Human Milk Whey Proteins.* (23595)

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The proteins of human milk whey have received relatively little study in comparison with the readily available bovine system. From a physical-chemical standpoint this system is quite complex and numerous electrophoretic(1,2) and ultracentrifugal(2) components are in evidence. It was of interest to determine whether any of these represented or contained the blood serum albumin and γ-globulins entities. These proteins have been found in bovine milk whey(3-6) and the γ-globulins in particular have received rather extensive study.

Methods. Human milk samples collected within a week postpartum were treated with rennin at pH 4.8-5.0. The casein was removed by centrifugation and the supernatants were either *lyophilized* following dialysis against distilled water or dialyzed against a given buffer preparatory to electrophoretic, ultracentrifugal or immunochemical assay. *Electrophoretic components* were numbered in order of increasing mobility in pH 8.6, $\mu = 0.1$, sodium diethylbarbiturate buffer. Ultracentrifuge components were designated alphabetically in terms of decreasing rates of sedimentation in pH 7.4, $\mu = 0.2$ potassium phosphate buffer. Samples of the *fastest and slowest migrating components* of the milk whey were separated by pipetting of the respective ascending and descending electrophoretic boundaries following maximum resolution. These samples were used for subsequent ultracentrifugal and qualitative immunochemical experiments. Quantitative immunochemical studies employed antibodies to purified human γ_2-globulin and to serum albumin. These showed no cross-reactions which indicated that they were not contaminated with each other. The quantitative precipitin reactions were carried out as previously described(7).

Results. A typical electrophoretic pattern of a pool of 4 human wheys is shown in Fig. 1 and is in keeping with previously reported results(2). Recent studies by Schulte and Müller(1) have indicated the presence of 7 electrophoretic components 2 of which were dialyzable.

Ultracentrifuge diagrams of pooled milk whey and material obtained by pipetting of electrophoretic boundaries 1 (descending) and into area 4 (ascending) are shown in Fig. 2, the analytical values being given in Table II. The milk shows at least 5 components, the major portion consisting of a relatively heterogeneous and slowly sedimenting portion.

The proteins comprising areas 4 and 5 of the electrophoretic pattern show a major component sedimenting near 4.4 S, the residual and slower sedimenting material being relatively heterogeneous (Fig. 2). The latter proteins are analogous to the 1.6 S component of the whey and these low molecular weight materials appear to be distributed throughout the electrophoretic range of the whey. The sedimentation constant of the major component (4.4 S) indicates that it could be serum albumin(8,9). The electrophoretic mobility of the proteins in areas 4 and 5 also covers the range of albumin(10,11). Area 4 exists as a shoulder of component 3; there is a considerable amount of rather heterogeneously charged protein migrating between

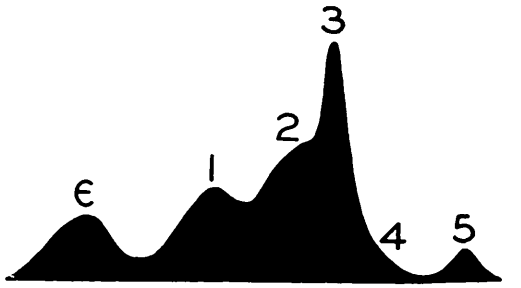

FIG. 1. Descending electrophoretic pattern of human milk whey in pH 8.6, $\mu = 0.1$ diethylbarbiturate buffer. Duration of experiment was 194 min at 4.1 volts cm^{-1}.

* This work was supported in part by a grant from the Wisconsin Alumni Research Fn.

TABLE I. Electrophoretic Composition of Human Milk Whey.

Sample	Component (%) 1	2	3	4	5
1	28	23	31	13	5
2	29	26	26	9	10
3	24	24	34	15	3
4	24	25	34	11	6
5	32	22	38	5	3
6	30	20	38	10	3
7	38	25	23	9	5
Range	24-38	20-26	23-38	5-15	3-10
Avg	29	24	32	10	5
Pool*	29	25	36	5	5
Avg mobilities†	2.4	3.7	4.6	5.6	7.1

* A pool of 4 samples not subjected to individual assay.

† Mobilities $\times 10^5$ cm^2 volt^{-1} sec.$^{-1}$.

areas 4 and 5.

Ultracentrifuge study of the material comprising area 1 of Fig. 1 (see lower diagram of Fig. 2) shows that the major portion consists of the above slow sedimenting material (near 1.6 S). However, 2 other components are seen. The faster of these sediments is near 17 S and is present in smaller amounts than the material sedimenting near 10.5 S. It may be analogous to the 18-19 S component of the γ_1-globulin fraction of human serum (12,13).

Immunochemical studies: Interfacial precipitin tests with the material of area 1 (Fig. 1) showed that it reacted with rabbit antibody to human serum γ_2-globulin but not with anti-human serum albumin. Attempts to demonstrate a significant amount of protein in the electrophoretic region between the salt boundary and area 1, which is the mobility region of human γ_2-globulin, were unsuccessful.

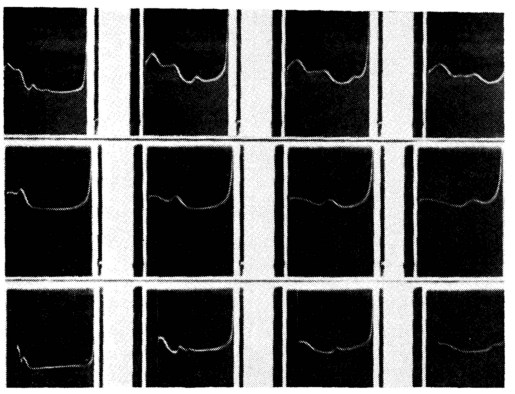

FIG. 2. Ultracentrifuge diagrams of human milk whey (upper), electrophoretic areas 4 and 5 (center) and area 1 (lower) separated by pipetting boundaries of Fig. 1. Direction of sedimentation at 59,780 r.p.m. is to the right.

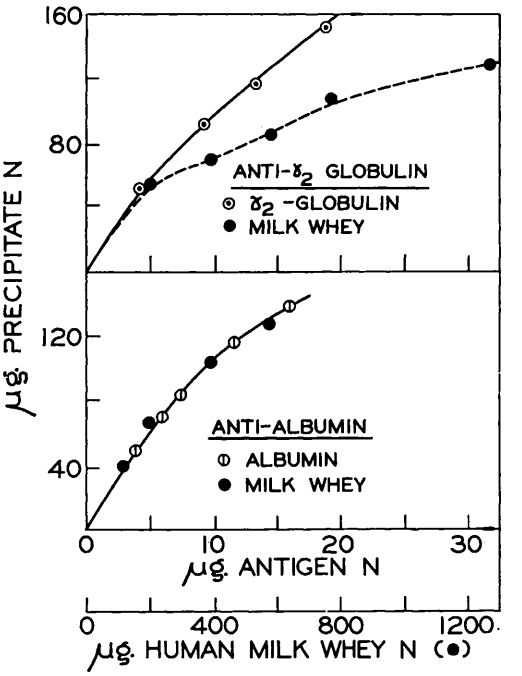

FIG. 3. Immunochemical reactions of human milk whey with a rabbit anti-human serum γ_2-globulin preparation (upper) and with a rabbit anti-human serum albumin preparation (lower).

The proteins of area 5 gave a weak reaction with rabbit antiserum albumin while those contained in areas 4 plus 5 gave a qualitatively stronger test. They did not contain components reacting with the antiserum to γ_2-globulin. The electrophoretic mobility range of these fractions would encompass serum albumin.

Quantitative precipitin studies employing pooled whey and the above antibody preparations gave the results plotted as Fig. 3. These immunochemical assays were carried out in

TABLE II. Ultracentrifugal Composition of Human Milk Whey.

Component	%	$s_{20,w}$*
a	3.0	11.7
b	6.6	10.0
c	17.5	6.0
d	17.4	4.6
e	55.5	1.6

* Values $\times 10^{13}$ cm sec.

the antibody excess region. It can be seen that the whey contains a component identical with serum albumin and that this component constitutes near 2.5% of the whey proteins The immunochemical reactions with antibody to γ_2-globulin gave assay values from an initial 2.5% to values near 1%. The data indicate that a cross-reactive protein(s) is present in the milk whey. It is known that γ_2-globulins show immunological cross-reactions with other proteins in the γ_1- and β-globulin areas(14-16) and the present results may indicate the presence of other serum proteins in the milk whey.

Summary. The results of electrophoretic, ultracentrifugal and immunochemical analyses indicate that serum albumin is present in human milk whey. The immunochemical assay shows that it comprises 2.5% of this system. Proteins with the physical properties of human γ_2-globulin could not be demonstrated although material showing immunological cross-reactions with such proteins is present.

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Received October 3, 1957. P.S.E.B.M., 1957, v96.

Inactivation of Biologically Active ("Endotoxic") Polysaccharides by Fresh Human Serum. (23596)

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Recent work in this laboratory concerned with the interaction of endotoxic polysaccharides with biological systems has utilized inactivation of T2r+ coli phage as an indicator for the reaction with properdin. As expected, such polysaccharides nullified the anti-viral effect of human serum properdin. The influence of serum on other biologic activities of these polysaccharides was next investigated. The first *in vivo* phenomenon examined was the response of mouse sarcoma to a tumor-necrotizing polysaccharide after incubation with serum of known properdin content. When it was found that fresh serum abolished this property, it was first thought that this was a consequence of complexing with properdin. Contrary to expectation, however, incubation with properdin-deficient serum also inactivated the polysaccharide. These findings brought to mind unpublished experiments (1955) designed by Pillemer and Landy to detect physicochemical changes in *Salmonella typhosa* endotoxin upon exposure to fresh human serum. This treatment greatly increased the dispersion of the polysaccharide: the turbidity markedly de-

* N.I.H., Public Health Service, U. S. Depart. of Health, Education and Welfare.