with biological, chemical, and physical properties identical to those of human interferon. Upon removal of PHA from human lymphocyte cultures interferon production ceased within 2 hours. Readdition of PHA resulted in reinitiation of interferon production.

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Neomycin Inhibition of Lipolysis *in vitro*.* (32236)

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Oral administration of neomycin may increase fecal fat excretion in normal human subjects(1). Histologic changes of the jejunal mucosa resulting from this antibiotic were reported by Jacobson, Prior and Faloon(2), but were not confirmed by Rubin and Dobbins(3). In a more recent study, defective hydrolysis of a long-chain unsaturated fat was demonstrated in 2 adults receiving oral neomycin(4). For this reason, the effect of neomycin and other antibiotics on pancreatic lipolysis was studied *in vitro*.

Methods. Pancreatic juice was collected at room temperature from a pancreaticocutaneous fistula in an asymptomatic non-fasting 32 year old white male. Injection of radiopaque material revealed that the fistula communicated directly with the main pancreatic duct. The colorless, slightly turbid, alkaline fluid (amylase activity 947 Somogyi units per ml) was centrifuged for 15 minutes at 2,500 g and 4°C, lyophilized, and stored in a vacuum desiccator at 4°C. Just prior to use, the lyophilized powder was dissolved in 1 ml of cold 0.05 M Tris (Trihydroxymethyl aminomethane) buffer, pH 7.0, and the final concentration of protein adjusted to 2 mg per ml. Protein was measured by the method of Warburg and Christian(5).

Lipolysis was measured at room temperature by a modification of the turbidimetric method of Vogel and Zieve(6). The triglyceride substrate was a stable emulsion of olive oil (0.04%) and sodium taurocholate (0.4%) in 0.05 M Tris buffer, pH 7.0. The reaction was started by adding 20 to 43 μg of pancreatic fluid protein to 2.8 ml of the olive oil emulsion. After inverting the mixture 3 times, lipolytic activity, reflected by clearing of the mixture, was measured by the change in absorbancy per minute between 2 and 5 minutes. Absorbancy was recorded in a 3 ml quartz cuvette with a 1 cm light path using a Zeiss spectrophotometer set at 650 mμ. No change in absorbancy was noted in a control cuvette when an equal volume of buffer without pancreatic enzyme was added to olive oil emulsion. The pH of the assay mixture remained constant during the initial

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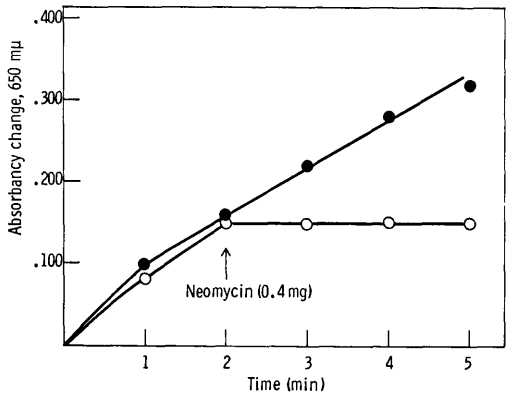

FIG. 1. Absorbancy change of olive oil emulsion after addition of pancreatic enzyme (●). Two minutes later, neomycin (0.4 mg) was added to reaction mixture (○). Assay conditions are as described in text.

5 minutes of reaction. No change in pH was detected after the addition of neomycin and other antibiotics to the reaction mixture. Acetone (0.1 ml) was added to the reaction mixture to stop lipolysis after 6 minutes of digestion. Silica gel chromatography of the reaction products was performed according to the method of Brown and Johnston(7).

Results. After addition of 20 μg of lyophilized pancreatic juice protein to the olive oil substrate, the decrease in absorbancy was linear from 2 to 5 minutes (Fig. 1). The rate of the reaction was proportional to the amount of added protein between absorbancy changes of 0.05 and 0.10 O.D. units per minute. When neomycin sulfate (0.4 mg) was added to the reaction mixture 2 minutes after pancreatic lipase, complete inhibition of lipolysis occurred (Fig. 1). There was no change in absorbancy of the triglyceride substrate when neomycin sulfate (2 mg per 100 ml) was added prior to pancreatic lipase. The calcium concentration of the mixture was less than 10^{-7} and potassium citrate (3×10^{-3} M) did not reverse the inhibition. Neither potassium citrate (3×10^{-3} M) nor potassium sulfate (3×10^{-3} M) alone produced any effect on lipolytic activity.

Six minutes after adding pancreatic enzyme, lipids were extracted from 3 ml of the reaction mixture and subjected to thin layer silica acid chromatography (Fig. 2). The olive oil emulsion yielded only one triglyceride spot; when larger quantities of emulsion were extracted, traces of fatty acid and glyceride were detected. The product from the reaction mixture containing emulsion and pancreatic enzyme was predominantly fatty acid with small amounts of mono and diglycerides. The spot for triglyceride was barely detectable. In the reaction mixture containing neomycin, the triglyceride spot remained mostly unhydrolyzed and much less fatty acid was detected.

Other antibiotics were tested for their ability to inhibit lipolysis. Polymyxin, streptomycin, kanamycin, bacitracin, and ampicillin all inhibited lipolysis at concentrations of less than 3 mg per 100 ml. No inhibition was seen with tetracycline, chlortetracycline, chloramphenicol, sulfisoxazole, or penicillin G at concentrations up to 50 mg per 100.

Discussion. The results of these studies suggest that neomycin inhibits pancreatic lipolysis *in vitro* at concentrations likely to be present in human intestine after usual oral doses of the drug. These findings are consistent with recent observations in man(4). Polymyxin and bacitracin have similar effects on serum cholesterol and bile acid excretion as neomycin(8). It was of interest therefore, to find that these antibiotics also inhibited lipolysis in relatively low concentrations.

Neomycin inhibition may result from a direct action on pancreatic lipase or physicochemical effects on the substrate system. Other enzymes, such as L-glutamic-decarboxylase have been reported to be inhibited by several antibiotics(9). Inhibition of hog and dog pancreatic lipase by chlorotetracycline was shown to be due to the presence of calcium in the antibiotic preparation(10). This possibility was excluded in our studies. Neomycin has also been reported to precipitate bile salts *in vitro* and alter micelle formation(11). No precipitation was noted in our experiments, possibly because we used lower concentrations of neomycin. Further studies with this *in vitro* system may prove useful in determining the mechanism by which neomycin inhibits lipolysis.

Summary. The effect of antibiotics on pancreatic lipolysis was studied *in vitro*. Neomycin and some other antibiotics inhibited

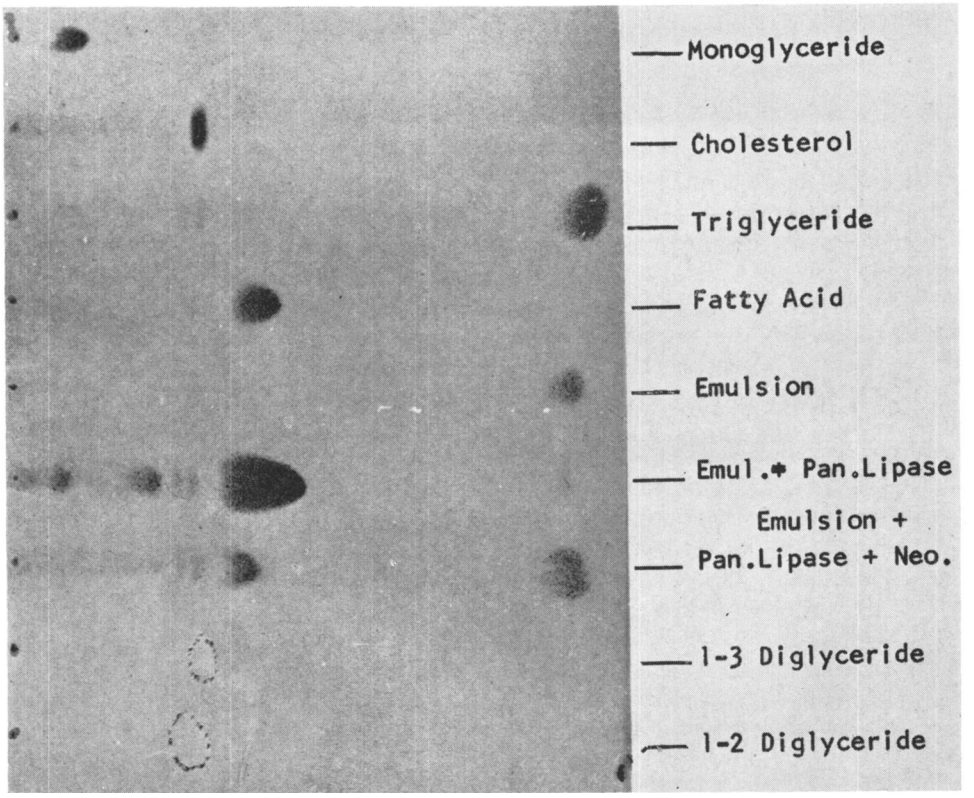

FIG. 2. Silicic acid chromatography of lipids recovered from reaction mixture 6 min, after addition of pancreatic enzyme. Reference compounds were chromatographed for comparison. Origin is at left. Solvent system was 90% hexane, 20% diethyl ether, 2% acetic acid, and 3% methanol. Plate sprayed with 10% phosphomolybdic acid in ethanol.

lipolysis at concentrations below 3 mg per 100 ml. These antibiotics may directly effect pancreatic lipase or alter the physico-chemical state of the substrate.

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