A STUDY OF BLOOD SERUM PROTEINS BY ELECTROPHORESIS[1]

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The primary object of this investigation was to study the changes in the proteins of the blood serum and other related organs caused by various physiological conditions, and in this manner gain a better understanding of the nature of these proteins and of the mechanism by which they are stored and utilized by the body. In particular, the experiments here recorded study the changes in the blood serum proteins of the adult of one species brought about by the ingestion of colostrum from an animal of a different species. To study these changes salting-out and electrophoretic experiments have been used.

Famulener, Little, Smith, and others (1, 6, 9, 10) found many years ago that antibodies are transferred from the mother to its young by colostrum. The literature is well reviewed by Traum (14). Howe (2) and Orcutt and Howe (7) paralleled this work by observing a simultaneous increase in globulins. Recently Schneider and Szathmary (8) confirmed earlier results with experiments on a number of different species. These experiments have been extended by the authors by feeding cow's colostrum to man and to the adult rat with a resulting increase in globulin concentration.

This appreciable change in the blood serum proteins of the adult rat was first noted in 1936 by one of the authors during a study of the building up of serum globulins in the newborn and adult animals. It was evident when a comparison was made of the salting-out curves made from blood serum of adult rats fed a normal stock diet and from the serum of adult rats fed colostrum. The curve obtained from the serum of the latter showed a protein fraction which was not visible in the former.

The procedure used in these experiments has been fully described in a previous communication (3). To weighed portions of the serum dialyzed against a 5 per cent potassium citrate solution at pH 6.8, dry potassium

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citrate was added to make the desired salt concentration. The pH was maintained by added citric acid. The precipitated protein was separated on filters, and the liquid phase was analyzed for protein and potassium. All analyses were made on weighed samples. Processes were carried out as rapidly as possible at 0°C.

Figure 1 shows the curve from normal rat serum. The results were plotted as per cent by weight on Gibbs' triangular phase rule diagrams. Protein dried at 110°C., potassium citrate, and water are to be found at the apices of the triangles. Only a portion of the diagram is given, as may be seen from the percentages along its sides. The heavy line rep-

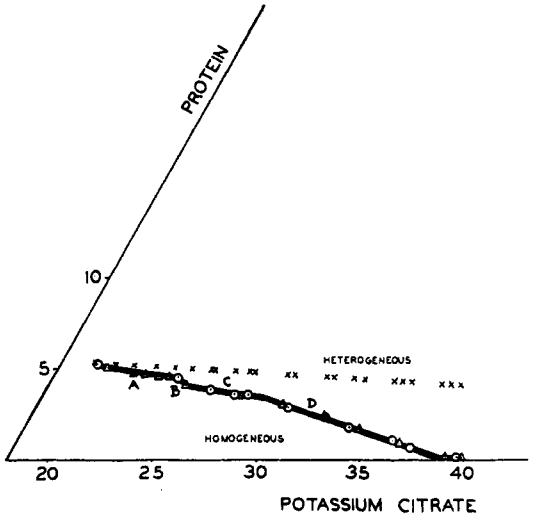

FIG. 1. Rat serum; stock diet. ×, total composition; ○, liquid phase; △, liquid phase

resents the analyses of the liquid phases in contact with the solid phases. *A* is considered the first fraction to appear as a solid phase on adding potassium citrate; *B* is precipitated on further addition, coming down in a very narrow range of salt concentration; and *C* is precipitated gradually as the salt concentration becomes higher. Finally *D*, the albumin fraction, appears.

In figure 1, the ○'s, and △'s are experimental points on a line separating the liquid phase from the heterogeneous mixture. They were obtained from two different pools of blood serum, each from thirty stock male rats, 100 days old. They coincide. There are four fractions, as shown by the changes in direction of the curve with increasing amounts of potassium citrate.

In figure 2, also from serum of thirty male rats, 100 days old but colostrum-fed for 2 days, an extra change of direction occurs, showing the separation of another solid phase, C_2.

Other proteins, such as casein, serum albumin, serum globulin, liver, and kidney, did not have this effect. After a 70 per cent protein diet of any of the above mentioned proteins is fed for 2 days, curves of the type found in figure 3 are found. This curve is made after feeding serum globulin.[2] The C_2 is missing or so small as to be negligible. Feeding of the dried colostrum[3] for 2 days gave a curve in which C_2 is high but not as greatly increased as in the serum where fresh colostrum was used

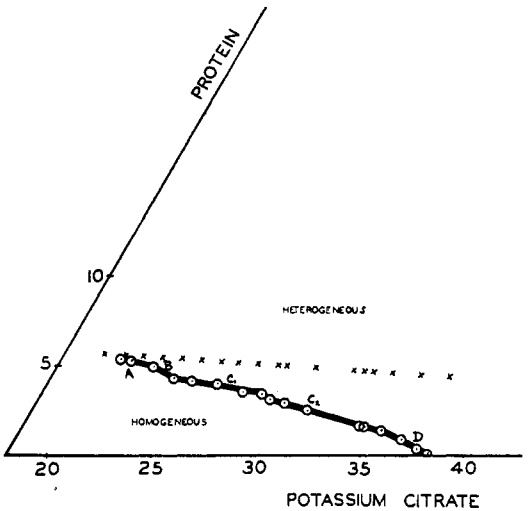

FIG. 2. Rat serum; diet of fresh cow's colostrum and milk for 2 days. ×, total composition; ○, liquid phase

After 7 days' feeding of dried colostrum the presence of the unusual fraction is proportionately less and is somewhat masked by the great increase of C_1. Figure 4 is made from such serum. This curve may be compared with those made from the serum of rats on other high-protein diets for the same length of time. In figure 5 the results are given after feeding a globulin diet for 7 days. The C_2 phase is still not very visible.

Electrophoretic study of the serum proteins, in serum both from stock rats and from those fed colostrum, was carried out in the Tiselius ap-

[2] Horse serum globulin was separated by half-saturation with ammonium sulfate, denatured, dried, and washed.

[3] The colostrum was spray-dried without preheating.

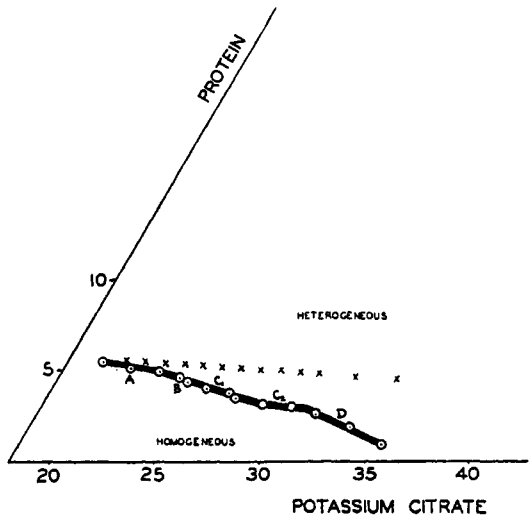

FIG. 3. Rat serum; diet of serum globulin for 2 days. ×, total composition; ○, liquid phase

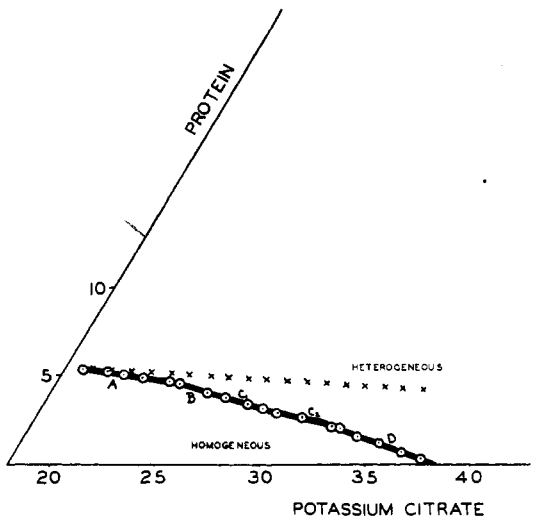

FIG. 4. Rat serum; diet of dried cow's colostrum for 7 days. ×, total composition; ○, liquid phase

paratus (11), using buffers 0.02 *M* in phosphate and 0.15 *M* in sodium chloride. Several pH values were used for the normal serum, but since it was found that the separation of the globulins is very satisfactory at a pH of 6.2, this value was used extensively. A potential gradient of 4.4 volts per centimeter was used in these experiments, since we had found that a higher voltage produces an increased number of protein fractions. This is probably due to the breaking of loose protein complexes by the application of a sufficiently high potential gradient (5).

The electrophoretic diagram for normal rat serum has been given by the authors in a previous article (5). In this communication it was

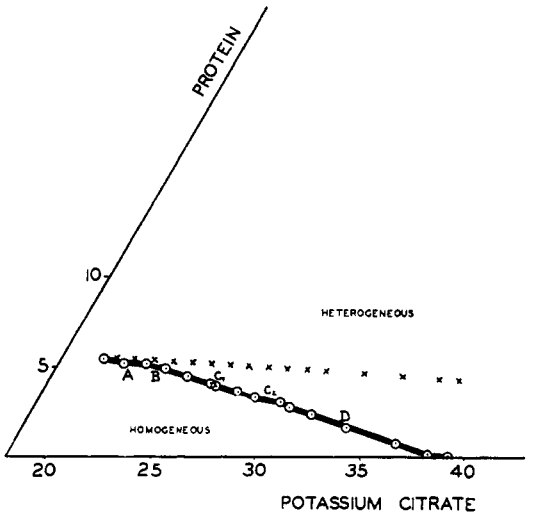

FIG. 5. Rat serum; diet of serum globulin for 7 days. ×, total composition; ○ liquid phase

reported that normal rat serum yields only three protein fractions,—an albumin and two globulins that were identified with the α- and β-fractions found by Tiselius and coworkers in horse serum. Further investigation has shown that a third globulin fraction is present in the rat serum, and that its concentration is normally so low that the *Schlieren* band corresponding to this globulin is visible only when undiluted serum is used, and then only faintly. Thus, when the serum is diluted with buffer, as it usually is for electrophoretic determinations, this fraction seems to be absent. The globulin that occurs at low concentration corresponds to the α-fraction described by Tiselius, so that the two fractions previously reported as α and β are found to correspond to Tiselius' β and γ.

The serum from colostrum-fed rats shows the line corresponding to the α-globulin quite strongly, even in serum diluted 1 to 3 with buffer. This protein becomes a considerable portion of the total globulins after 24 hr. of feeding colostrum. This is, then, one of the changes that occur in rat serum, owing to the ingestion of colostrum. The increase in the concentration of one of the naturally occurring blood serum proteins is similarly found to occur in man. Eighty grams of dried colostrum, ingested during 2 days, noticeably increases the concentration of α-globulin in human serum. Figure 6 shows reproductions of electrophoretic diagrams, illustrating this effect in rat serum (diluted 1 to 3 with buffer) and human serum (diluted 1 to 4).

Tiselius (12) has found that the best source of the α-globulin in horse serum is the pseudoglobulin in which the γ-fraction also appears. This is comparable to the *C*-fraction of the salting-out curves. If this is also true for rat serum, and there are indications that it is, then the α-fraction may be the same fraction as the C_2 visible in the salting-out curves afte

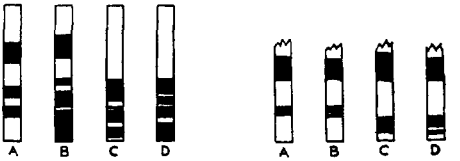

FIG. 6. (a) Electrophoretic diagrams showing increase in α-fraction. A and B, rat serum before and after ingestion of colostrum; C and D, human serum. (b) Electrophoretic diagrams showing division of the γ-fraction. A and B, rat serum; C and D, human serum.

2 days of feeding colostrum. Tiselius' β-fraction probably corresponds to our *A*-fraction.

A second and subsequent effect is the division of the γ-fraction or the appearance of a new protein fraction that has almost the same mobility as the γ-fraction in normal serum. The effect is somewhat similar to that obtained with immune sera. In immune horse serum, Tiselius and Kabat (13) found the γ-fraction to occur in two parts of rather different mobilities, so that a good separation of these two fractions was obtained. In the serum of colostrum-fed rats, the mobilities are more nearly the same; thus the separation becomes more difficult. At pH 7.0 the two fractions can not be separated, and the apparent effect is the increase in the concentration of the γ-globulin. In immune rabbit serum, this concentration change appears to be the only effect, since no pH value could be found at which the γ-fraction could be separated. Our experiments on immune rabbit serum confirmed in this respect the observations of Kabat. The immune rabbit serum was obtained from Dr. S. Raffel of the Department of Bacteriology of Stanford University.

Since it seemed advisable to compare the effect produced by the colostrum diet with any effect that immunization would have on the rat serum, the electrophoretic diagrams of serum of rats that had been made highly immune to *Proteus vulgaris* and to sheep red cells were obtained through the coöperation of Dr. S. Raffel. In each case a similar division of the fraction was observed. As has been shown above, the two main effects on the serum protein content caused by the ingestion of colostrum are the increase in the concentration of the α-fraction and the division of the γ-globulin. To determine which of these two effects is the one more directly connected with the presence of agglutinins in the rat serum, samples of serum rendered highly immune by infections of killed bacteria and sheep's erythrocytes were fractionated by electrophoresis, and each fraction titrated for agglutinin contents. In this manner it was found that the more rapidly moving part of the γ-fraction has the highest titer per gram of protein present. In no instance was the separation of the protein fractions absolutely complete, so that no samples containing a single protein fraction were titrated.

It should be possible to determine whether the γ-fraction is split or a new fraction has appeared by a more complete study of the mobilities. A pH will have to be found at which greater mobility and consequently more accuracy can be obtained in the study of the original γ-fraction and the two subsequent γ-fractions.

The idea occurred to the authors that the new fraction might have some relation to the antibodies, in view of the work of Famulener and others on antibodies in the newborn, Howe's and our own (4) on the increase in globulins in the newborn,—changes directly traceable to the ingestion of colostrum,—as well as the similarity of electrophoretic diagrams of immune sera and sera after the ingestion of colostrum. Experiments are still in progress to determine this point. In five cases agglutinins to *Brucella* have been found in adult rat serum in a dilution of 1 to 8 and less after feeding colostrum having a low antibody titre. The controls in each case were negative in all dilutions. In one case where the colostrum had a high titre to *Brucella* no agglutinins were found in that serum after its ingestion. Previously, sera of the newborn had been tested in dilutions of 1 to 10 or 1 to 20 and above. Since the mechanism of the process is doubtful, no explanation can be offered at present.

CONCLUSIONS

Changes in rat and human serum take place after feeding cow's colostrum. The α-fraction is greatly increased, while the γ-fraction is either split or a new fraction appears. The change in the number of fractions is visible both in the salting-out curves and by electrophoresis.

Similar changes in the γ-fraction occur in highly immune rat serum.

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