture heated on the water-bath. The volume of the acid is maintained by further additions until the solution becomes clear, when the whole of the acid is allowed to evaporate off and the residue subsequently reduced to an ash.

This ash is dissolved in the smallest possible amount of 50 % trichloroacetic acid (usually 1 cc. is sufficient), and the solution transferred to a graduated flask and made to a convenient volume with distilled water. The amount of material to be taken and the ultimate dilution depend upon the phosphorus content of the material; using 5 cc. milk the final solution is made to 250 cc. The phosphorus determination is carried out by the same method as that used for the inorganic phosphorus.

(e) Method of preparing collodion thimbles.

The special collodion solution prepared by the British Drug Houses is diluted with half its volume of ether. A suitable volume of this solution is poured into a cut-down boiling-tube (of internal diameter slightly larger than the size of thimbles required) and the inside completely coated by careful rotation of the tube, finally allowing the excess liquid to drain away by inverting the tube. The rotation is continued for a further 5 minutes to allow some evaporation of the ether, and the tube is then carefully filled with 70 % alcohol and allowed to stand for 10 minutes. The alcohol is poured out and the tube and membrane

placed in running water; after a short while the thimble may be disengaged and allowed to remain in the water until required (within 1 or 2 days). The thimbles are attached to suitable glass holders by sliding the latter into them for a distance of about half an inch and then drying the top of the thimble with a small gas flame. In this manner the top of the thimble is made to contract and hold firmly on the glass holder.

(f) *Comparison of static dialysis with ultrafiltration.*

A bulk of separated milk powder "solution" was prepared and eight lots of 900 cc. of this solution were each used for a "static" dialysis experiment at 0° to 5°. This number of dialyses was set up in order to provide sufficient material for the numerous determinations. After 2 days the solutions in the eight collodion sacs (now reduced from 25 cc. to approximately 15 cc.) were bulked as also were the milks remaining in the eight flasks. A sample of the bulked dialysate was removed for analysis, and of the remainder 15 cc. were replaced in each of six of the thimbles and the thimbles suspended in six flasks each containing 910 cc. of the bulked milk solution. This procedure was repeated on two further occasions.

At the intervals of time when the bulked static dialysates were sampled, samples of the original milk powder "solution," which had been stored at 0° to 5°, were subjected to ultrafiltration, and the ultrafiltrates analysed in a similar manner to the dialysates mentioned above.

This procedure ensured that any changes induced in the milk during storage would be the same in the two types of sera obtained. Typical results of two such experiments (Exps. 1 and 2) are recorded in Table III.

Table III. *Results calculated as percentage on non-fatty solids of original solution of spray-dried separated milk.*

Time of storage at 0° to 5° before treatment (days)	Exp. 1				Exp. 2			
	Static dialysate		Ultrafiltrate		Static dialysate		Ultrafiltrate	
	Total P	Inorg. P	Total P	Inorg. P	Total P	Inorg. P	Total P	Inorg. P
0	—	—	0.40	0.35	—	—	0.37	0.30
2	0.42	0.34	0.40	0.32	0.39	0.32	0.36	0.29
3	0.40	0.34	0.42	0.34	—	—	—	—
4	0.41	0.34	0.41	0.34	0.38	0.31	0.38	0.31
6	—	—	—	—	0.39	0.31	0.38	0.31
8	—	—	—	—	0.39	0.31	0.38	0.31
Average	0.41	0.34	0.41	0.34	0.39	0.31	0.37	0.30

A further somewhat similar series of experiments was carried out in order to compare different batches of spray-dried separated milk. In these experiments the static dialysis experiments were continued for 48 hours. The results are recorded in Table VII. Comment on this and on Table III is reserved for the general discussion of all the results.

(g) *Comparison of continuous dialysis with static dialysis.*

In this experiment (Exp. 3) Grade A.TT milk, separated at 32° and kept at 0° to 5°, was employed. After various times of storage, samples were subjected to continuous and to static dialysis, each process being allowed to proceed for 48 hours. In addition, the acid changes of the stored milk were determined [Lampitt and Bogod, 1930; 1932] by analysis of samples of the bulked milk at

various times, with the results recorded in Table V; phosphorus analyses are given in Table IV.

Table IV. *Exp. 3. Percentage phosphorus calculated on non-fatty solids of original liquid separated milk.*

Time of storage before dialysing for 48 hours (days)	Phosphorus in combination as	Continuous dialysis					Static dialysis	
		Original milk	Residue (determined)	Dialysate (by difference)	Dialysate (determined)	Residue + dialysate (determined)	Residue (by difference)	Dialysate (determined)
0	Inorg.	0.71	0.11	0.60	0.51	0.62	0.30	0.42
	Org. (by diff.)	0.35	0.33	0.02	0.05	0.38	0.28	0.06
	Total	1.06	0.44	0.62	0.56	1.00	0.58	0.48
2	Inorg.	0.72	0.18	0.54	0.46	0.64	0.31	0.41
	Org. (by diff.)	0.36	0.26	0.11	0.01	0.26	0.29	0.07
	Total	1.08	0.44	0.65	0.47	0.90	0.60	0.48
10	Inorg.	0.72	0.15	0.56	0.56	0.71	0.28	0.44
	Org. (by diff.)	0.29	0.25	0.04	0.03	0.28	0.24	0.05
	Total	1.01	0.40	0.60	0.59	0.99	0.52	0.49
Average of 3 sets of results	Inorg.	0.72	0.15	0.57	0.51	0.65	0.30	0.42
	Org. (by diff.)	0.33	0.28	0.06	0.03	0.31	0.27	0.06
	Total	1.05	0.43	0.63	0.54	0.96	0.57	0.48

Table V. *The acidity of the separated milk of Exp. 3 during storage at 0-5°.*

Time of storage (days)	Total acidity (N/4 NaOH per 100 cc. milk)	Lactic acid %	Citric acid %	Volatile acidity (N/4 NaOH per 100 cc. milk)
	cc.			cc.
0	5.96	0.020	0.194	0.64
2	6.16	0.045	0.186	0.80
10	6.60	0.015	0.163	0.64

It will be observed that the residue and the dialysate obtained by the continuous dialysis method have both been analysed, whilst the composition of the dialysate has also been calculated from the difference between the analyses of the original milk and that of the residue. These calculated figures for the dialysate are considered more accurate than the determined values, which were subject to inaccuracies incurred in the bulking of numerous samples. Nevertheless, in the circumstances there is satisfactory agreement in most cases between the calculated and the determined values for the dialysate. In the case of the static dialysis the phosphorus distribution of the non-dialysable portion cannot be determined and therefore only the calculated composition from the difference between the determinations made on the dialysate and on the original milk is given.

As before, comment on these results is reserved for the general discussion.

DISCUSSION.

The phosphorus contents recorded in Tables III and IV are calculated as percentages of the non-fatty solids of the separated milk used, in order to facilitate comparison between the different milks.

Examination of the results both of the static dialysis and of the ultrafiltration, demonstrates that when separated milk powder "solution" is stored at 0° to 5° no significant change occurs in the phosphorus distribution, for after 2, 3, 4, 6 and 8 days the figures are materially the same; they have therefore been averaged. The figures so obtained show that the sera resulting from the two methods are similar in their organic and inorganic phosphorus contents. This is confirmed by the results obtained with the further series of milk powders (Table VI).

Table VI. *Comparison of the phosphorus distribution of different batches of spray-dried milk powder.*

Figures represent percentages.

		Exp. 4				Exp. 5			
				Ultrafiltrate				Ultrafiltrate	
Time of storage at 0° to 5° (hrs.) ...		O.M.	S.D.	0	48	O.M.	S.D.	0	48
Calculated on non-fatty solids	Inorg. P	0.65	0.34	0.34	0.33	0.64	0.34	0.32	0.34
	Org. P (by diff.)	0.35	0.08	0.06	0.07	0.35	0.10	0.09	0.09
	Total P	1.00	0.42	0.40	0.40	0.99	0.44	0.41	0.43
Calculated on total phosphorus of original milk	Inorg. P	65	34	34	33	65	34	32	34
	Org. P (by diff.)	35	8	6	7	35	10	9	9
	Total P	100	42	40	40	100	44	41	43

		Exp. 6				Exp. 7			
				Ultrafiltrate				Ultrafiltrate	
Time of storage at 0° to 5° (hrs.) ...		O.M.	S.D.	0	48	O.M.	S.D.	0	48
Calculated on non-fatty solids	Inorg. P	0.69	0.34	0.32	0.34	0.68	0.33	0.32	0.33
	Org. P (by diff.)	0.31	0.09	0.09	0.08	0.31	0.08	0.09	0.09
	Total P	1.00	0.43	0.41	0.42	0.99	0.41	0.41	0.42
Calculated on total phosphorus of original milk	Inorg. P	69	34	32	34	68	33	32	33
	Org. P (by diff.)	31	9	9	8	32	8	9	9
	Total P	100	43	41	42	100	41	41	42

O.M. = original milk.

S.D. = static dialysate.

Examination of the figures obtained for the dialysate by static dialysis of freshly separated milk, Table IV, shows that no apparent change occurs on storing at 0° to 5° for 10 days, a result in agreement with the findings obtained using separated milk powder "solution." This is confirmed also by the results of the continuous dialysis, although the figures are subject to greater variation due to the more complicated technique.

The acid contents of the milk show very little change during the time of storage (Table V) and although variations in acidity do not necessarily indicate the presence or absence of proteolytic bacteria, it can be assumed from the results that little change of the type usually experienced in non-pasteurised milk had taken place.

These facts, together with the apparent constancy of the phosphorus results, were sufficient justification for averaging the various inorganic, organic and total phosphorus contents of the dialysate, Table IV.

On comparing these averaged results of the continuous dialysis with those of the static dialysis, it is evident that much more inorganic phosphorus is

dialysed by the former method, while the amounts of dialysable organic phosphorus are identical in both cases. This is taken to indicate that the continuous method disturbs the salt equilibrium by dilution causing increased solution of inorganic phosphate, presumably calcium phosphate, but does not so affect the phosphorus organically combined.

When the static dialysis or ultrafiltration experiments of the "solution" of dried milk, Tables III and VI, are compared with the static dialysis experiment of liquid milk, Table IV, it is seen that much more phosphorus, both total and inorganic, occurs in the dialysate from the liquid milk. This observation appears to be in agreement with the findings of other workers; Magee and Harvey [1926] and Bell [1925] may be cited, who state that the action of heat on milk is to cause a reduction in soluble calcium and phosphorus probably by the production of colloidal calcium phosphate. This change might possibly be induced by the condensing and drying processes in the manufacture of powder. Obviously one cannot make any general statement as the original milks were all different, but it is to be remembered that in each case the experiment was carried out on samples of bulked milk.

Caution must be observed in commenting on the amount of the organic phosphorus (the difference between total and inorganic) but certainly it is remarkably constant in all instances even under the drastic treatment of continuous dialysis.

Comparison of the results here recorded with those of previous workers is practically impossible since no really adequate series of results is available. The results collected in Table I are abstracted from papers, the chief purport of which was not necessarily that of the distribution of phosphorus in milk, and therefore the figures are not at all complete. The figures reported herein (Tables VI and VII) show that 37-44 % of the phosphorus is in the dialysable form in separated milk powder "solution" and 46 % in liquid separated milk (Table VII), while 60 % becomes dialysable under the conditions of continuous dialysis.

The phosphorus analyses of sera obtained by other workers (Table I) fall between our values for ultrafiltrate or static dialysate and those for dialysate by the continuous method. This is what would be expected since certain of the

Table VII.

		Solution of spray-dried separated milk powder						Freshly separated liquid milk Experiment III		
		Experiment I			Experiment II			Average of three sets of results		
		Original milk %	Static dialysate %	Ultrafiltrate %	Original milk %	Static dialysate %	Ultrafiltrate %	Original milk %	Static dialysate %	Continuous dialysate %
Calculated on non-fatty solids	Total solids	9.85	—	—	9.32	—	—	8.80	—	—
	Fat	0.10	—	—	0.09	—	—	0.08	—	—
	Inorg. P	0.71	0.34	0.34	0.70	0.31	0.30	0.71	0.42	0.57
	Org. P (by diff.)	0.30	0.07	0.07	0.36	0.08	0.07	0.34	0.06	0.06
	Total P	1.01	0.41	0.41	1.06	0.39	0.37	1.05	0.48	0.63
Calculated on total phosphorus of original milk	Inorg. P	70.00	34.00	34.00	66.00	30.00	29.00	68.00	40.00	54.00
	Org. P (by diff.)	30.00	7.00	7.00	34.00	7.00	6.00	32.00	6.00	6.00
	Total P	100.00	41.00	41.00	100.00	37.00	35.00	100.00	46.00	60.00

methods adopted involve either direct or indirect dilution of the milk with consequent increase in the inorganic phosphorus of the serum.

It appears justifiable to average the results obtained from the six experiments using dried milk "solution" in view of the smallness of the differences observed. The phosphorus calculated on the total non-fatty solids is therefore:

	Total P %	Inorganic P %	Organic P %
Original milk	1.01	0.68	0.33
Serum	0.40	0.33	0.07

In other words, either by static dialysis or by ultrafiltration using collodion thimbles prepared as described, about 40 % of the total phosphorus, nearly 50 % of the inorganic phosphorus and a little more than 20 % of the organic phosphorus passes into the serum.

SUMMARY.

1. A continuous dialysis apparatus using collodion thimbles has been described which enables dialysis to be carried out at a low temperature (0° to 5°) and by means of which the dialysate can be concentrated to a convenient volume and the volume of distilled water used brought to a minimum. By applying a negative pressure to the outside of the dialysing sac, ultrafiltration is combined with dialysis.

2. Dialysis of milk by this means has been compared with that by a "static" dialysis method in which minimum dilution of the milk occurs; the dialysate so obtained has been compared with an ultrafiltrate. In all cases collodion thimbles were used. The comparisons have been made between the determined amounts of total and of inorganic phosphorus in the dialysis fractions and in the ultrafiltrates.

3. It has been shown that the serum obtained by "static" dialysis of a solution of spray-dried separated milk powder has approximately the same total and inorganic phosphorus content as has that of an ultrafiltrate from the same solution.

4. Continuous dialysis compared in a similar manner with static dialysis using fresh separated milk shows that the former method causes much more phosphorus to be dialysable and that this increase is confined entirely to the inorganic phosphorus, the dialysable organic phosphorus being the same in both cases.

5. The results recorded show a certain constancy in the phosphorus distribution of the particular brand of spray-dried separated milk used. These results when compared with similar ones using bulked liquid separated milk support the contention of other workers that heat treatment reduces the inorganic phosphorus of the serum obtained by dialysis or by ultrafiltration.

6. Sufficient evidence has not yet been obtained to make a definite statement regarding the distribution of phosphorus in milk and spray process powder made therefrom, but there are indications that the heating, or it may be the desiccation, causes slight alterations in the relative amount of phosphorus dialysable.

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