PHOSPHORUS, CALCIUM AND MAGNESIUM IN MILK—II

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Calcium, magnesium and phosphorus are capable of forming both soluble and insoluble salts, but the mode of formation of the latter will be of great interest. Some workers have drawn their conclusions regarding the salt formation in milk from the analysis of the scum formed after heating the milk; others generalised merely on the strength of their experiments of dialysis or of ultra-centrifuging and so on.

Van Slyke and Bosworth[1] have shown that milk contains $CaHPO_4$ on the results of their ultra-centrifuge experiments.

Soldner and others[2] have found that on heating milk a scum is deposited which is mostly tricalcium phosphate $Ca_3(PO_4)_2$, concluding its presence originally in milk.

Palmer[3] however thinks that $CaHPO_4$, stabilised with gelatine, forms a precipitate of colloidal calcium phosphate on heating.

Various workers[4] have studied the effect of dialysis of milk and tried to draw inferences about the composition of salts in milk which do not seem to be definite. Considerable divergence in their observations is to be attributed to the fact that the experimental conditions were different in each case.

Casein—a phospho-protein body in milk was also subjected to various experiments. Somer and Hart[5] state that casein forms some kind of loose

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[1] *J. Biol. Chem.*, 1915, **20**, 135; 1916, **24**, 199.

[2] Soldner, *Landw. Versuchs-Stat*, 1885, **35**, 351; de Vries and Boekhout, *Ibid.*, 1901, **55**, 201; Purvis, Brehaut and M'Hattie, *J. Roy. San. Inst.*, 1912, **33**, 154 Grosser, *Biochem. Z.*, 1913, **48**, 422; Diffloth, *Bull. Sci. Pharmacol.*, 1904, **10**, 278.

[3] *Proc. Soc. Exp. Biol. Med.*, 1921.

[4] Gyorgy, *Biochem. Z.*, 1923, **142**, 1; Mattick and Hallett, *J. Agric. Sci.*, 1929, **19**, 452; Wardlaw, *J. Roy. Soc., N.S.W.*, 1914, **48**, 253, etc.

[5] *J. Biol. Chem.*, 1919, **40**, 137.

compound with calcium. It is reported that casein on suspension in water, or on heating for some time, loses some of its phosphorus.[6] Berggren[7] is of opinion that the phosphorus of casein is more loosely bound than is usually supposed.

To get an insight into the distribution of calcium, magnesium and phosphorus in milk the problem of the composition of milk is to be approached in several ways. In the present investigation estimations of phosphorus, calcium and magnesium were made under varying conditions. First of all milk was divided into two portions soluble and insoluble in trichloracetic acid (2 c.c. of 25% acid for 0·5 c.c. of milk diluted to 3 c.c. with water). Next the amounts of the constituents of whole milk which are dialysable were differentiated from the undialysable. Lastly fresh milk was heated after removing the fat, till scum was formed. The scum was analysed. The residual milk free from scum was divided into two portions—soluble and insoluble—in trichloracetic acid.

For the estimation of phosphorus Brigg's method was used. For calcium, the method of Masayoshi Sato and Kuchi Murata[8] with a few modifications was adopted. Magnesium was estimated by the usual gravimetric method.

Results and Discussions

TABLE I

Amount of Total, Acid-Soluble and Acid-Insoluble Phosphorus, Calcium and Magnesium

(In mg. per 100 c.c. of Milk)

No.	1			2			3		
	Total			Acid-Soluble			Acid-Insoluble		
	P	Ca	Mg	P	Ca	Mg	P	Ca	Mg
1	126·2	148·2	18·42	100·30	99·26	17·23	25·36	48·00	1·19
2	113·4	146·3	14·31	92·36	98·24	13·30	22·03	48·28	1·01
3	128·6	152·4	19·63	97·10	101·34	18·31	30·08	50·48	1·32
4	123·4	149·2	18·83	96·50	98·34	17·52	25·23	50·50	1·31
	122·95	149·0	18·00	96·57	99·30	16·59	25·68	49·32	1·21

[6] Loubavin, *Ber.*, 1877, **10**, 2237; 1879, **12**, 1021.

[7] *J. Biol. Chem.*, 1922, **95**, 451.

[8] *J. Agr. Chem. Soc. Japan*, 1933, 334–36.

TABLE II

Analysis of the Scum and the Milk after removal of Scum

(In mg. per 100 c.c. of Milk)

	1			2			3		
	Total			Acid-Soluble			Acid-Insoluble		
	P	Ca	Mg	P	Ca	Mg	P	Ca	Mg
Whole milk ..	126·4	148·20	18·42	100·30	99·26	17·23	25·36	48·60	1·89
Milk heated and scum removed	99·64	86·20	10·10	78·64	71·63	9·60	21·06	15·60	0·50
Scum ..	27·40	64·00	8·00	..	..	..	..	..	..

Scum is derived from the acid-soluble and acid-insoluble fractions.

TABLE III

Amounts of Dialysable and Undialysable Phosphorus, Calcium and Magnesium

(In mg. per 100 c.c. of Milk)

Time in hours	I			II			III		
	Total			Dialysable			Undialysable		
	P	Ca	Mg	P	Ca	Mg	P	Ca	Mg
0	126·4	148·2	18·42						
6				43·20	44·32	10·42	82·68	102·94	8·00
12				47·63	48·06	12·39	77·35	99·83	6·03
18				53·93	53·58	14·00	71·96	94·63	4·42
24				60·32	60·00	16·10	64·90	98·32	2·32
30				60·32	60·20	16·32	63·19	86·28	2·10

The above results show that even before twenty-four hours all the (maximum) dialysable constituents have passed out in the dialysate.

TABLE IV

Amounts of Dialysable and Undialysable Phosphorus, Calcium and Magnesium

(In mg. per 100 c.c. of Milk)

(*Results of 30 hours Dialysis of the same Four Samples of Milk used in Table I, respectively*)

	1			2			3			4		
	DIALYSABLE			UNDIALYSABLE			UNDIALYSABLE ACID-SOLUBLE			UNDIALYSABLE ACID-INSOLUBLE		
No.	P	Ca	Mg	P	Ca	Mg	P	Ca	Mg	P	Ca	Mg
1	62·23	60·20	16·32	63·13	86·28	2·10	48·86	51·48	1·02	14·20	33·46	Traces
2	52·90	56·30	12·23	59·17	89·10	2·08	46·38	47·96	1·07	12·20	40·34	,,
3	62·40	60·40	16·86	65·32	91·60	2·77	48·20	50·56	1·16	16·87	40·83	,,
4	55·35	55·10	15·93	66·98	82·40	2·90	47·86	49·26	1·20	18·80	32·82	,,
	58·32	58·00	15·36	63·65	87·35	2·46	47·83	49·82	1·11	15·52	36·86	..

N.B.—Sum of 1 and 3 is more than the acid-soluble of column 2 in Table I. For explanation see text.

Similarly 4, acid-insoluble contents on dialysis are less than that in column 3, Table I.

TABLE V

Analysis of Milk Dialysed for 6 and 30 Hours. (Undialysed Acid-Soluble and Insoluble Fractions.) Concentration of Phosphorus, Calcium and Magnesium

(In mg. per 100 c.c. of Milk)

	1	2			3			4		
	Casein in gm.	TOTAL			ACID-SOLUBLE			ACID-INSOLUBLE		
		P	Ca	Mg	P	Ca	Mg	P	Ca	Mg
Whole milk ..	3·2	126·4	148·2	18·42	100·30	99·26	17·23	25·36	48·00	1·19
Milk dialysed for 6 hrs. ..	2·9	78·46	103·64	..	61·00	63·00	..	16·86	40·14	..
Dialysate after 6 hrs. ..	..	44·46	44·62	10·42	..	..	..	..	..	..
Milk dialysed for 30 hrs. ..	..	63·13	86·28	2·10	48·86	51·48	1·20	14·13	33·46	..
Dialysate ..	..	62·23	60·20	16·32	..	..	..	..	..	..

N.B.—In Tables II, III and V, the values obtained in the case of an experiment, *i.e.*, one sample of milk are given. The process of heating and dialysis being semi-quantitative, the other three sets of readings obtained are not identical with these values as is to be expected, but are similar to them and lead to the same conclusions. They are not reproduced here.

TABLE VI

Amount of Phosphorus in the Undialysable Acid-Soluble Fraction

(In mg. per 100 c.c. of Milk)

As Tricalcium and Magnesium Phosphate

Expt.	*a*	*b*		*a* − *b* = *c*
	Free phosphorus, as undialysable acid-soluble phosphorus	Phosphorus as $Ca_3(PO_4)_2$	Phosphorus as as $Mg_3(PO_4)_2$	Phosphorus in organic compounds
1	48·86	26·61	1·030	21·22
2	46·38	24·77	0·922	21·56
3	48·20	26·12	0·999	21·08
4	47·86	25·45	1·030	21·38

1. *Acid-insoluble Phosphorus, Calcium and Magnesium.*—The amounts of phosphorus, calcium and magnesium in milk, acid-soluble, and acid-insoluble fractions are recorded in Table I. It can be seen that the amounts of acid-insoluble phosphorus, calcium and magnesium are 25·68 mg., 49·32 mg. and 1·21 mg. respectively. It is clear that the concentration of the acid, or more accurately pH of the resulting solution is such that all the calcium and the magnesium salts such as carbonates, phosphates, etc., must be soluble. The only source of phosphorus, calcium and magnesium must be, therefore, from those substances which are precipitated by the acid, *i.e.*, the proteins mainly casein (lactoalbumin and lactoglobulin). It has been shown that the acid-insoluble phosphorus consists of casein phosphorus *plus* lipoid phosphorus.[9]

The next question is to trace the source of calcium and magnesium. It is suggested that calcium must be in the form of a loose compound like calcium caseinate.[10] But if we look at the molecular proportions of calcium and magnesium and that of casein, no direct relationship is apparent. A more plausible explanation, however, appears to be the adsorption of the calcium and the magnesium ions by casein complex molecules, to form a colloid system and that the acid coagulates this colloid and the ions of calcium and magnesium are adsorbed by the coagulum.

[9] Acharya and Devadatta, *Proc. Ind. Acad. Sci.*, same volume.

[10] Van Slyke and Bosworth, *J. Biol. Chem.*, 1915, **20**, 135 ; 1916, **24**, 199.

2. *Scum of the Milk.*—Fresh Milk on mere heating gives rise to a scum which when dry has the following composition :—Phosphorus 27·4 mg., Calcium 64·00 mg., and Magnesium 8·00 mg. per 100 c.c. of milk (refer Table II). The explanation of this scum formation is (1) the coagulation of some constituents which are dispersed colloidally and (2) the formation of insoluble salts—both as a result of heating. The former phenomenon is evident and needs no comment. If this scum was derived exclusively from the colloidally dispersed constituents, we should expect that the acid-insoluble portion of milk, free from scum on analysis, should show a decrease, corresponding to the amounts of phosphorus, calcium and magnesium found in the scum ; but it should not affect these in that portion of milk which is acid-soluble. It is observed that there is a decrease in the acid-soluble constitutents also. Some colloidal substances like calcium and magnesium phosphates have been coagulated. There is also the precipitation of acid phosphates, and carbonates of calcium and magnesium, which before heating, in the form of acid salts, are more soluble in water than the normal salts. On heating milk for a long time in presence of few drops of methyl orange, a decrease of acidity was observed.

3. *Water-soluble and Water-insoluble Constituents.*—It is seen from Table I that the amounts of phosphorus, calcium and magnesium that are soluble in acid are respectively 96·67 mg., 99·30 mg., and 16·59 mg. per 100 c.c. of milk. Milk may contain calcium and magnesium as carbonates, phosphates, lactates, etc., both in the form of normal salts and of acid salts. To estimate the amounts of acid salts—soluble in water, milk was dialysed for 30 hours against distilled water, and the dialysate was analysed. In Table IV are given the amounts of dialysable and undialysable phosphorus, calcium, magnesium, in mg. for 100 c.c. of milk, *i.e.*, soluble salts P = 58·32, Ca = 58·00 and Mg = 15·36 mg. ; and insoluble salts P = 63·65, Ca = 87·35 and Mg = 2·46 mg.

For a particular sample shown in Table V it can be seen that the amounts of phosphorus, calcium and magnesium that pass out of the bag during dialysis for six hours are, 44·46 mg., 44·62 mg. and 10·42 mg. respectively. The constitutents which are not dialysable but soluble in acid are :—phosphorus 61·00 mg., calcium 63·00 mg. and magnesium only traces. Similar values are given for 30 hours dialysis. On comparing the total of dialysable and acid-soluble undialysed constituents, Table IV, columns 1 and 3, with the total acid-soluble constituents, Table I, column 2, there is an increase in the amounts of phosphorus, calcium and magnesium present in the acid-soluble (dialysable *plus* acid-soluble undialysable) portion, on dialysis. It may be noted also that there is a corresponding

decrease in the acid-insoluble portion (undialysable). The increase in the amounts of acid-soluble constituents of phosphorus, calcium and magnesium amounts to 9·73, 8·52, and 1·11 mg. respectively, on dialysing milk for 30 hours (Table V). This is due to the fact that casein, which had adsorbed calcium and magnesium ions, on coming in contact with water becomes partly soluble, otherwise quite insoluble in acid. Further, it can be seen that casein decreases in undialysable milk from 3·2 to 2·9 gm. per cent. It is also possible to detect some casein in the dialysate. Therefore our assumption that the acid-insoluble phosphorus, calcium and magnesium as being associated with casein is corroborated (*vide* below).[9]

4. *Acid-soluble Phosphorus, Calcium and Magnesium.*—The insoluble or undialysable fraction is further subdivided into acid-soluble and acid-insoluble parts. From Table IV, column 3, it will be seen that the amounts of phosphorus, calcium and magnesium which are soluble in acid but insoluble in water are as follows : phosphorus 47·83 mg., calcium 49·82 mg. and magnesium 1·11 mg. per 100 c.c. of milk (*cf.* Table V). These amounts refer to such salts as tricalcium and magnesium phosphates. If we look to the amounts of phosphorus we find that it is much more than what should correspond to tricalcium and magnesium phosphates as noted in Table VI. That surplus phosphorus may be the portion of phosphorus combined to the acid-soluble organic phosphorus discussed elsewhere.[9]

Summary and Conclusions

In conclusion, it may be said that the portion of milk insoluble in acid is mostly casein which had adsorbed some ions of calcium and magnesium. The portion soluble in acid but insoluble in water consists of tricalcium and magnesium phosphates and organic phosphorus. Scum which is collected after heating the milk is derived both from the acid-insoluble and from the acid-soluble portions. On dialysis some acid-insoluble portion becomes water soluble. The water-soluble portion of milk consists of acid phosphates of calcium and magnesium. The amounts of phosphorus, calcium and magnesium present in water, acid-soluble and insoluble fractions—and in scum of 100 c.c. of milk are recorded.

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