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Effect of High Lipid Diets on Normal and Traumatized Rats.* (31576)

ELIZABETH LETITIA BEARD† AND JOHN K. HAMPTON, JR.‡
Department of Physiology, Tulane University School of Medicine, New Orleans, La.

Diets rich in saturated fat are reported to diminish blood proteolytic activity in a variety of mammals(1-9). Such a depression could enhance blood coagulability by inhibiting A) the blood proteolytic enzyme, plasmin; B) the activation of its precursor, plasminogen; or C) normal maintenance of blood plasminogen levels. In seemingly unrelated studies, Duguid(10) has reported that thrombi lodged at the intimal surface of arteries may readily become endothelialized and undergo necrotic conversion into atheromatous plaques. These findings suggest that lipemia might induce the development of atherosclerosis by depressing proteolytic activity and thereby enhance the production of thrombi.

In this study, direct inhibition of plasmin activity by lipemia, particularly hypercholesterolemia, was investigated by measuring serum proteolytic activities in rats maintained on diets rich in a highly saturated fat (butter), a relatively unsaturated fat (corn oil) and a normal diet containing lipid levels of 1/10 the experimental diets.

Plasminogen reserves in the lipemic and normal rats were assessed using trauma as the means of plasminogen activation. Rats from each diet category were subjected to doses of trauma in a Noble-Collip Drum shown to yield 2- and 4-fold increases in the plasmin levels of normal rats(11). If trauma failed to activate similar plasmin levels in lipemic rats one would assume that lipemia inhibited plasminogen activation or in some way depressed body plasminogen concentrations.

Methods. Ninety male Holtzman rats were maintained on one of the following experimental diets: A) 40% whole butter diet, B) 40% corn oil diet and C) normal diet of Wayne Lab Blox containing 4.9% lipid by weight.

These diets were compounded as follows:

40% lipid diet[1]	g %
Cholesterol	5.0
Choline chloride	.2
Sodium cholate	2.0
Salt mixture[2]	4.0
Vitamin mixture[3]	2.0
Casein	20.0
Sucrose	26.8
Whole butter or corn oil	40.0
Normal diet[4]	
Crude protein (min)	26.0
Crude fat	4.9
Crude fiber (max)	6.5
Crude ash (min)	7.5

[1] Purchased from Nutritional Biochemicals Corp. —approximately 610 calories/100 g.

[2] Salt mixture U.S.P. XIV from N.B.C.

	g	
Cupric sulfate	0.48	16.2 g
Ferric ammonium citrate	94.33	
Manganese sulfate	1.24	
Ammonium alum.	0.57	
Potassium iodide	0.25	
Sodium fluoride	3.13	
to make	100.00	
Calcium carbonate	68.6	
" citrate	308.3	
" biphosphate	112.8	
Magnesium carbonate	35.2	
" sulfate	38.3	
Potassium chloride	124.7	
Dibasic potassium phosphate	218.8	
Sodium chloride	77.1	
	1000.00	

[3] Vitamin fortification mixture from N.B.C. without choline chloride.

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† Present address: Department of Biological Sciences, Loyola Univ. of the South, New Orleans, La.

‡ Present address: Inst. for Dental Science, Univ. of Texas Dental Branch, Houston.

	g/100 lb diet
Vitamin A concentrate, 2,000 units/g	4.5
Vitamin D concentrate, 400,000 units/g	.25
Alpha tocopherol	5.0
Menadione	2.25
P aminobenzoic acid	5.0
Niacin	4.5
Riboflavin	1.0
Pyridoxine hydrochloride	1.0
Thiamine hydrochloride	1.0
	mg/100 lb diet
Biotin	20
Folic acid	90
Vitamin B_{12}	1.35

[4] Wayne Lab Blox—approximately 168 calories/100 g.

The lipid diets differed in their degree of saturation. Butter is a highly saturated lipid with an iodine number range from 26-28(11). Corn oil has an iodine number range of 115-124(11) with an 85.6% content of unsaturated fatty acids(12). The 40% butter diet then was 3-4 times more saturated than the corn oil diet. The 40% lipid diet used was essentially that described by O'Neal and coworkers(13) to produce atherosclerosis in albino rats. The diet used here had 5% more sucrose replacing Alphacel in O'Neal's formula. The 5% cholesterol was added by these workers and earlier investigators(14) to increase rat plasma cholesterol levels. The bile salt was used to initiate atherosclerotic development. These workers found elevated serum cholesterol levels to correlate with the development of atherosclerosis in the rat.

Each diet was administered for 2-, 4- and 6-week periods to 30 rats, approximately 3 months of age. At the end of the feeding period, 10 were retained as nontraumatized normal rats, 10 were rotated in the Noble-Collip Drum for 240 revolutions and 10 were rotated for 480 turns. These levels of trauma had previously produced 2- and 4-fold increases in rat plasmin levels, presumably by activating circulating plasminogen(15). Whole blood was drawn from the traumatized rats 30 minutes post trauma. The rats were anesthetized with 6% Nembutal just prior to removing blood from the abdominal aorta.

Serum proteolytic activity, reflecting blood fibrinolytic or plasmin activity, was determined for all rats by a Casein I^{131} lysis method(15). Serum cholesterol concentrations were determined for all rats by the method of Abell(16).

Results. In most groups, food consumption increased gradually with time. The average food intake per rat per 24 hours was 25.00 g for the normal diet rat, 12.9 g for those on the 40% corn oil diet, and 15.2 g for those on the 40% butter diet. Weight differences among rats dieted for comparable periods were slight despite differences in diet and food intake.

Serum cholesterol levels of the rats fed butter or corn oil diets each containing 5% cholesterol were markedly higher than those of the controls (Table I). The intensity of lipemia from the high lipid dieted rats was evidenced by the milky white appearance of their sera. Trauma in the Noble-Collip Drum usually caused increased serum cholesterol. MacLaughlin and Gray have reported marked increases in the adrenal cortical steroid concentrations of rat plasma following tumbling

TABLE I. Effect of High Fat Diets and Noble-Collip Drum Trauma on Rat Serum Cholesterol Levels.

Drum rev†	Weeks dieted	Blood serum cholesterol levels (mg %)* Normal diet‡	40% corn oil diet	40% butter diet
0	2	78.70 ± 19	101.90 ± 19	91.88 ± 29
	4	60.25 ± 20	92.70 ± 20	124.50 ± 32
	6	73.95 ± 10	131.50 ± 34	98.94 ± 15
240	2	85.15 ± 31	123.55 ± 39	103.00 ± 30
	4	63.31 ± 12	117.88 ± 19	125.28 ± 37
	6	61.13 ± 14	121.44 ± 20	112.06 ± 33
480	2	86.71 ± 14	127.68 ± 42	156.19 ± 39
	4	84.92 ± 13	145.55 ± 31	141.22 ± 34
	6	83.79 ± 15	108.56 ± 34	138.50 ± 31

* Mean values of approximately 10 rats/datum.
† No. of revolutions in Noble-Collip Drum.
‡ Wayne Lab Blox (4.9% fat content).

TABLE II. Effect of High Fat Diet and Noble-Collip Drum Trauma on Rat Serum Proteolytic Activity.

		Normal diet*		40% corn oil diet		40% butter diet	
Drum rev§	Weeks dieted	% Substrate digested†‡	P value‖	% Substrate digested	P value	% Substrate digested	P value
0	2	3.65 ± 2.46		3.81 ± 2.91		3.55 ± 2.09	
	4	1.93 ± .68		1.98 ± .76		2.53 ± 1.89	
	6	1.94 ± .68		1.99 ± .68		2.49 ± 1.27	
240	2	4.22 ± .99	<.5	4.50 ± 1.31	<.5	4.39 ± 1.37	<.5
	4	4.65 ± 3.16	<.02	4.93 ± 1.82	<.01	4.94 ± 1.82	<.05
	6	4.38 ± 1.94	<.05	4.51 ± 1.54	<.01	5.41 ± 1.84	<.001
480	2	8.29 ± 2.09	<.001	8.29 ± 1.38	<.001	9.95 ± 2.17	<.001
	4	8.18 ± 1.66	<.001	9.21 ± 1.64	<.001	9.66 ± 2.67	<.001
	6	10.77 ± 2.62	<.001	10.37 ± 3.88	<.001	10.05 ± 1.64	<.001

* Wayne Lab Blox (4.9% fat content).
† Mean values of 10 rats per datum.
‡ % Casein I^{131} digestion by serum sample.
§ No. of revolutions in the Noble-Collip Drum.
‖ Significance of differences between traumatized and nontraumatized members of the same diet groups. P values of Student "t" test.

trauma(17). The mean serum proteolytic activity data for each subgroup of dieted rat listed in Table II were analyzed statistically by a 3 × 3 × 3 factorial design. Trauma was found to be the only factor which significantly altered rat serum proteolytic activity and it increased maximally following maximum trauma administration. The increase was highly significant in the rats drummed for 480 revolutions ($P<0.001$). In those drummed for 240 revolutions, the increase was significant in the rats dieted for 4- and 6-week periods ($P<0.05$).

The type of diet administered and the length of its administration did not significantly alter serum proteolytic activity of normal or traumatized rats. Interaction effects of diet type, duration of diet, and extent of trauma levels were statistically negligible.

The mild hypercholesterolemia produced in the rats studied chronically (2-6 weeks) did not appear to depress the plasmin content in blood plasma. Proteolytic activity was essentially the same in sera from the 3 dieted groups of non-traumatized rats. Trauma was used as a means of activating plasminogen *in vivo* in each rat diet group. Since plasmin levels following trauma were similar in the lipemic and normal dieted rats we concluded that the cholesterolemia produced by the conditions described did not inhibit the concentration or activity of the blood components responsible for plasminogen activation.

In view of these findings, the depression of blood proteolytic activity widely reported to be induced by lipemia(1-8,18) and to lead to thromboembolic abnormalities(10) and eventually to the development of atherosclerosis(1,4) is curious. It appears that moderate hypercholesterolemia in rats does not significantly alter levels of blood proteolytic activity by depressing plasmin or plasminogen activator activity.

The possibility exists that high serum lipid levels which develop in rats dieted on high lipid diets for longer periods might interfere with the union of plasminogen activator or plasmin with fibrin and thus inhibit blood fibrinolytic activity. The nature of such an interference is being investigated.

Summary and conclusions. Rats were maintained on 40% butter, 40% corn oil and control diets for 2, 4 and 6 weeks. Rats from each diet were traumatized in the Noble-Collip Drum so as to compare plasminogen activation. Serum proteolytic activity and cholesterol levels were determined. Differences in weights of the comparably dieted animals were negligible. Serum cholesterol levels were markedly higher in those fed high fat diets with 5% cholesterol than in rats on control diets. Trauma caused significantly increased serum proteolytic activity and serum cholesterol levels in all groups. The diet administered produced moderate hypercholesterolemia which did not significantly alter the serum

proteolytic activator or plasmin activity of normal or traumatized rats.

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Determination of Total Cation-Forming Mineral Elements in Feces And Urine and Its Relation to Renal "Net Acid" Excretion.* (31577)

MERRILL N. CAMIEN, LEO M. SMITH,† THOMAS J. REILLY, AND DANIEL H. SIMMONS
Cedars-Sinai Medical Research Institute, and Departments of Medicine and Physiology, University of California Medical Center, Los Angeles

The significance of renal "net acid" excretion, calculated as the sum of urinary titratable acid (TA) and ammonium minus urinary bicarbonate ("net acid" = TA + NH_4^+ — HCO_3^-), in relation to hydrogen ion regulation in the mammalian organism is widely recognized, and a detailed discussion of this subject has been presented by Elkinton(1). Questions concerning the ultimate sources of urinary "acid" have long been of considerable interest. Early investigations reviewed by Shohl(2) tended to emphasize dietary ash as a source of this "acid"; however, a relatively recent study reported by Hunt(3) indicated that it was "impossible to demonstrate any relation between the urinary output of acid and the acidity or alkalinity of the ash of the diet, apart from its sulphur content." This observation has apparently considerably influenced current concepts of acid production as discussed by Relman *et al*(4-6) in their studies concerning "net balance of acid." On the other hand, the present authors(7) have indicated that urinary mineral composition and renal "net acid" excretion are markedly interdependent and that the extent to which cation-forming‡ elements are excreted in the feces can affect the extent of renal "net acid" excretion. The latter observation suggests, since cation-forming elements constitute a major component of both dietary and fecal ash, that the conclu-

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‡ Replaces the anachronistic and partly inaccurate term, "base-forming," in reference to elements such as Na, K, Mg and Ca, which exist in biological fluids solely as either free or bound cations.