

therefore extraneous carbohydrate contamination was assumed in these cases.

In Fig. 1 is shown the colour reaction of dextran solutions in water after hydrolysis. There is good agreement with Beer's law within the concentration-range 25-400 mgm./100 ml. Dilution of serum is recommended for higher concentrations.

The coefficient of variation (per cent standard deviation) obtained by 20 parallel determinations on rat serum was 2.7 per cent for glucose at a level of 194 mgm./100 ml. and 2.5 per cent for dextran at a level of 184 mgm./100 ml.

We are indebted to Glaxo Laboratories, Ltd., for a gift of 'Clinical Dextran' used in this work. Dr. G. Szepessy, of the Central Research Laboratory of this University, kindly determined the optical densities.

A. RÉDEI
S. NAGY

Institute of Pathophysiology,
Medical University of Szeged.

¹ Hultman, E., *Nature*, **183**, 108 (1959).

² Dimler, R. J., Davis, H. A., Gill, G. J., and Rist, C. E., *Anal. Chem.*, **26**, 1142 (1954).

³ Földes, J., *Kisérlet. Orvostud.*, **11**, 1 (1959).

Emulsifying Capacity of Blood Sera

IN view of the suspected link between fat metabolism and atherosclerotic disease, the state and behaviour of the emulsified fats in the blood serum have been the object of numerous investigations. Thus, for example, stabilized emulsions of lipoproteins, of coconut oil, and of olive oil have been added to sera in order to demonstrate the clearing reaction of heparin¹. It seems, however, that the ability of different sera to emulsify various fats has never been investigated systematically.

In order to test the emulsifying ability of different sera, a simple, reproducible turbidimetric method of measurement has been developed. Briefly, the serum is shaken under controlled conditions with 0.5 per cent olive oil. The optical extinction rises to a maximum with time and then begins to fall; at 37° C. the maximum extinction is reached in about 20 min. The maximum extinction value we termed the 'emulsifying capacity' of the serum. The dispersion of 0.5 per cent olive oil in 0.005 per cent 'Lissapol' (run parallel with the experiments) served as control.

The emulsifying ability of a given serum has been found to be reproducible to within about ± 2 per cent.

Microscopic investigation showed that greater turbidity corresponds to a smaller average oil-droplet diameter.

After the reproducibility of the results was proved beyond doubt, studies have been initiated of the influence of various factors on the maximum turbidity obtained. The following is a summary of the results:

(1) There is an optimum shaking rate of about 200 beats/min. for the development of high turbidity emulsions.

(2) At oil concentrations between 0.1 and 1 per cent the maximum turbidity has been found to be proportional to the logarithm of the oil concentration.

(3) The emulsifying ability of sera from different animals has been found to vary both from animal to animal, as well as from species to species. Thus, sheep and cattle sera show higher emulsifying ability than pig sera; this variation in emulsifying capacity is well outside the variations found with sera from individual animals of the same species.

(4) The type of oil used determines, to a large extent, the maximum turbidity. In general, oils yielding high-turbidity emulsions have high acetyl and iodine numbers, and low viscosity. It must be stressed, however, that these differences are substantially smaller when the experiments are conducted at 37° C., although some differences still persist; thus, coconut oil yields substantially coarser emulsions both at room temperature and at 37° C.

(5) The addition of surface-active compounds exerts a strong influence on the maximum turbidity obtained. I found that in the presence of small concentrations ($5 \times 10^{-4} M$) of surfactive acids (for example, oleic acid) the emulsification decreases, while the addition of the same amount of surfactive bases (for example, long-chain amines) or of neutral surfactants (for example, cetyl alcohol) increases the maximum turbidity. The effect of the surfactant is the same, whether it is added to the serum or to the oil phase.

(6) Diluting the serum up to ten-fold with isotonic phosphate buffer (pH 7.2) increases the maximum obtainable turbidity; further dilution results in a decrease of the maximum turbidity. The reasons for this particular behaviour remain to be investigated; they could be attributed tentatively to either changes in the protein surface and/or to viscosity changes.

Examination of some of the physico-chemical properties of the resulting emulsions have shown that:

(1) The electrophoretic mobilities of the oil droplets seemed unchanged by the nature of the surfactant added, a result which warrants further investigation.

(2) The interfacial surface tension is only slightly lowered in the presence of surface-active compounds; the degree of emulsification obtained seems unrelated to the interfacial surface tension.

Clinical investigations are at present being carried out in the Lidcombe State Hospital, Sydney. Although it is too early at present to draw conclusions from the results so far obtained, it is already evident that there are individual variations in the emulsifying abilities of human sera; sera obtained at different times from one and the same person can also show marked variations. Thus, the emulsifying ability obtained with the serum of a given individual invariably decreases after the ingestion of fats. Interesting effects have been observed on intravenous injection of heparin; they will form the subject of a separate communication, as will the details of the work presented here.

B. BREYER

Section of Agricultural Chemistry,
Department of Agriculture,
University of Sydney,
New South Wales.

¹ Shore, B., Nichols, A. V., and Freeman, N. K., *Proc. Soc. Exp. Biol.*, New York, **83**, 216 (1953). Brown, R. K., Boyle, E., and Antinsen, G. B., *J. Biol. Chem.*, **204**, 423 (1953). Meng, H. C., Hollett, C., and Cole, W. E., *Amer. J. Physiol.*, **179**, 314 (1954).

Hydrolysis of Fatty Acid Esters of Riboflavin by Pancreatic Lipase

FATTY acid esters of riboflavin, fat-soluble riboflavin derivatives, have been synthesized in our laboratory to widen the application of riboflavin to pharmaceutical and nutritional fields¹⁻³. As a fundamental problem of this application, the possibility of enzymic hydrolysis of these esters to riboflavin and fatty acids has to be investigated first. This communi-