The low packing fraction of iron and the apparent abundance of the element in the supernovae seem possibly to be related.[18]

The authors are especially indebted to Dr. L. Goldberg for advice with regard to the theory of line intensities, and to Drs. B. Edlén and P. Swings for use of their unpublished data on the spectrum of Fe III.

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MICELLE FORMATION IN AQUEOUS SOLUTIONS OF DIGITONIN

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Communicated February 16, 1940

(1) The well-known experiments of McBain and his collaborators[1] established that micelles are formed in aqueous solutions of electrolytes such as soaps and other paraffin-chain salts, particularly those of the sulphonic acids.[2] The methods of detecting micelle formation have usually consisted in showing that measurements of conductivity, freezing point, dew point, etc., deviate from those predicted for the individual ions. Since such methods portray only the average behavior of many particles, they have yielded no information regarding the size or quantity of the micelles in solution.

Using the ultracentrifuge to investigate particle size, we have found that a nonelectrolyte, the glucoside digitonin, forms large micelles in aqueous solution. It is known that the digitonin molecule possesses a

hydrophobic nucleus similar to that of the sterols, and several carbohydrate side-chains of hydrophylic nature. It is probable that micelle formation may occur in aqueous solutions of many other substances which are partly hydrophobic and partly hydrophylic.

(2) The digitonin was obtained from Eimer and Amend, New York, as "crystalline digitalin" and should not be confused with the true digitalin,

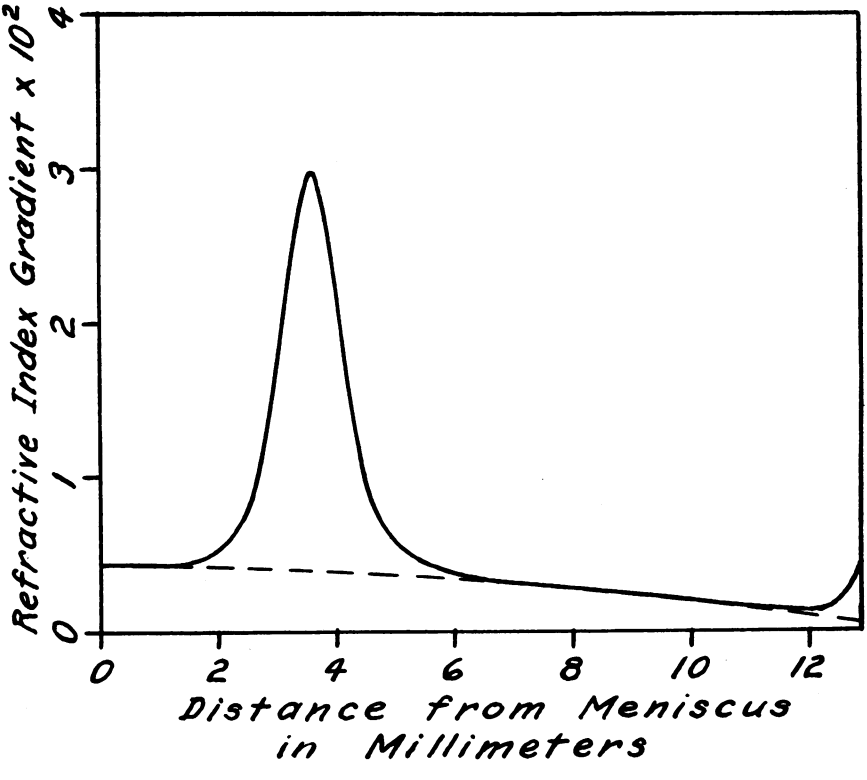

FIGURE 1

Sedimenting boundary of 2.5 per cent digitonin after 1 hour of centrifugation at 780 revolutions per second. The dotted line indicates the base line for water at the same speed.

a cardiac glycoside. The preparation used in this study gave the familiar digitonide precipitation reaction when it was added in alcoholic solution to cholesterol. For centrifugation the digitonin was dissolved in distilled water by slowly heating to boiling, and then cooling to room temperature. A 5 per cent solution showed only a faint opalescence.

All of the measurements were made using the air-driven ultracentrifuge

of Bauer and Pickels.[3] The sedimentation velocity of the digitonin was determined by centrifuging at 780 revolutions per second, which was equivalent to an average force of 160,000 gravity. Observations of the sedimentating material were made by a direct-reading refractive index method designed especially for the ultracentrifuge and utilizing a scanning system similar to that described by Longsworth[4] for electrophoresis measurements. The distribution of the micelles was recorded at 20-minute intervals by photographing the refractive index diagram. The runs were carried out at temperatures in the neighborhood of 25°C.

Figure 1 shows a tracing of a magnified refractive index photograph made with a 2.5 per cent digitonin solution 60 minutes after the centrifuge was brought to full speed. All of the photographs have shown only one discrete sedimenting boundary which was characteristic of an approximately homogeneous group of particles.

The sedimentation velocity of the micelles was estimated from curves such as the one illustrated by measuring as a function of time the successive displacements from the meniscus of the peak, which corresponds to the mean position of the diffuse boundary. Using the values for the viscosity and density of water and correcting the data to 20°C., the sedimentation constants were computed from the sedimentation velocities. Six independent determinations gave the following values: 5.35, 6.15, 5.33, 5.67, 6.36 and 6.41 $\times 10^{-13}$ cm. sec.$^{-1}$ dyne^{-1}. These yield an average value for S_{20} of 5.88 $\times 10^{-13}$ cm. sec.$^{-1}$ dyne^{-1} with an average deviation of less than 7 per cent.

One of the runs was made with 0.63 per cent digitonin; its sedimentation constant lies within the range of the other determinations which were made with 2.5 per cent solutions. Freshly prepared solutions or those several weeks old gave similar values. Some of the observations were made while studying the effect of digitonin on the chloroplast protein of spinach; no correlation was found between the sedimentation velocity of the digitonin micelle and the protein concentration, which varied up to about 1 per cent, even though the chlorophyll migrated together with the digitonin micelles.

(3) The concentration of material sedimenting at a measured rate can be estimated by measuring the area under the respective refractive index curve, if the refractive indices of solution and solvent are known. The differential refractive index, and hence the concentration, is directly proportional to the area. The solutions used were all originally made up to a concentration of 5 per cent. With one of these solutions, a refractive index determination was made which in itself had no significance as an absolute value since the material was of unknown purity and contained some water of crystallization. However, this determination could be used for estimating the relative concentration of the sedimenting material since the same solution was used for both measurements.

The refractive index of the 5 per cent solution at 20°C. was 1.3402; that of water measured with the same refractometer was 1.3328. Therefore, the differential refractive index of 2.5 per cent digitonin was 0.0037. Measurements on the photographs taken during centrifugation indicated a differential refractive index of 0.00368 for the sedimenting boundary. This shows that in a solution of this concentration practically all of the

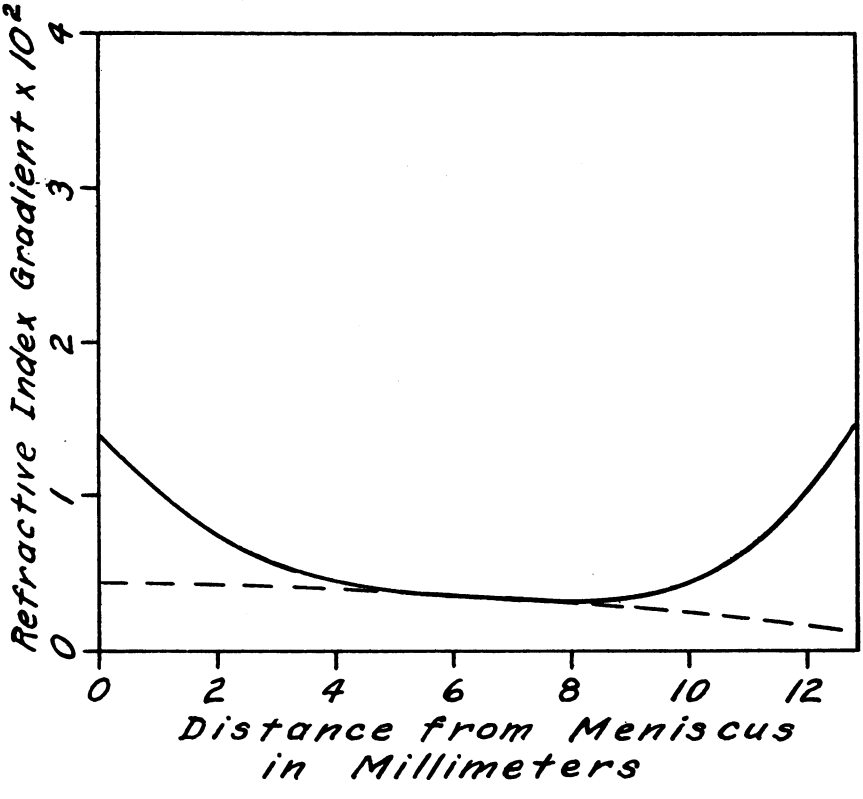

FIGURE 2

Refractive index curve of 5 per cent sodium desoxycholate after centrifuging for 90 minutes at 780 revolutions per second.

digitonin molecules are in the form of micelles of only one well-defined size. Nevertheless, there must be an equilibrium between these large micelles and a few smaller particles, probably the individual molecules, since the digitonin can be completely dialyzed through a cellophane membrane which will not permit the passage of particles even a tenth the probable size of these micelles.

(4) The exact micellar size cannot be computed because the shape and

density of the particles are unknown. A minimum value of the micellar weight can be obtained by assigning a maximum possible density value and applying Stokes' law. Since approximately half of the digitonin molecule is lipoidal in character, the average density is quite unlikely to be higher than that of most proteins, i.e., 1.33. With this value as an upper limit, the micellar weight is computed to be approximately 75,000. Since the molecular weight of digitonin is 1228, the micelles are extremely large, involving at least 60 of the primary molecules. The true micellar weight is undoubtedly larger than 75,000 since hydration, a lower density or any deviation from spherical shape would yield a larger size as computed from the observed sedimentation constant.

From the shapes of the refractive index curves of the sedimenting micelles, it is possible to determine the homogeneity of the particles and, if homogeneous, to obtain an approximate diffusion constant. The curves obtained were nearly symmetrical about the peak positions throughout the centrifugation, and showed a smaller spread than that expected for a micellar weight of 75,000. This shows not only that the particles are homogeneous, but that the true micellar weight must be higher than 75,000.

The diffusion constant estimated from the spread of the sedimenting boundary was 4.0×10^{-7} cm.^{2} per sec., it being fully recognized that values obtained in this way from sedimentation curves are only approximations. If the particles are spherical, this indicates that the true micellar weight may be as high as 400,000 and the density as low as 1.10.

(5) Aqueous 5 per cent solutions of sodium desoxycholate and a crystalline preparation of bile salts (mostly sodium glycocholate) were also studied. It was of considerable interest to test these substances since they possess a hydrophobic nucleus similar to that of digitonin, but differ in that they are electrolytes. The refractive index curves of these substances showed no detectable quantities of micelles of more than a few thousand in molecular weight. The type of curve obtained (Fig. 2) was characteristic of particles of relatively low molecular weight, there being no boundary but only a decrease of concentration in the upper part of the solution and some increase in the lower section. The shape of the curve changed little even on prolonged centrifugation. A similar result was obtained with solutions of sodium dodecyl sulphate.

(6) The random spread of particle size exhibited by the more familiar colloidal aggregates such as gold sols has usually been accepted as a distinguishing characteristic of colloids in general, in contrast to the well-defined molecular sizes of pure protein preparations. The homogeneous micelles of digitonin provide an interesting example of a "colloidal" particle, which is not consistent with this viewpoint, and it is quite possible that other substances of mixed hydrophobic and hydrophylic nature which act as detergents may also show this property. Large micelles are not

likely to be found among the electrolytes; this is indicated by the three studied by us, and also by those studied by McBain and Laing-McBain.[5]

Detergents have long been used for the dispersal of various types of substances of biological interest, particularly the proteins. It is important to emphasize that the detergent may not only affect the proteins studied but that some of its properties such as the molecular size may fall within that range usually considered to be characteristic of proteins alone.

Summary.—Ultracentrifugal observations using a direct reading refractive index method have been made on aqueous solutions of digitonin. Practically all of the digitonin exists in the form of micelles of homogeneous size, with an average sedimentation constant of 5.88×10^{-13} cm. per dyne per sec. The micellar weight is likely to lie within the range of 75,000 to 400,000, as contrasted with a molecular weight of 1228.

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A SET OF POSTULATES FOR BOLYAI-LOBATCHEVSKY GEOMETRY

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Communicated March 12, 1940

1. *Introduction.*—In some recent papers[1] Menger proved that all concepts of the Bolyai-Lobatchevsky geometry can be defined in terms of the operations "joining" and "intersecting," basic to his algebra of projective and affine geometry, as well as to G. Birkhoff's lattice theory. It follows that a complete foundation of non-Euclidean geometry can be given in terms of these two concepts, e.g., by substituting into the ordinary postulates Menger's definitions of the concepts "between," "parallel," "congruent," etc., in terms of joining and intersecting. Since postulates obtained in this way would be very cumbersome, there arose the problem of establishing some simple direct postulates concerning the two operations