feces, and where a small number of units was fed the absolute amount in the feces was small. Direct irradiation of the infant resulted in a moderate amount of antirachitic in the blood and no excretion of vitamin in the feces.

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Comparative Analysis of Forms of Calcium and Inorganic Phosphorus in Human and Cow's Milk.

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It is a common observation that rickets occurs less frequently in breast-fed infants than in those which are artificially fed. The antirachitic superiority of human milk is not due to a greater content of vitamin D.[1] Moreover, cow's milk contains more Ca and P than does breast milk and the ratio of Ca:P is lower in the former. It was thought that, in spite of its lower content of total Ca and P, human milk might contain a larger amount of the most effective forms of these elements and that therein might lie an explanation for the difference in antirachitic value of the 2 kinds of milk.

In a recent communication,[2] a technic was described which rendered it possible to separate the Ca of serum into 4 forms and the P into 2. The technic involved removal, by means of dry $BaSO_4$, of the adsorbable forms of Ca and of inorganic P from the serum and from the corresponding ultrafiltrate. It was shown[3] further that one of the forms, namely the filtrable, adsorbable Ca-P complex, was the one primarily involved in bone formation. Gyorgy,[4] as well as others, have suggested that the Ca and P of milk exist in the form of complex salts and Klinke[5] has demonstrated that more than half of the Ca of cow's milk can be adsorbed with $Al(OH)_3$. There is, thus, some indication that milk may contain a fraction similar to the filtrable, adsorbable Ca-P complex of serum. Accordingly, the above

[1] Hess, A. F., and Weinstock, M., *Am. J. Dis. Child.*, 1927, **34**, 845. Outhouse, J., Macy, I. G., and Brekke, V., *J. Biol. Chem.*, 1928, **78**, 129.

[2] Benjamin, H. R., and Hess, A. F., *J. Biol. Chem.*, 1933, **100**, 27.

[3] Benjamin, H. R., *J. Biol. Chem.*, 1933, **100**, 57.

[4] Gyorgy, P., *Biochem. Z.*, 1923, **142**, 1.

[5] Klinke, K., *Ergeb. d. Physiol.*, 1928, **26**, 235.

mentioned technic was applied to a study of the forms of the Ca and inorganic P in human and in cow's milk. Irradiated cow's milk was also included in the investigation because of its increasing importance as an antirachitic agent.

It should be stated at once that, as regards its forms of Ca and P, milk represents a more complicated system than serum. It has been shown,[6] for example, that casein is capable of adsorbing Ca added to milk and the possibility has been raised[7] that some of the Ca and P normally present in milk is adsorbed to casein. The presence of citrates has also been suggested (Klinke) as a factor in the formation of Ca complexes in milk. The fractions separated from milk by means of $BaSO_4$ adsorption and ultrafiltration are, therefore, probably not single entities and the results are presented, not as indicating the complete partition of Ca and inorganic P but rather as a comparative analysis of different kinds of milk.

Method. The methods used in this study were those described for serum, with the following exceptions. For the adsorption test the milk or ultrafiltrate was diluted so as to contain about 10 mg. % of Ca. With human milk it was found that the same results were obtained when undiluted milk was used. For the determination of inorganic P the Fiske-Subbarow method[8] was used. The results were compared to those obtained by the use of a titrimetric method and found to be identical. Total Ca was determined by the method of Rothwell.[9] Ultrafiltration of the human milk was continued overnight. This was necessary because it was found that in order to obtain 3 cc. of ultrafiltrate from human milk 6 to 24 hours were required, whereas this amount was obtained with cow's milk in less than 3 hours. The explanation for this phenomenon is wanting. Skimming the human milk did not hasten ultrafiltration. The viscosity of this skimmed milk was found to be less than that of skimmed cow's milk. After removal of the casein with acid and rennin the whey of human milk still filtered more slowly than that of cow's milk. This experiment was not entirely satisfactory, however, since it was found impossible to obtain entirely clear whey from human milk by this means. That the prolonged ultrafiltration did not alter the composition of the ultrafiltrate was ascertained by a comparison of ultrafiltrates of the same sample of cow's milk obtained after 3 and after 24 hours.

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[6] Ballowitz, K., *Biochem. Z.*, 1932, **256**, 64.

[7] Marui, S., *Biochem. Z.*, 1927, **173**, 381.

[8] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1926, **66**, 375.

[9] Rothwell, C. S., *J. Biol. Chem.*, 1925, **65**, 129; 1927, **75**, 23.

TABLE I.

Samples of milk*	Ca mg. per 100 cc. Milk Total	Milk Adsorbable	Ultrafiltrate Total	Ultrafiltrate Adsorbable	Inorganic P mg. per 100 cc. Milk Total	Milk Adsorbable	Ultrafiltrate Total	Ultrafiltrate Adsorbable
Human A—Whole	25.2	14.0	17.9	7.1	8.4	5.1	5.1	2.2
" B "	23.4	11.6	14.4	5.3	7.4	5.4	2.5	1.1
" C "	—	—	14.8	5.5	3.6	—	2.9	1.8
" D "	34.8	16.8	21.8	14.1	7.2	3.8	6.1	3.1
" E "	24.6	10.0	19.4	7.2	5.1	3.6	3.8	2.9
" C—Skimmed	23.0	11.1	15.1	4.8	3.5	2.6	3.0	1.8
" D "	32.8	13.4	21.6	14.3	8.3	—	4.2	1.9
" E "	25.5	10.7	18.2	7.8	5.3	—	3.1	2.7
Cow's A—Raw	129.6	—	37.2	—	66.3	21.2	34.1	13.5
" B "	118.8	70.5	38.5	21.4	69.4	32.3	34.3	6.1
" C "	129.3	90.9	41.9	17.0	73.4	39.8	38.6	11.6
" D "	129.9	76.0	35.7	10.7	76.0	29.8	37.3	9.6
" B—Pasteurized	119.3	50.8	38.8	23.2	63.4	30.1	34.7	10.3
" E "	122.4	61.6	38.4	16.3	74.5	39.9	34.8	11.5
" F "	133.4	89.0	33.8	18.0	79.7	38.6	36.4	14.7
" G—Raw, irradiated	131.4	85.3	35.1	17.3	73.0	46.9	39.1	14.9
" H—Pasteurized, irradiated	131.9	99.3	34.6	18.1	76.0	41.3	36.6	14.7
" I " "	108.0	53.5	33.3	15.7	68.8	28.2	29.1	5.9
Averages		%	%	%		%	%	%
Human	27.2	12.5 (47)	17.9 (66)	8.3 (30)	6.1	4.1 (67)	3.8 (62)	2.2 (36)
Cow's, raw	126.9	79.1 (62)	38.3 (30)	16.3 (13)	71.3	30.9 (43)	36.1 (51)	10.2 (14)
" pasteurized	125.0	67.1 (54)	37.0 (30)	19.2 (15)	72.5	36.2 (50)	35.3 (49)	12.2 (17)
" irradiated	123.8	79.3 (64)	34.3 (28)	17.0 (14)	72.6	38.8 (54)	33.9 (47)	11.8 (16)

*The samples of human milk were all individual, and varied from 10 days to 5 months postpartum. Those of cow's milk were all composite.

It may be seen from the table that a partition somewhat similar to that of the Ca of serum may be made of the Ca in milk. The total may be divided into (1) a filtrable adsorbable fraction; (2) a filtrable remainder, probably containing the ionized fraction (column 3 minus column 4); (3) a non-filtrable, adsorbable fraction (column 2 minus column 4), and (4) a non-filtrable remainder, possibly bound to protein (column 1 minus column 3). In respect to the relative amounts of these fractions, human milk resembles serum, whether human or bovine, more closely than does cow's milk. In human serum 45% of the Ca is capable of adsorption by $BaSO_4$, 55% is ultrafiltrable, the filtrable adsorbable Ca-P complex constituting 35% of the total. These figures compare closely with the corresponding values for human milk: 47, 66, and 30%, respectively. *The filtrability of the Ca in human milk is more than twice as great as that in cow's milk, and it contains a much larger proportion of the filtrable adsorbable Ca fraction.* Thus it would seem that, for purposes of bone formation, the Ca in human milk is in a more available form than that in cow's milk. On the other hand, the absolute amount of the filtrable adsorbable fraction of Ca is small in human milk in comparison to that in cow's milk, so that even a two-thirds dilution of the latter would supply the infant with a greater amount of this particular form than undiluted human milk. It is, therefore, unlikely that these differences in composition are directly responsible for the greater efficacy of human milk in the prevention of rickets.

As in the case of serum, irradiation of cow's milk had no effect on the Ca partition. This held true also for pasteurization.

The partition of inorganic P in milk is different from that in serum. In the latter, the inorganic P normally is entirely ultrafiltrable and only 2 fractions can be separated by the use of the technic employed. In milk, on the other hand, only about one-half to one-third of the total P is found in the ultrafiltrate. Furthermore, not all of the adsorbable P is ultrafiltrable so that the P of milk can be separated into 4 fractions, corresponding to the 4 calcium fractions. Indeed, the relative concentration of the filtrable adsorbable P in the various samples of milk closely parallels that of the filtrable adsorbable Ca.

As with the Ca, the absolute amount of all fractions of P was greater in cow's than in human milk and likewise, the partition was not altered either by irradiation or by pasteurization.