that in a lyophobic colloid.

We found that similar coagulation curves could be produced with sodium, calcium, and aluminum salts and that the only real difference was in the position of the vertical region of the sigmoid curve (12). The effect of valency was approximately as would be predicted by the Schulze-Hardy rule. The suspensions coagulated with the calcium chloride were particularly interesting since, with the highest concentration of electrolyte used, a further step in the sedimentation height curve was observed. Compatibility tests between the electrolyte and surfactant showed that, at this concentration, slight precipitation occurred. We interpret this as being perhaps the point of transition between coagulation and flocculation.

Ecanow *et al.* (5, 7, 18) appear to argue that since coagulation and flocculation must produce qualitatively different results and that we merely found quantitative differences, we could not be studying two different phenomena. We find it difficult to answer such circular reasoning. We would still maintain, on the basis of carefully controlled experiments whose results are compared with fundamental theory, that both coagulation and flocculation can produce suspensions that remain free from caking on storage.

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Effect of Macromolecules on Aqueous Solubility of Cholesterol

Keyphrases ☐ Cholesterol, cholesterol-26-^{14}C solubility—macromolecule effects ☐ Macromolecular substances—cholesterol solubility effect ☐ Pectin, acacia, dextrans effect—cholesterol solubility ☐ Scintillometry—analysis

Sir:

Intravenous administration of dextran solution has been suggested for treatment of hypercholesterolemia and atherosclerosis (1), and numerous investigations have been carried out to determine the effect of dextran on experimental hypercholesterolemia (2–6). Oral administrations of pectin and acacia have been studied for their hypocholesterolemic effects (7–14). The cited reports contain various and conflicting statements concerning the *in vivo* effect, value, and mechanisms of action of macromolecular substances for lowering serum cholesterol. However, there have been no reports of studies conducted to determine the *in vitro* effects of these carbohydrate macromolecules on the solubilization of cholesterol. It was, therefore, considered desirable to study the effect of these macromolecules on the aqueous solubility of cholesterol.

Cholesterol concentrations were determined using a radioactive technique suitable for very low amounts of cholesterol in water. A 10.0-ml. volume of a benzene stock solution, containing 10 mcg. of cholesterol and 0.1 μc. of cholesterol-26-^{14}C/ml., was transferred to a 125-ml. iodine flask, and the solvent was evaporated under a mild stream of nitrogen with constant shaking. A 50-ml. volume of the aqueous solution of the particular macromolecular substance was added to the flask. The dissolution studies were carried out at 30 ± 0.5°, and the test solutions were agitated using magnetic stirrers. At predetermined intervals of time, samples were withdrawn from the system and filtered immediately through a Millipore filtration assembly containing 0.45-μ filter paper. An accurately measured 0.20-ml. volume of the particle-free filtrate was pipeted into a liquid-scintillation vial. To this was added 15 ml. of dioxane–naphthalene phosphor (15), and the vials were shaken for 30 sec. to ensure thorough mixing. Duplicate samples, along with appropriate standards and blanks, were counted directly using a liquid-scintillation system.[1] The counts per minute involved after 12 hr. were between 150 and 415.

The data from the solubility studies are illustrated in Fig. 1. Each point on the solubility curves represents an average of at least eight determinations. Pectin (0.5%) and acacia (0.5%) solutions significantly increased the aqueous solubility of cholesterol, while slight increases were observed for the solubility of cholesterol in dextran solutions. The results for both high and low molecular weight dextrans[2] were similar.

[1] Unilux II, Nuclear Chicago.
[2] Dextran T70 and T40, Pharmacia Laboratories.

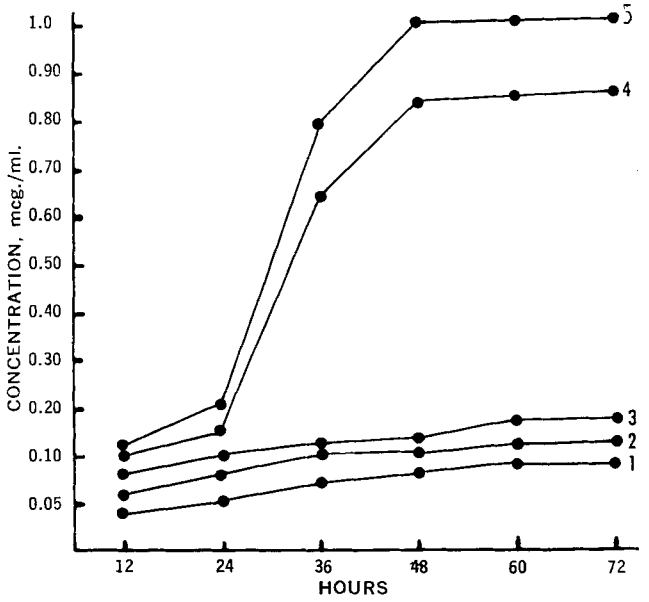

Figure 1—*Effect of various macromolecules on the aqueous solubility of cholesterol at 30°. Key: 1, water; 2, 6% dextran; 3, 10% dextran; 4, 0.5% acacia; and 5, 0.5% pectin.*

Statistical analyses of the data by means of t and F tests at 95% confidence levels indicate the differences in solubilities between various test solutions to be significant.

It is well known that dextran, pectin, and acacia form colloidal solutions. It is possible that cholesterol might be suspended, dispersed, or adsorbed on these colloidal particles. Another possibility is that these macromolecules might entrap the relatively smaller molecule of cholesterol. Brintzinger and Beier (16) suggested a number of mechanisms for the ability of gum acacia to increase the solubility of substances such as benzoic acid, anisic acid, sulfanilic acid, strychnine, and nitroaniline. The suggested mechanisms included adsorption on the acacia micelle, solution in the micelle, adsorption exchange between the added substance and water of hydration, and formation of chemical bonds.

The large increase in the apparent solubility of cholesterol in 0.5% pectin solution is most interesting when related to the findings of Wells and Ershoff (8). They found that pectin inhibited the increase in liver cholesterol following cholesterol feeding of rats, even when the rats were fed on alternate days with cholesterol. These authors suggested that more is involved than the simple tying up of cholesterol (possibly by the formation of a nonabsorbable pectin–cholesterol complex). Another possibility they suggested was that pectin induces changes in the intestinal flora which result in greater degradation of cholesterol, thereby leaving less of this material available for absorption. The present finding that pectin increases cholesterol solubility suggests the possibility that cholesterol in a solubilized form might be more susceptible to degradation in the intestine. Other possibilities are that cholesterol is adsorbed or dispersed in the colloidal particles formed by pectin or that a water-soluble complex is formed which is nonabsorbable. The fact that significant increases in the solubility of cholesterol in acacia and pectin solutions were observed after 24 hr. suggests that there may be more than one mechanism.

Since in the current studies the solubility of cholesterol was greatly increased in acacia and pectin solutions, it would appear that acacia might have the same *in vivo* cholesterol-lowering effect as pectin. However, Lin *et al.* (7) reported that addition of pectin to a basal diet containing cholesterol increased the excretion of exogenous cholesterol, while the addition of acacia or arabinose to this ration produced practically no change in the amount of fecal lipids and in the recovery of exogenous cholesterol in the feces.

Flotte and Buxton (2, 3) and Ditzel and Dyerberg (6) observed that administration of dextran has a profound effect in lowering serum cholesterol. Ditzel and Dyerberg discussed the possible modes of action of dextran on plasma lipids and cholesterol. They postulated that a dextran–lipid complex might influence cholesterol synthesis or breakdown or facilitate cholesterol transport through the cell membranes by altering its solubility characteristics or charge. Rothschild *et al.* (17) postulated that the mode of action of dextran might be explained by a stimulating effect on the reticuloendothelial system. The current findings suggest that dextran might facilitate at least part of the transport redistribution of cholesterol by an increase in the apparent solubility of cholesterol.

Further investigations are being conducted to determine the effect of a variety of macromolecules on the aqueous solubility of cholesterol and related hormone drugs and to elucidate the mechanism(s) of action of solubilization phenomena.

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