Summary. The buoyant density of rubella virus HA was determined to be 1.23 in Cs_2SO_4. A density of 1.33 was obtained in CsCl. Rubella virus HA, either Tween-80, ether-treated or untreated, and infectivity could not be separated on sucrose by rate zonal or density gradient techniques. These activities also could not be separated on CsCl and Cs_2SO_4 density gradients. The banding density of rubella virus HA preparations was unaffected by the type of cell culture used for antigen production; neither the passage level nor the strain of virus altered the point at which rubella virus HA banded in CsCl.

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The Effect of Neomycin Sulfate on Pancreatic Lipase Activity (32918)

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Oral neomycin sulfate administration to man frequently leads to steatorrhea (1,2). The mechanism responsible for this association is not yet known. Investigations directed toward elucidating its pathogenesis have logically been centered around digestive and absorptive stages involved in lipid assimilation. There is evidence suggesting that neomycin sulfate may adversely affect both stages (3–5). Recent observations have yielded contrasting results regarding the *in vitro* effect of the antibiotic on lipase activity. Conditions of reported experiments have varied considerably (6,7). Because of the importance of determining what effect neomycin sulfate has on lipase activity, we have

undertaken a series of *in vitro* studies utilizing both porcine and human lipase under similar experimental conditions.

Methods. All reactions utilized the same incubation system which included 1 ml substrate (9% triolein) made up in 0.05 *M* Tris buffer containing 10 m*M* taurocholic acid and 1 ml 0.05 *M* Tris buffer containing enzyme. In test solutions neomycin sulfate was added to give final antibiotic concentrations of 0.25, 0.50, and 1.00 mg/ml. Systems were incubated at 37°C for 1 hour at pH 7.0, the reaction stopped with three drops of concentrated HCl and the lipid extracted into chloroform according to the method of Folch *et al.* (8). Titration of liberated fatty acids was performed in 95% ethanol to pH 9.6, using 0.01 *N* NaOH and a Metrohm pH meter equipped with a combination glass electrode.

Neomycin sulfate (Upjohn—bulk) solutions were made up in 0.05 *M* Tris buffer and adjusted to pH 7.0. The *N*-acetyl neomycin was prepared according to the method of Rinehart *et al.* (9) and treated as neomycin.

Porcine pancreatic lipase was obtained from Nutritional Biochemicals (lot no. 9975) and used without further purification. Solutions of the enzyme were made up with 0.05 *M* Tris and adjusted to pH 7.0.

Human lipase was obtained in the following manner: A specially prepared meal containing 11.2% powdered milk, 11% triolein and 9.2% dextrose in water was fed to previously intubated, fasting healthy male volunteers. Tubes were fluoroscopically localized at the duodenojejunal junction. Once satisfactory flow by gravity was established (usually within 30 min), 20–30 ml of the intestinal aspirate, which was visibly bile stained, was collected into a flask immersed in ice and water. The juice temperature was thus maintained at 2–3°C during collection. The intestinal aspirate was centrifuged at 1200 rpm for 10 min and the resulting supernatant utilized as a source of lipase.

Studies on emulsion stability were performed as follows: Assay systems identical in composition to the basic system utilized throughout were incubated with and without neomycin for 1 hour at 37°C and then centrifuged at 1200 rpm for 10 min. Two ml of a resulting infranatant were removed and total fat was determined by the method of Van de Kamer (10). Emulsion stability was expressed as the amount of fat remaining in the infranatant portion of the centrifuged sample.

Results. Figure 1 (A,C) illustrates that significant enhancement of both porcine and human lipase activity was evident at the lowest concentration of neomycin utilized (0.25 mg/ml), and that the degree of enhancement decreased with progressive increases in concentration of the antibiotic (0.50, 1.0 mg/ml). Emulsion instability, evident in all neomycin-containing systems, was greatest at the highest antibiotic concentrations. Figure 2 demonstrates that neomycin adversely affected the stability of the emulsions utilized, that the acetylated form of the antibiotic exerted no such effect and that the addition of gum acacia (2%) reduced this property considerably.

In subsequent experiments in which triolein was prepared in 2% gum acacia, the enhancing effect of increasing concentrations of neomycin sulfate was noted on both porcine and human lipase preparations and was linear with respect to concentration (Fig. 1B and D). In systems employing the acetylated form of neomycin, no effect on enzyme activity was observed nor was any degree of emulsion instability noted (Figs. 1A–D and 2).

In order to simulate more closely the actual physiologic circumstances existing in the human intestinal lumen during lipid assimilation, control meal (see "Methods") was incubated for 1 hour at 37°C with stimulated digestive juices in a volume ratio of 1:1. The effect on lipase activity of adding neomycin sulfate (0.5, 1.0, and 1.5 mg/ml) to the systems was determined. Figure 3 illustrates the results of such an experiment performed in two persons. In one, significant enhancement of lipase activity is apparent; in the other, slight enhancement was noted.

Discussion. It is apparent that under the present experimental conditions neomycin sulfate enhanced the activity of both porcine and human lipase against an insoluble sub-

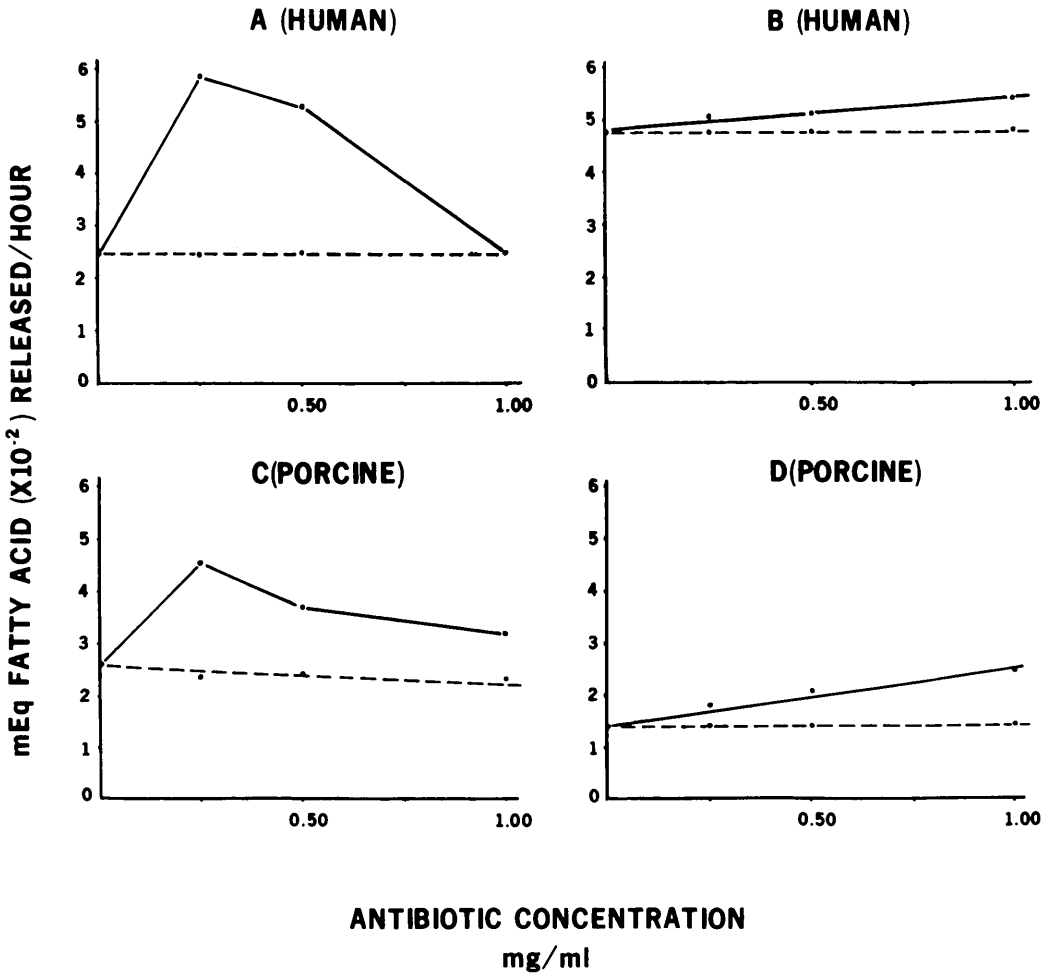

FIG. 1. Antibiotic effect on porcine and human lipase activity with and without gum acacia (2%). Assay conditions are as described in text. Neomycin sulfate (——); *N*-acetyl neomycin(- - -). A and C, no gum acacia; B and D, added gum acacia.

strate. Why does this take place? Yamamoto has previously observed that certain compounds were capable of enhancing the activity of porcine lipase against olive oil and that most of these compounds were characterized by the presence of one or two free amino groups. He further noted that when lysine was the enchancing agent, the elimination of its free amino groups by conversion to α-ε-dibenzyl lysine, resulted in abolition of its lipase-enchancing properties (11). In view of Yamamoto's observations, it seemed appropriate to determine the effect of eliminating the 6 free amino groups of neomycin by acetylation. This alteration of the neomycin molecule abolished its enzyme-enchancing properties.

It has been demonstrated previously that when a lipid substrate is prepared at constant concentrations but with varying total surface area, a decrease in lipase activity is seen to be proportional to the reduction in surface area (12). The present study demonstrates that neomycin affects emulsion instability, thereby leading to a reduction in effective substrate concentration. This reduction in available substrate is reflected in the decreased enhancement of lipase activity evident at the higher concentrations of neomycin sulfate. Emulsion stabilization with gum acacia re-

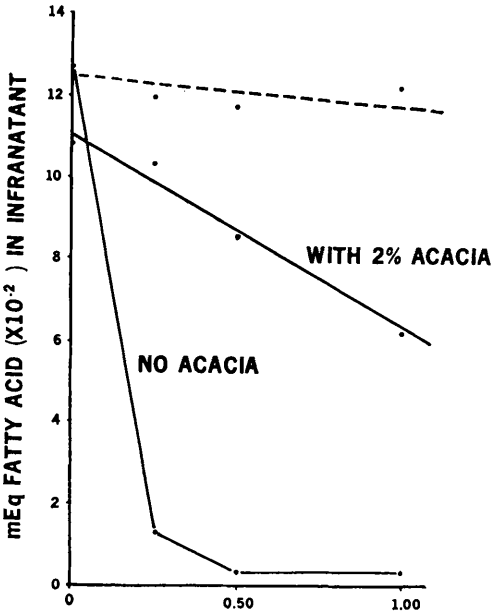

FIG. 2. Effect of neomycin on emulsion stability with and without added gum acacia. Neomycin (—); N-acetyl neomycin (---). Ordinate represents total lipid remaining in stable suspension in 2 ml reaction system.

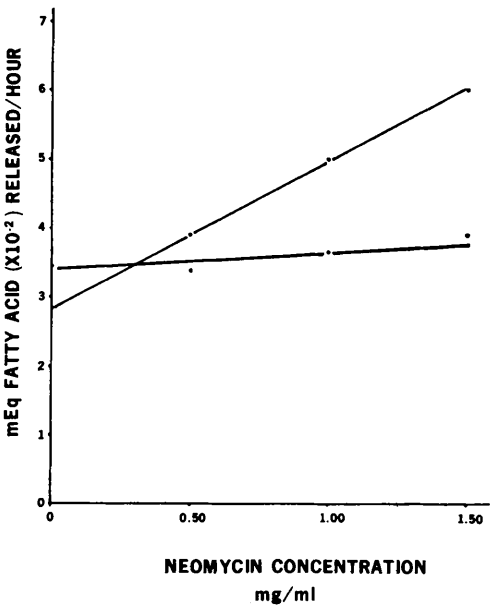

FIG. 3. Influence of neomycin sulfate on human lipase activity. Fat stimulated duodenal juice incubated 5 min at 37°C in a 1:1 volume ratio with control meal (described in text). Each line represents a separate patient.

sulted in enhancement of lipase activity by the same concentrations of neomycin. The acetylated form of the antibiotic did not appear to affect emulsion stability.

In the light of these observations, it is suggested that the free amino groups in the unaltered antibiotic molecule exert opposite effects on lipase activity. They can impair emulsion stability and reduce lipase efficiency; when emulsion stability is maintained, however, enzyme activity is enhanced in their presence. It appears, then, that experimental conditions would be expected to influence the effect neomycin sulfate has on the activity of pancreatic lipase.

Our results using porcine lipase parallel those obtained by Urban and Kern who stabilized their olive oil substrate with gum acacia (6). It is difficult to compare our observations with those of Mehta *et al.* (7). Utilizing human lipase obtained via pancreatic fistula, they demonstrated that neomycin sulfate inhibited the activity of lipase on an olive oil substrate. One further obvious difference in methodology which might have accounted for opposite results is the fact that, in proportion, the concentrations of neomycin employed by Mehta *et al.* with respect to substrate exceeded ours by a factor of 200. Furthermore, no mention is made regarding stability of emulsions to which neomycin was added (7). Krondl *et al.* have demonstrated previously emulsion unstabilizing properties of neomycin using fasting human duodenal juice and olive oil substrate (13). Utilizing isolated loops of rat small intestine (which included the common bile duct) in a separate experiment, the same workers demonstrated that high concentrations of neomycin (20 mg/ml) added to unemulsified olive oil exerted inhibition of lipase activity *in vivo* and that the degree of inhibition was less at 120 min than at 30 min of incubation. Though they did not attempt to explain this difference in lipolysis rates with respect to time, it seems reasonable to postulate that the constant addition of bile salts in response to fat in the

intestinal loop led to emulsion stabilization and reduced neomycin effects on lipase.

Previous studies from our unit have demonstrated that neomycin administration to humans resulted in apparent impairment of the *in vivo* hydrolysis of a lipid meal (14). Inhibition of lipase activity was suggested by these observations, although it was not known whether the inhibition was a direct or indirect effect of the neomycin. Interpreted in the light of present data, it is possible that the decreased lipolysis observed *in vivo* can be accounted for on the basis of induced emulsion instability. The known bile-salt precipitating properties of neomycin may contribute to its emulsion unstabilizing potential *in vivo* (3).

Summary. In separating the various effects of neomycin sulfate which may be exerted in systems designed to measure the activity of lipase, it is apparent that the antibiotic enhances the activity of both porcine and human lipase on an insoluble substrate. Additionally, it has the propensity to unstabilize emulsions. These effects are antagonistic and may account for divergent results previously recorded regarding neomycin–lipase interrelationships. Both the enzyme enchancing and emulsion unstabilizing properties of the antibiotic seem to reside in the presence of its free amino groups.

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On the Effects of Tannic Acid on Erythrocyte Membrane Acetylcholinesterase* (32919)

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The occurrence in human serum of agglutinating factors specific for erythrocytes pretreated with tannic acid (TA) has been recently reported from three different laboratories (1–3). Treatment of red cells with tannic acid has been widely used in passive hemagglutination procedures since Boyden made the observation that TA renders erythrocytes capable of adsorbing protein molecues from solution (4). Although it has been shown that this kind of treatment causes reduction in anion permeability (5), alterations in osmotic resistance (6), agglutination (7), loss of agglutinability by blood-group specific antibodies (8), the mechanism by which TA exerts its action on the red cell is not understood. Whereas Bohlmann (6) has

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