COMPOUNDS OF PHOSPHORUS IN CAMEL MILK

By

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Camel milk is a good source of phosphorus and its pattern of distribution along with calcium forms a major inorganic nutrient for the developing young one of the species. In contrast to the abundance of information regarding the distribution of phosphorus compounds in muscle, relatively little is known about the distribution of phosphorus compounds in other body fluids and secretions like milk. Vertebrate and invertebrate tissues and muscle have been investigated for these compounds and their distribution pattern studied by many workers, (4,7,9). In India too, similar studies have been undertaken on the milk of buffalo and muscle of prawn; (1, 2). The present investigation was undertaken to determine the type of distribution of compounds of phosphorus in camel milk.

MATERIALS AND METHODS

Careful sampling was important in the investigation. Uniformity was maintained when drawing the sample. In all seven samples of camel milk (individual) were obtained for the investigation from the Government controlled camel breeding station.

Trichloracetic acid extract

The acid extract was prepared on similar lines adopted by Acharya and Devadatta (1). The quantity of milk taken for analysis was 2 *ml.* in each instance. The concentration of the acid and the working temperature were so controlled as not to interfere with the hydrolysis of esters of phosphoric acid. It was found that a 20% acid was preferable to 5% acid on account of minimum volume and so it was used for deproteinizing the milk. The findings of Kay, (6) that when trichloracetic acid extract was kept in contact with the acid, there occurred a progressive increase in the phosphate fraction, was also confirmed by us.

Fractionation of Acid soluble Phosphorus in Barium Hydroxide

The method of fractionation of acid soluble phosphorus in camel milk was on similar lines adopted by Eggleton and Eggleton (4) with slight modification. A barium precipitate was obtained by adding crystalline barium hydroxide to the acid extract to the turning point of phenolphthalein (*p*H 9) (3). Neutralization of the extract was carried out at low temperature. The precipitate and filtrate were quantitatively collected by repeated neutralization and centrifugation. The entire precipitate was dissolved in 0.1N hydrochloric acid and made to volume to constitute fraction '*A*', while the filtrate was likewise collected, made to volume to constitute fraction '*B*'. Fraction '*A*' consisted of mostly organic and inorganic phosphorus compounds insoluble at *p*H 9, while fraction '*B*' consisted of organic phosphorus compounds like esters of phosphoric acid with carbohydrates, barium hydroxide soluble nucleotides and some in-, organic phosphates escaping preciptiation by barium hydroxide. The estimation of phosphorus compounds was confined to the following fractions. In fraction '*A*' total acid soluble phosphorus was determined after digestion, oxidation and hydrolysis with sulphuric acid and hydrogen peroxide. This method was found to be more convenient than that of Lowry and Lopez (8) for the estimation of inorganic phosphorus compounds in presence of labile phosphates.

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The *ortho* and *pyor* phosphates where estimated prior to digestion, the former directly in the precipitate and the latter after 7 minutes hydrolysis in acid.

The fraction 'B' of the soluble barium salts consisted of organic phsophorus compounds like esters of phosphoric acid with carbohydrates, barium hydroxide soluble nucleotides and some inorganic phosphates escaping preciptiation by barium hydroxide. An aliquot of the soluble barium salts was used for the estimation of directly determinable phosphorus by the procedures of Fiske and Subba Row (5). This fraction corresponded to the easily hydrolysable phosphorus compound of organic type. The non-hydrolysable organic phosphorus compound of '*B*' fraction was obtained by determing the total phosphorus by digestion and hydrolysis and by difference between total phosphorus and easily hydrolysable phosphorus compound. (Table I)

Acid Insoluble fraction

This fraction mainly consisted of lipoid phosphorus and Casein phosphorus. The lipoid phosphorus was estimated by adopting the methods of Youngburg and Youngburg (10) and Fiske and Subba Row (5). The phosphorus bound to Casein was determined by Fiske and Subba Row (5) procedure. The casein was prepared in a pure state and a small quantity was used for the estimation (0.05 *gm*). Since there was little organic matter associated with casein, igniition with sodium carbonate was not done. (Table I).

Table I

Distribution of phosphorus compounds in the acid soluble fraction of camel milk. Phosphorus Values are given in mg per 100 ml of fresh milk (individual sample) Measure of Variability is standard error of the mean.

Sample No.	Total Phosphorus	Total acid soluble phosphorus	Fraction 'A' INSOLUBLE IN $Ba(OH)_2$			Fraction 'B' soluble in $Ba(OH)_2$	
			Inorganic		Organic	Organic	
			Ortho P;	Pyre P	Non-hydro-lysable P	Non-hydro-lysable P	Easily hydrolys-able P
1	96.41	76.44	50.44	11.60	4.26	4.85	8.80
2	97.01	75.25	50.09	11.24	4.50	4.10	9.01
3	96.96	77.06	50.19	10.96	4.16	4.09	8.67
4	95.86	76.58	51.04	10.56	4.18	1.56	8.56
5	95.47	78.04	52.02	10.46	4.95	5.02	8.71
6	95.81	74.51	51.94	11.90	5.01	5.04	9.01
7	96.21	76.04	51.09	12.10	5.34	4.02	9.02
Mean	96.10	76.28	50.67	11.25	4.61	4.52	8.81
S.E.	1.32	1.02	0.92	1.10	0.85	0.99	1.20

Results and Discussion

It was of interest to compare our method with that devised by Acharya and Devadatta (1) for the separation of acid soluble phosphates in buffalo milk. The distribution of the organic and inorganic phosphorus compounds was more or less on similar pattern.

In Table I are given the concentration of different types of phosphorus compounds in camel milk. Table III indicates the maximum concentration of trichloracetic acid that could be employed to extract all the acid soluble phosphorus besides deproteinizing the milk. In our experiments 20% acid was found to be the proper concentration that could be employed

Table II

Distribution of phosphorus compounds in acid insoluble fraction of fresh camel milk.

Measure of Variability is standard error of mean

Sample Number	Total Phosphorus	Total acid insoluble phosphorus	Liqoid phosphorus	Casein Phosphorus
1	96.41	20.84	3.40	17.89
2	97.01	20.89	3.67	17.01
3	95.98	20.91	4.02	16.21
4	95.86	20.10	3.89	17.91
5	95.47	20.24	4.02	16.59
6	95.81	20.26	3.47	16.66
7	96.21	20.17	3.50	17.01
Mean	66.10	20.48	3.71	17.04
S.E.	1.32	1.18	1.25	1.02

Table III

Recovery of acid soluble phosphorus compounds using various concentration of trichloracetic acid

(Quantity of milk used 1 ml.)

Concentration of 2 *ml* trichloracetic acid used %	Phosphorus in *mg.* percent of milk
0.5	32.20
13.0	42.80
18.0	68.50
20.0	85.60
22.0	90.00
25.0	98.20

for the fractionation of acid soluble phosphorus compounds in milk. It is seen that t phosphorus exists to the extent of 96.10 *mg.*%. The total acid soluble phosphorus% is see be 78 *mg.*%of the total phosphorus and the remaining 22 *mg*% is acid insoluble. The total soluble phosphorus of camel milk is 72.20 *mg* % and 20.20 *mg*% is acidinsoluble. In the soluble fraction the phosphorus occurs both as organic and inorganic variety. Nearly 1 of the total phosphorus is in the organic form and 63% in the inorganic form.

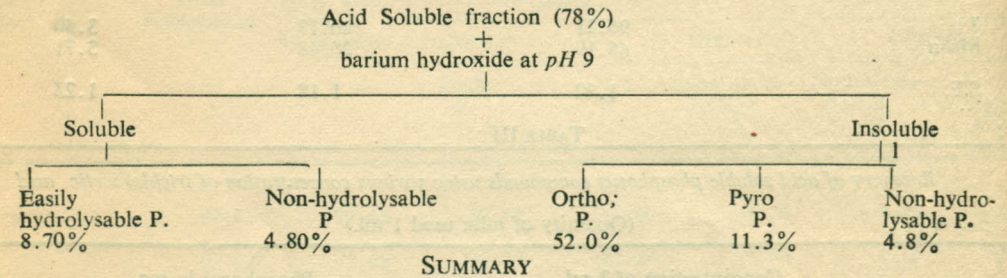
When trichloracetic acid extract of milk is treated with crystalline barium hydroxide *pH* 9, three types of organic phosphorus compounds could be estimated. (i) Phospho compounds soluble in barium hydroxide at *pH* 9 which could be estimated by the applicati of Fiske and Subba Row procdure (5), (ii) compounds of phosphorus soluble in barium hydr xide at *pH* 9 which could only be estimated after oxidation and hydrolysis and (iii) compoun of phosphorus insoluble in barium hydroxide which could be estimated after oxidation a hydrolysis.

The inorganic phosphorus compounds of camel milk exists in two different forms : (*a*) the ortho and (*b*) the pyro phosphorus respectively, being 52.00 and 11.50% of the total phos phorus.

Acid insoluble phosphorus compounds in milk mainly consist of lipoid and casein phos phorus. 4% of the total phosphorus constituted lipoid phosphorus and 18.75% makes up the casein phosphorus.

It is felt that this scheme of fractionation can be incorporated and used as a general system of analysis for the sepration of various compounds of phosphorus in the acid soluble fraction.

Scheme of distribution of phosphorus in milk



SUMMARY

A Scheme is given for the separation of the acid soluble phosphates in camel milk. Acid soluble inorganic phosphorus in camel milk exists in ortho and pyro forms and the organic phosphorus in two forms i.e., soluble in barium hydroxide at pH 9 and the insoluble phosphaphate in barium hydroxide at pH 9. Acid insoluble phosphorus compounds are lipoid and casein phosphorus compounds.

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