anode, but in agarose this component moved a much greater distance toward the anode in the same period of time.

Summary. Several antigen-antibody precipitin systems were compared by immunoelectrophoretic analysis using agar and agarose as the supporting medium. Electroendosmosis was reduced in agarose, mobility of components was more rapid, lateral separation of alpha and beta globulin components was increased and better definition of individual precipitin arcs in this medium was observed. Some precipitin reactions which failed to develop in agar gave clear reactions in agarose. The reverse was never observed. There were some individual antigen-antibody reactions in which there appeared to be little change in either the shape or position of the precipitin arcs on either medium, but increased clarity was always observed on the agarose medium. The advantages of agarose for I.E.A. when increased sensitivity is required, as in quantitating antigens or in testing for homogeneity of purified proteins, should be considered.

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Influence of Some Nonionic Surfactants on Pancreatic Lipase Activity. (30521)

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Earlier investigations have demonstrated that Triton WR-1339, a nonionic surfactant, induced hyperlipemia when injected into laboratory animals(1-5). The elevation of blood lipids is believed to result from inhibition of the host's lipid catabolic processes(2-3). It has been reported that plasma lipoproteins of Triton-treated rabbits(6) and guinea pigs (7) are resistant to the action of lipoprotein lipase. Furthermore, normal guinea pig plasma lipoproteins which are incubated with Triton are also resistant to the enzyme(7). Similar treatment of a coconut oil emulsion with Triton shows that the clearing action of pancreatic lipase is impaired(8). These results suggest that the detergent might alter the original substrate molecule so that an entirely different substrate is formed that would be resistant to the action of a lipolytic enzyme. The present study was conducted to evaluate this possibility and to gain similar information concerning two other chemically related nonionic surfactants, Macrocyclon and HOC-60.

Materials and methods. A 50% coconut oil emulsion, (Ediol, Schenlabs Pharmaceuticals, Inc., New York), was used as the substrate in all experiments; the stock solution was diluted to 1% with 0.15 M saline-phosphate buffer (pH 7.4) to prepare daily working solutions. Commercial pancreatic lipase (General Biochemicals, Chagrin Falls, Ohio), prepared as a 20 mg/ml solution in saline-phosphate buffer, was used as the enzyme. Three nonionic surfactants (polyoxyethylene ethers of octyl phenol) were used. Triton WR-1339 was obtained from Winthrop Laboratories, Rensselaer, N. Y.; Macrocyclon and HOC-60 were obtained from Dr. R. J. W. Rees (National Institute for Medical Research, Mill Hill, London). In the report describing the preparation of the latter two surfactants(9), Macrocyclon was listed as HOC-20 (GN/57) and HOC-60 was coded as GN/102. Work-

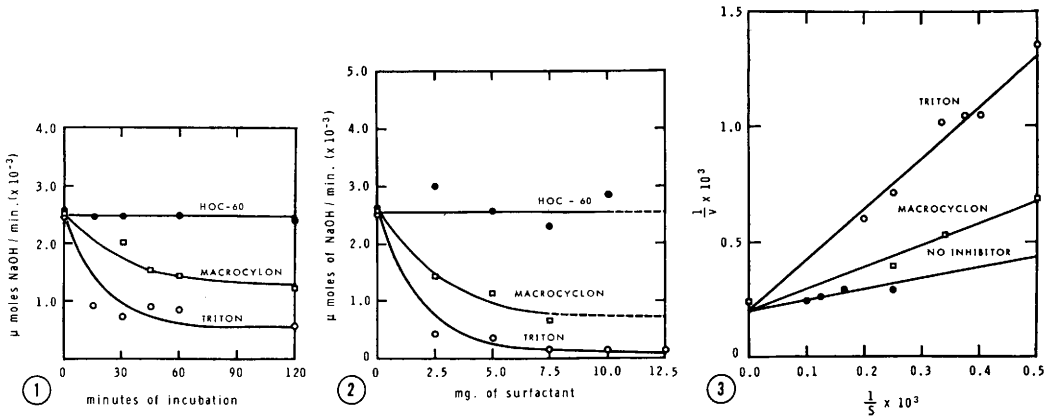

FIG. 1. Effect of varying incubation time of Ediol with surfactant (2.5 mg) on hydrolysis by pancreatic lipase.

FIG. 2. Effect of varying surfactant concentration on hydrolysis of Ediol by pancreatic lipase.

FIG. 3. Lineweaver-Burk plots of the effect of various surfactants on hydrolysis of Ediol by pancreatic lipase.

ing solutions (2.5 mg/ml) were prepared daily from 12.5% stock solutions of each surfactant by dilution with the buffer.

Titration was performed using a pH-Stat (Titrator TTT1, Radiometer, Copenhagen, Denmark) which was connected to a titrigraph (Type SBR2/SBU1, Radiometer, Copenhagen, Denmark) and to a 0.5 ml syringe burette. The instrument was standardized daily with pH 6.5 buffer. A 1.0 N stock solution of sodium hydroxide was prepared from pellets and standardized against potassium acid phthalate; for titration, the stock solution was diluted to 0.01 N. All titrations were conducted in a water-jacketed vessel that was maintained at 37°C. The reaction mixture consisted of 0.5 ml of substrate, 2.5 ml of saline-phosphate buffer, and 1 ml of enzyme. The substrate-buffer mixture was allowed to equilibrate in the vessel after which the enzyme was added quickly and the titration was recorded. Duplicate titration curves were obtained for each mixture. In the inhibition studies, the reaction mixture consisted of 0.5 ml of substrate, 1 ml of surfactant solution, 1.5 ml of saline-phosphate buffer, and 1 ml of enzyme; the substrate and the surfactant were incubated together at 37°C for the required period of time before adding the enzyme.

Results. Effect of time of incubation. The results, plotted in Fig. 1, demonstrate that in the absence of a period of incubation (0 minutes) of substrate with surfactant before addition of enzyme, no inhibition occurred; incubation of the substrate-surfactant mixture prior to addition of enzyme obviously was necessary to inhibit hydrolysis. Triton and Macrocyclon showed parallel inhibition but HOC-60 did not influence the rate of hydrolysis even after 120 minutes of incubation. Since no further inhibition occurred after 30 minutes of incubation, subsequent inhibition studies were carried out using 1-hour incubation periods before addition of enzyme.

Effect of concentration of surfactant. In Fig. 2, it can be seen that as the concentration of Triton and Macrocyclon increased, the rate of hydrolysis of Ediol decreased, levelling off at a concentration of about 2.5 mg/ml. However, a comparison of the 2 curves suggests that Triton is a greater inhibitor than Macrocyclon. The Figure also shows that, regardless of concentration, HOC-60 does not affect the rate of hydrolysis.

Lineweaver-Burk plots. The double reciprocal plots of 1/v against 1/S exhibited a pattern of competitive inhibition (Fig. 3). The data yielded K_i constants of 0.66×10^3 M for Triton and 1.22×10^3 M for Macrocyclon; the affinity constants which are the reciprocals of the K_i values were found

to be 1.51×10^{-3} M and 0.82×10^{-3} M for Triton and Macrocyclon, respectively. These values suggest that Triton is approximately twice as inhibitory as Macrocyclon.

Discussion. The results of the present study demonstrate that, of the 3 nonionic surfactants used in the inhibition experiments, Triton WR-1339 and Macrocyclon but not HOC-60 inhibit the hydrolysis of the triglycerides found in a coconut oil emulsion by pancreatic lipase (Fig. 3). Hydrolysis was not inhibited when substrate, surfactant and enzyme were added to the reaction vessel essentially simultaneously; however, when the mixture was incubated at 37°C for at least 30 minutes before adding the enzyme, hydrolysis was inhibited (Fig. 1). Furthermore, upon reversing the addition sequence by adding the substrate to a preincubated enzyme-surfactant mixture, hydrolysis was not inhibited, indicating that there was no direct inactivation of the enzyme and that the surfactant does not compete with the substrate for sites on the enzyme. Therefore, the surfactant is inhibiting the reaction through action on the substrate rather than on the enzyme, and it appears that the surfactant alters the substrate molecule during the preincubation period in such a way that it becomes less reactive to pancreatic lipase.

If indeed the substrate molecule was altered during incubation, it is suggested that the surfactant may combine with the substrate during this period to form a substrate-surfactant structure so that the substrate is presented to the enzyme in an entirely different form. At this time, the nature of the substrate-surfactant structure is not known; however, either an irreversible complex or a micellar aggregate appears to be a valid possibility. The former would be in agreement with the suggestion of Brown, Boyle and Anfinsen(10) that Triton WR-1339 formed an irreversible complex with serum lipoproteins *in vitro*. On the other hand, a micellar structure may induce conformational changes in the substrate molecule which could render certain functional groups unavailable to the enzyme and result in inhibition of enzymatic action. Whichever mechanism occurs, it is probable that the substrate molecule was altered to cause the observed resistance to pancreatic lipase. The experimental results indicate that the inhibitory effect of Triton and Macrocyclon may be explained as due to the action of the surfactant on the substrate rather than on the enzyme.

It is of interest that these observations are consistent with the results of a study of the inhibitory effect of Triton on the action of lipoprotein lipase on plasma lipoproteins(7). From this, it is reasonable to believe that the hyperlipemia induced by Triton results from the impairment of lipid catabolism caused by the inability of the lipolytic enzymes of the host to act on the Triton-altered plasma triglycerides. In this respect, it should be noted that only the hyperlipemia-inducing surfactant, Triton in this case, was found to inhibit the action of pancreatic lipase on the triglycerides of Ediol; HOC-60 which does not induce hyperlipemia(9), was not inhibitory. As for Macrocyclon, although Cornforth *et al*(9) reported that it did not induce hyperlipemia in rabbits and mice at the same dosage as Triton, Boyd and his coworkers indicated that it increased serum cholesterol levels in man and rhesus monkeys (11). As suggested earlier in the results (Fig. 2 and 3), it appears that Macrocyclon would require approximately twice the dose of Triton to induce hyperlipemia. On this basis it seems that good agreement was obtained between the ability of an agent to induce hyperlipemia and to inhibit enzymatic lipolysis *in vitro*.

Summary. Two nonionic surfactants, Triton WR-1339 and Macrocyclon inhibit the hydrolysis of triglycerides in a coconut oil emulsion (Ediol) by pancreatic lipase, Triton inhibiting to a greater extent than Macrocyclon in a ratio of approximately 2:1. HOC-60, also a nonionic surfactant, is not inhibitory. The results of the present study indicate that during its incubation with the surfactant at 37°C for 1 hour before adding the enzyme, the substrate may undergo a change which would result in an altered substrate molecule that is less reactive to pancreatic lipase. Two suggested mechanisms of the inhibitory action of the nonionic surfactants are discussed: (1) formation of an

irreversible complex and (2) formation of a micelle. The ability of these agents to induce hyperlipemia in animals was reflected by inhibition of enzymatic lipolysis *in vitro*.

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Increased Urinary Pressor Activity (Aortic Strip Assay) in Tolbutamide-Treated Diabetes.*† (30522)

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Measurements of urinary pressor activity (aortic strip assay) in healthy subjects, in patients with newly diagnosed or known and treated diabetes mellitus, and in non-diabetic adults with symptoms suggestive of endocrinopathy have revealed differences which are statistically significant. The data and possible interpretations are herein presented.

Materials and methods. Pressor activity (aortic strip assay) has been measured in a total of 692 twenty-four hour specimens of urine collected with refrigeration from the same number of subjects and patients. The results are expressed as gamma epinephrine equivalents or g.e.e.(1).

The presence or absence of diabetes mellitus was determined in each subject by means of one or more oral glucose tolerance tests(2).

The results have been grouped into 5 categories: a) healthy adult controls, male and female, b) ambulatory non-diabetic patients with symptoms or signs indicative of possible endocrinopathy, c) adults with newly detected and untreated diabetes, d) adults with known diabetes under diet and insulin treatment, and e) adults with known diabetes under diet and tolbutamide therapy (0.5 to 1.5 g per day) (Table I).

Each subgroup was in turn divided into age categories by successive decades between 20 and 70 years, and then by weight, *i.e.*, 169 lb or less and 170 lb or higher to determine whether these variables were related to urine pressor activity.

The statistical significance of any differences between each of the 4 groups of patients and the control subjects, and within each patient group subdivided as to age and body weight was evaluated by T test ($p<0.05$ indicated statistically significant differences).

Results. Urinary pressor activity in 116 healthy adult subjects ranged between 8.1 and 158.8 g.e.e., averaging 27.8 $\pm$ 27.5 g.e.e. (Table I).

Urinary pressor activity was higher by about one-half in a series of adults with symptoms, signs and laboratory findings indicative of an endocrinopathy or a metabolic disorder other than diabetes mellitus (Table I). This group of 312 patients comprised

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