perimental tube as opposed to the survival control(20). The *in vitro* cytotoxic test not only provides a convenient assay of cell injury but, as pointed out by Winn(21), it enables the investigator to detect destruction of fractions of cell populations.

Summary. Evidence is presented indicating that viable cells, freshly prepared from a solid mouse melanoma, are killed when reacted with normal human serum. Serum cytotoxicity was shown to be complement dependent by 1) heat lability, 2) residual complement testing, and 3) through diminution by reacting the serum with a secondary antigen for which it has a demonstrable antibody. Apparent absorption of toxicity from VDRL-reactive human sera by VDRL antigen was shown to be due to concomitant depletion of complement. The toxicity factor could not be correlated with "Wassermann" antibody or the sheep red blood cell antibody content of the sera. Furthermore, the cytotoxic activity could not be related to recent antibiotic therapy or clinically manifest venereal disease of the serum donors.

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Effect of Nicotinic Acid on Serum Triglyceride, Cholesterol and Chylomicrons in Rats. (30391)

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The serum cholesterol lowering effect of nicotinic acid has been studied in various animal species since it was first reported to be of interest in the treatment of hypercholesterolemia in man(1). Recent clinical studies have shown that, in addition to reduced serum cholesterol, nicotinic acid treatment resulted in lowered blood triglyceride(2). In this paper are reported experiments in which reductions in serum cholesterol and triglyceride were obtained in the rat, and data are presented on the effects of nicotinic acid in reducing the number of serum chylomicrons present after fat ingestion.

Methods. A) *Serum triglyceride and cholesterol in fasted rats.* Individually housed male Charles River rats (150-180 g) were fasted 24 hours. Water was given *ad libitum.* Nicotinic acid suspended in distilled water was administered intragastrically to treat-

ment groups (12 rats/group) in geometric doses ranging from 5 to 320 mg/kg. A comparable control group received distilled water only. After 4 hours, arterial blood samples were collected and serum extraction of triglycerides carried out in duplicate using a modification of the method of VanHandel and Zilversmit(3). Colorimetric determinations for triglyceride were made by the method of Blankenhorn *et al*(4). Cholesterol analyses were carried out with an auto-analyzer using the method of Zlatkis *et al*(5). The number of determinations varied within a treatment group due to an occasional insufficient sample volume for both analyses. This experiment was performed twice. The median response with 95% confidence limits was calculated for each dosage level. Straight lines were fitted to the median responses on the log dose scale by the method of least squares and significance of the slopes determined by F-tests. Groups treated with nicotonic acid were compared with the control group by means of the Wilcoxon Rank Sum Test(6).

B) *Four hour chylomicronemia.* In this procedure the strain of rat and weight range were the same as for the method outlined above. Water was given *ad libitum*. Each rat, fasted 24 hours, received an olive oil emulsion* at an oral dose (25 ml/kg) preselected to give optimum chylomicronemia for counting purposes. Nicotinic acid suspended in distilled water or 0.85% saline was given either intragastrically or subcutaneously in geometric doses ranging from 10 to 160 mg/kg (4 rats/treatment) immediately after administration of the olive oil emulsion. Comparable control groups received distilled water. After 4 hours arterial blood samples were collected, centrifuged, and serum pipetted off for chylomicron counting. Chylomicron counts were made under blind experimental conditions. In this procedure, duplicate coded plasma samples were counted under oil immersion (mag. = 1400 ×) in a standard field using a Bausch and Lomb long-working distance phase contrast microscope with a Whipple disc and Spencer phase hemacytometer.

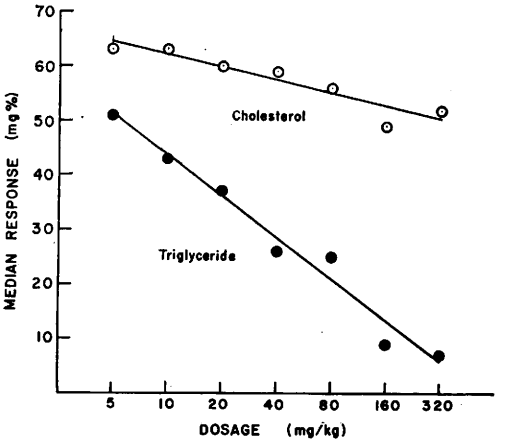

FIG. 1. Effects of oral administration of nicotinic acid in reducing serum triglyceride and cholesterol levels in fasted rats. (Median values in rats not treated with nicotinic acid were 51 and 71 mg% for triglyceride and cholesterol respectively. Number of animals per dose level ranged from 22 to 24.)

This experiment was performed 3 times. After averaging duplicate chylomicron counts for each rat, the data were pooled for the 3 tests and the medians determined. Ninety-five per cent confidence limits were estimated as the range of the 12 responses after deleting the lowest and the highest values.† Groups treated with nicotinic acid were compared with the control group by means of the Wilcoxon Rank Sum Test(6).

Results. A) *Triglyceride and cholesterol experiment.* Oral administration of nicotinic acid to fasted rats resulted in a linear regression of serum triglyceride and cholesterol levels with increasing dosage increments (Fig. 1). F-tests showed the regression slopes to be statistically significant ($P<0.001$) for both triglyceride and cholesterol ($F = 195.09$ and 28.34 respectively). Based on the mg% scale used in describing the response levels, greater reductions occurred in triglyceride than in cholesterol (slope = −25.22 and −7.66 respectively). At the highest dose tested (320 mg/kg) serum triglyceride was reduced 87% whereas cholesterol was reduced by only 27%. Significant differences

* 60 ml olive oil + 120 ml 10% gum acacia + 300 ml water.

† The true significance level is $P = 0.036$. Thus the declared 95% confidence limits are conservative.

TABLE I. Effect of Oral Administration of Nicotinic Acid on Number of Serum Chylomicrons Present After Fat Ingestion.

Treatment mg/kg	Chylomicron count*	95% Confidence limits
(Controls)	106	63-143
10	66†	30-92
20	38†	30-54
40	30†	19-49
80	21†	12-44
160	12†	8-36

* Median, 12 rats per treatment.
† Significantly different from control group ($P<.05$) by Wilcoxon Rank Sum Test(6).

between control and treated groups ($P< 0.05$) were realized at dosage levels $\geqq$ 10 mg/kg for triglyceride and at all doses tested for cholesterol.

B) *Chylomicron experiment.* Oral administration of nicotinic acid to groups of rats immediately following a standard oral dose of olive oil, resulted in a reduction in the number of chylomicrons present in the serum at 4 hours. Decreases in median counts were observed as the dose of nicotinic acid increased (Table I). Qualitatively similar results were obtained in an experiment in which nicotinic acid was administered subcutaneously (Table II). Significant differences in chylomicron counts occurred between control and treated groups at all dosage levels tested by both routes of administration ($P< 0.05$).

Discussion. Duncan *et al*(7) have reported a reduction in serum cholesterol by addition of 1% nicotinic acid to a diet fed *ad libitum* to rats for 8 and 42 days. When weight gain was controlled by paired-feeding in another experiment, no significant difference in cholesterol between control and treated groups was observed. Garattini *et al*(8) have reported reductions in serum cholesterol in rats following treatment with nicotinic acid administered subcutaneously for 6 days at high doses (600 mg/kg). In addition, these investigators reported inhibition of Triton-induced hypercholesterolemia at 1000 mg/kg by intraperitoneal injections. Data on serum triglyceride were not reported in the above experiments. By comparison, in the present study, nicotinic acid significantly lowered both serum cholesterol and triglyceride by oral administration over a low dosage range and within a relatively short post-treatment time interval. It should be noted that a greater reduction in triglyceride occurred than in cholesterol.

TABLE II. Effect of Subcutaneous Administration of Nicotinic Acid on Number of Serum Chylomicrons Present After Fat Ingestion.

Treatment mg/kg	Chylomicron count*	95% Confidence limits
(Controls)	94	63-133
10	48†	29-62
20	19†	12-37
40	14†	5-25
80	12†	5-27
160	6†	4-18

* Median, 12 rats per treatment.
† Significantly different from control group ($P<.05$) by Wilcoxon Rank Sum Test(6).

The inhibition of chylomicronemia occurring with nicotinic acid treatment has not previously been reported. Whether this is an effect on fat absorption, or on the synthesis, mobilization or metabolism of chylomicrons is yet to be determined. The possibility that nicotinic acid interferes with intraluminal fat digestion seems to be excluded by the fact that reductions in chylomicron counts were obtained following parenteral administration. The marked effect of nicotinic acid on serum triglyceride and chylomicrons in rats suggests that in these experiments the reduction in serum cholesterol obtained may be secondary to a general hypolipemic response.

Summary. The effects of nicotinic acid on serum lipids have been studied in rats. Significant decreases in serum triglyceride and cholesterol were observed in fasted rats 4 hours after oral administration of nicotinic acid. Greater reductions in triglyceride occurred than in cholesterol. In addition, in another study, oral or parenteral administration of nicotinic acid significantly inhibited fat-induced chylomicronemia.

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Pancreatitis in the Rat. (30392)

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Most experimental work on pancreatitis has been done in the dog, usually with bile injected under pressure into the major pancreatic duct. The rat, a smaller and more economical animal, has many pancreatic ducts which open into the lower part of the common bile duct, simulating a common channel where biliary and pancreatic secretions can mix. However, earlier efforts to produce pancreatitis in the rat by ligation of the lower common bile duct were unsuccessful. Baggenstoss(1), Edlund(2), Nestel(3), and others reported that simple occlusion of the common bile duct at the duodenum of the rat produced edema of the pancreas and fat necrosis, but no parenchymal hemorrhagic necrosis.

We have found earlier(4) that in the rat ligation of the common bile duct at the duodenum produced parenchymatous necrosis, hemorrhage and fat necrosis within a few days. We have now established this on a larger series of animals.

Methods. Sprague-Dawley male and female rats with average weight of 222 g, fasted for 24 or 48 hours (with water available), were anesthetized with ether. In one group (Group A, 45 animals; Tables I and II, Group A-1, 28; Group A-2, 12; Group A-3, 5 animals), the common bile duct was ligated on the duodenum. It was important to employ fine silk sutures (size 5-0), because coarse thread may permit leakage. Blood vessels on and near the duct were excluded from the ligatures. Groups A-2 and A-3 received secretin Vitrum i.v. After 3-10 days, the contents of the dilated duct were aspirated and examined for enzymes; since the amount of fluid from a single rat was small, fluids obtained from 2-5 animals were pooled. Trypsin and trypsinogen were estimated by the method of Anson(5) and amylase by the method of Somogyi(6). Most animals died (38), and those in critical condition were killed. All animals were autopsied immediately; those that died during the night were discarded. Tissue sections from parts of the pancreas were stained with H.E. In 5 rats (Group B), the bile duct was ligated above and below the entrance of the pancreatic ducts. Pancreatic secretion which accumulated in the interligature segment was used for assay of enzymes. Histological examinations of 5 parts of the pancreas, and of the liver and omentum, were performed; classification of pancreatic pathology is based on histologic and gross observations.

In another group, the common duct was cut above and below the entrance of the pancreatic ducts, and plastic tubing was introduced into each end, thus permitting pure biliary and pancreatic secretion to be obtained or to determine secretory pressures.

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