saturation of the inulin spaces occurs in approximately 4 hr.

The results for the normal Krebs solution in Fig. 1 show that the presence of glyceryl trinitrate increases the permeability of the cell membrane in the tissue as represented by the increase in ECF. The increase was significant from the first to the fourth hour. The values obtained were high relative to those reported in the literature by the use of other methods. It was not intended that the measurement would be an exact value of the extracellular fluid space since the incomplete penetration of inulin into the connective tissue has not been considered. Nichols and co-workers have shown that the ECF space measurements obtained with chloride are better estimations, since chloride penetrates much more rapidly than inulin (6).

The muscle exposed to glyceryl trinitrate in a calcium-free medium also shows a significant increase in the extracellular fluid (Fig. 2). The results indicate that absence of calcium does not affect the increased ECF space induced by the presence of glyceryl trinitrate.

The magnesium-free Krebs media produced an inhibition of the ability of glyceryl trinitrate to increase the ECF space of the smooth muscle over most of the 4-hr. period (Fig. 3). The Krebs media without calcium or magnesium showed no change in the ECF space for the first 2 hr., then a significant increase in ECF in the last 2 hr. as seen in the unmodified Krebs medium (Fig. 4).

The approximately $\pm 5\%$ of the calculated mean in the ECF space determination reflects some variability among the multiple samples. That may be attributed to an actual section-to-section variation within the aorta, with respect to the inulin space or endogenous carbohydrates.

DISCUSSION

Although the velocity of inulin distribution is not affected by incubation of the muscle with glyceryl trinitrate, the apparent volume of distribution of inulin is increased. This indicates an increased permeability of the tissue in the presence of nitrate and nitrite. The increased permeability was not observed in the absence of magnesium with calcium present.

In all the bathing solutions, an early rapid phase and a late slow phase was noted before equilibrium was attained. This could be attributed to different water compartments, but recent studies have indicated that there are more than the two classic water compartments (7) indicated in this study. Possibly, the phases noted could be characteristic of the tissue investigated, indicative of latent penetration of inulin into more complex extracellular structures as suggested by Page (8) for heart muscle.

Saturation of the inulin space occurred in 3 to 4 hr. in these studies. Even after this prolonged time, nitrates and nitrites can still exert an effect on the cell membrane (9).

SUMMARY

Glyceryl trinitrate increases the extracellular fluid space of rabbit aortic tissue in normal Krebs and calcium–magnesium-free Krebs solutions. Only the magnesium-free Krebs solution failed to produce an elevation of the extracellular space of aortic tissue after treatment with glyceryl trinitrate.

REFERENCES

(1) Bohr, D. F., *Pharmacol. Rev.*, **16**, 85(1964).
(2) Bohr, D. F., Filo, R. S., and Guthe, K. F., *Physiol. Rev., Suppl. 98*, 42, 1962.
(3) Dreywood, R., *Ind. Eng. Chem., Anal. Ed.*, **18**, 499 (1946).
(4) Furchgott, R. F., *Methods Med. Res.*, **8**, 177(1960).
(5) Normal, A., Rondell, P. A., and Bohr, D. F., *Am. J. Clin. Pathol.*, **32**, 465(1959).
(6) Nichols, G., Jr., Nichols, N., Weil, W. B., and Wallace, W. M., *J. Clin. Invest.*, **32**, 1299(1953).
(7) Bozler, E., *Am. J. Physiol.*, **200**, 651(1961).
(8) Page, E., *J. Gen. Physiol.*, **46**, 201(1962).
(9) Lorenzetti, O. J., Tye, A., and Nelson, J. W., *J. Pharm. Sci.*, **55**, 105(1966).

Interactions of Surfactants with Lipoproteins

By H. TAKRURI and B. ECANOW

The interactions of benzalkonium chloride and sodium lauryl sulfate with α- and β-serum lipoproteins have been studied. Both fractions, below their isoelectric points, form insoluble complexes with the anionic surfactant. At higher concentrations of the surfactant the insoluble complexes are resolubilized completely. Above the isoelectric point, the lipoprotein fractions exhibit the same phenomenon with benzalkonium chloride. The charge and the hydrophilic nature of the macromolecules are the major factors in these interactions. The formation of insoluble complexes and the resolubilization of these complexes are modified considerably by the addition of urea to the systems.

THE INTERACTIONS of benzalkonium chloride and sodium lauryl sulfate with α- and β-serum lipoproteins have been studied. Both fractions, below their isoelectric points, form insoluble complexes with the anionic surfactant. At higher concentrations of the surfactant the insoluble complexes are resolubilized completely. Above the isoelectric point, the lipoprotein fractions exhibit the same phenomenon with benzalkonium chloride. The charge and the hydrophilic nature of the macromolecules are the major factors in these interactions. The formation of insoluble complexes and the resolubilization of these complexes are modified considerably by the addition of urea to the systems.

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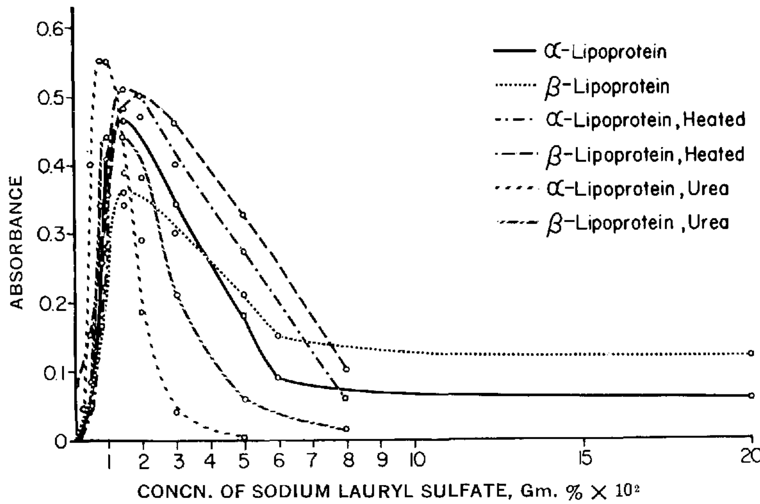

Fig. 1.—Turbidity of various lipoprotein systems as a function of the concentration of sodium lauryl sulfate.

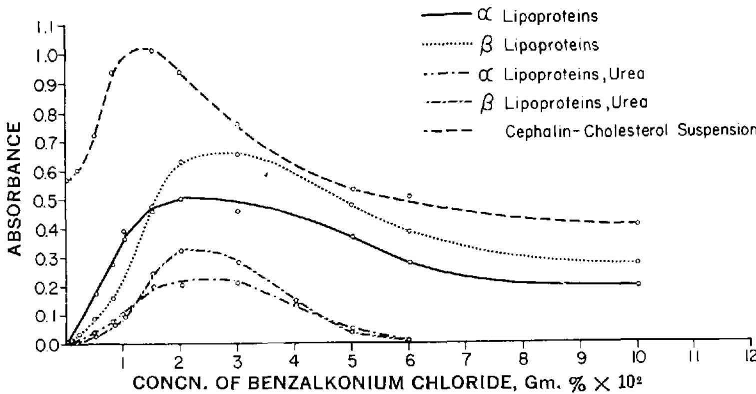

Fig. 2.—Turbidity of various lipoprotein systems as a function of the concentration of benzalkonium chloride.

The lipoproteins have been studied recently in connection with their possible role in the pathogenesis of atherosclerosis and other diseases. Certain subfractions of β lipoprotein were found to be definitely linked to plaque formation (4, 5). The effects of nonionic surfactants on lipoproteins were studied *in vivo* and *in vitro* (6–8). The effects of ionic surfactants on the electrophoretic mobility of these fractions were also studied (1). β Lipoproteins have the property of forming several complexes, soluble and insoluble, with heparin and other polysaccharide sulfates. These interactions depend on several factors, including pH, ionic strength, and metal ions. These interactions are utilized in the separation and characterization of β lipoproteins. Cornwell and Kruger (3) reviewed these interactions in detail.

This paper is a report on a study of the interactions of an anionic and a cationic surfactant with α and β lipoproteins carrying charges opposite to that of the surfactant. The influence of urea on these interactions was also studied.

EXPERIMENTAL

Reagents

Human α lipoprotein, fraction IV-1 (Nutritional Biochemical Corp.), human β lipoprotein, fraction III-0 (Nutritional Biochemical Corp.), bovine α lipoprotein fraction IV-1 (Pentex), cephalin-cholesterol reagent (Wilson), sodium lauryl sulfate (Fisher), benzalkonium chloride solution, 17% (Winthrop Laboratories).

Procedure

Interaction with Sodium Lauryl Sulfate.—A stock solution of 1% sodium lauryl sulfate in distilled water was prepared. Dilutions were made of this stock solution by adding a specified volume to 200-ml. portions of 0.04 *M* sodium chloride solution. One milliliter of 0.1 *N* hydrochloric acid was added to each solution to give a uniform pH of all the solutions. The solutions of the lipoproteins were prepared by dissolving 250 mg. of human β lipoprotein or bovine α lipoprotein in 50 ml. of 0.04 *M* saline. These solutions were dialyzed for 4 hr. at room temperature against a large volume of 0.04 *M* saline. After dialysis, 1.5 ml. of 0.1 *N* HCl was added to each solution and each was made up to 60 ml.

Three milliliters of the lipoprotein solution were added to 22 ml. of the various dilute solutions of sodium lauryl sulfate. This gives a concentration of 50 mg. % of the lipoprotein. This concentration was found to be the optimum concentration for

the determination of turbidity. After the surfactant–lipoprotein solutions were vigorously shaken, they were left to stand for 30–60 min. before the measurements were performed. The interaction was instantaneous and the only observation that required time was the sedimentation of the floccules. The turbidity was measured in a Coleman junior spectrophotometer. The measurements were performed at 450 mμ. The choice of this wavelength was arbitrary. In the study of urea-containing systems, the lipoprotein solutions were subjected to the action of 6 *M* urea for 1 hr. before they were added to the surfactant solutions.

Interactions with Benzalkonium Chloride.—Human lipoprotein fractions, 125 mg. of each, were dissolved in 50 ml. of 0.04 *M* saline, dialyzed for 4 hr. at room temperature against 0.04 *M* saline, and then diluted to 250 ml. with the same solvent. The pH was adjusted to 7.5 $\mp$ 0.2 with 0.1 *N* NaOH. Twenty milliliters was measured out in 1-oz. bottles and specified volumes of benzalkonium chloride stock solutions (0.1, 1, and 10%) were added to the individual bottles to give the desired concentration of the surfactant. In studying the effect of urea, the lipoprotein solutions were subjected to the action of 3 *M* urea for 36 hr. before adding the surfactant.

The interaction of cephalin–cholesterol reagent and benzalkonium chloride was also studied. This reagent is employed as a liver-function test. The mechanism of the test was reviewed by Bauer (2).

RESULTS

Figure 1 shows the turbidity of the various lipoprotein–sodium lauryl sulfate systems as a function of the concentration of the surfactant. The curves show that α lipoprotein complexes are solubilized at a lower concentration than β lipoprotein complexes. Also they show the negligible effect of heating the lipoproteins solutions for 4 hr. at 60° and the significant effect produced by urea.

Figure 2 shows the turbidity of various lipoprotein–benzalkonium chloride systems and the system benzalkonium chloride–cephalin–cholesterol. The turbidity curves resemble in shape those obtained with sodium lauryl sulfate.

DISCUSSION AND SUMMARY

The plasma concentration of most lipids, including cholesterol, glycerides, and phospholipids, has been studied extensively in relation to many diseases and pathological conditions. But the fact remains that practically all these lipids are found in blood as lipoproteins. The physical-chemical properties of these colloids are very important in determining their behavior and interactions in the body regardless of the total concentration of a particular component, *e.g.*, cholesterol or phospholipids. α and β lipoproteins differ considerably in their colloidal properties. The main difference could be expressed in terms of their hydrophilic nature.

The results of this study show that negatively charged lipoproteins, both α and β, are precipitated and then resolubilized by cationic surfactants. Positively charged lipoproteins are, on the other hand, precipitated and resolubilized by anionic surfactants. The interaction does not require di- or polyvalent metal ions and the insoluble complexes are not dissociated at very high ionic strength (up to 20% NaCl). These results are in contrast to the interactions of polysaccharide sulfates with lipoproteins. The results show that the more hydrophilic α fraction is solubilized at a lower concentration than the β fraction. Heating the lipoproteins causes a slight modification of these interactions. Urea produces a major change in these interactions. The concentration at which no observable turbidity is attained is considerably lowered in the presence of urea. The effect is related to the action of urea on the lipoprotein structure, the water structure, and the critical micelle concentration of the surfactant. The pH profiles of the lipoprotein–surfactant systems as a function of the concentration of the surfactant throw a light on the mechanism of these interactions. This aspect will be the subject of a future communication.

REFERENCES

(1) Ayrault-Jarrier, M., Wald, R., and Polonovski, J., *Bull. Soc. Chim. Biol.*, **41**, 753(1959).
(2) Bauer, R., *Rev. Gastroenterology*, **6**, 158(1949).
(3) Cornwell, D. G., and Kruger, F. A., *J. Lipid Res.*, **2**, 110(1961).
(4) Gofman, J. W., and Young, W., "Atherosclerosis and Its Origin," Sandler, M., and Bourne, G. H., eds., Academic Press Inc., New York, N. Y., 1963, p. 208.
(5) Hanig, M., "The Lipoproteins, Methods and Clinical Significance," Homburger, F., and Bernfeld, P., eds., S. Karger, Basel, Switzerland, 1958, p. 72.
(6) Kellner, A., Correll, J., and Ladd, A. T., *J. Exptl. Med.*, **93**, 385(1951).
(7) Kellner, A., and Hirsch, R., *ibid.*, **104**, 15(1956).
(8) Scanu, A., and Oriente, P., *ibid.*, **113**, 735(1961).