ready and easily available source of this material for various *in vitro* studies.

Although the present experiments suggested that gruel from an experimental atherosclerotic plaque did hasten the formation of a thrombus in the Chandler apparatus(3), its thrombogenic propensity was no greater than that of fibrous tissue obtained from simple thrombosclerotic plaques, and very much less than that of normal aortic tissue. Moreover, the lipids extracted from experimental plaque gruel did not possess significant thrombogenic activity. Finally, native gruel obtained from human coronary plaques had no discernible thrombogenic properties.

These results suggest to us that the lipid component of the gruel of an atherosclerotic plaque even in the human subject probably does not play an important part in the occurrence of luminal thrombosis. However, this does not mean that the luminal rupture of an atherosclerotic process may not initiate thrombosis. Substances other than lipid may be present which activate the process of platelet aggregation or some other phase of the thrombosis phenomenon. Then too, luminal blood rushing into the cavity walls of a ruptured plaque may expose such blood to still living but badly damaged and possibly potently thromboplastic tissue.

Summary. Gruel obtained from experimental thromboatherosclerotic plaques and from human coronary artery plaques was tested in *in vitro* studies for its possible thromboplastic potency. Gruel from experimental thromboatherosclerotic plaques was moderately thrombogenic, but that obtained from the human plaque appeared to be non-thrombogenic. The lipid extract of the experimental gruel failed to exhibit any thrombogenic propensity.

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Glucose Metabolism in the Rat During Starvation and Refeeding Following Starvation.* (28935)

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Reports of impaired glucose tolerance in fasting and starved animals have led to a popular concept of "starvation diabetes"(1), although impaired glucose tolerance does not necessarily reflect diabetes nor does starvation necessarily decrease glucose tolerance(2). There are certainly profound changes in the metabolism of glucose in starvation, and a marked "supernormal lipogenesis" in refeeding following starvation(3) which is apparently related primarily to abnormal glucose metabolism. Refeeding following starvation often results in fatty livers which may be sufficiently severe to result in degenerative changes(4-6). These, and perhaps other metabolic abnormalities which accompany refeeding following starvation, often cause physiological "stresses" which may be manifested either acutely or chronically. In the cardiovascular system particularly, tissue damage in refeeding might be expected to be manifested chronically in terms of hypertensive and/or atherosclerotic cardiovascular disease(7).

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Methods and materials. Experiment 1.

Male albino rats were fed *ad libitum* a semi-purified diet† from weanling weight until they weighed approximately 200 g. They were subjected to starvation and refeeding with groups of 2 individuals selected for study at 0, 4, 6, 8, and 10 days of starvation and at 4 and 6 days of refeeding following 10 days starvation. Drinking water was available at all times. A "tolerance-type" dose of glucose (400 mg/100 g body weight) containing 10 μc of uniformly labeled glucose-C^{14} was injected intraperitoneally, and total respiratory CO_2 was collected hourly in 5 N NaOH for 5 hours following glucose injection. The rats were then sacrificed, and hearts, livers and kidneys were collected, weighed and instantly frozen.

Samples of liver were analyzed for content of glycogen, total lipid extractable into ethanol-"hexane" (petroleum ether, b.p. 60-68°C), (10:8.5 v/v), cholesterol, and TCA-precipitable nitrogen. Hearts, kidneys, and samples of livers were homogenized in 10% trichloroacetic acid (TCA) and centrifuged. The insoluble residue was extracted with the ethanol-petroleum ether mixture. For hearts and kidneys the solvent was evaporated from aliquots of the lipid extract, and the lipid residue was reextracted with acetone. The lipid-free residues from the tissue homogenates were assayed for TCA-precipitable nitrogen by Kjeldahl analysis. Glycogen was precipitated from the TCA extract of liver by addition of ethanol. Glycogen was purified by reprecipitation from 10% TCA and measured colorimetrically by reaction with anthrone reagent. Lipid content of the various extracts was estimated gravimetrically, except that cholesterol in liver extracts was determined colorimetrically after precipitation with digitonin and reaction with p-toluene sulfonic acid in acetic anhydride and acetic acid. Total respiratory CO_2 was determined by differential titration(8) of aliquots from the alkaline collections. Radioactivity of respiratory CO_2 was assayed by counting of 50 μl aliquots of the alkaline collections in 6 ml absolute ethanol and 8 ml toluene containing diphenyloxazole (0.3% w/v). Emulsions of the alkaline aliquot were stabilized for liquid scintillation counting by addition of natural lecithin ("Thixcin," Baker Castor Oil Co., New York). Radioactivity of all lipids, including cholesterol digitonides redissolved in glacial acetic acid, was assayed by liquid scintillation counting in solutions of absolute ethanol and toluene containing 0.3% diphenyloxazole. Appropriate corrections for counting efficiencies and quenching effects were made through addition of internal standards and recounting of all samples(9). Radioactivity of liver glycogen was assayed by direct counting of planchets with infinitely thin samples, in a windowless gas-flow counter.

Experiment 2. Young adult male rats of the Sprague-Dawley strain were fed *ad libitum* a commercial diet (Rockland Rat Diet, A. E. Staley Co., Decatur, Ill.) continuously or were subjected to 4 episodes of starvation and refeeding involving 20% losses in body weight during starvation. On the third day of refeeding in the final episode, 5 starved-refed rats and 5 non-starved "control" rats each were injected intraperitoneally with 400 mg of glucose per 100 g body weight. One hour after injection the rats were killed by decapitation and blood was collected into 2 pooled samples (control *vs* starved-refed) and chilled. Hearts were weighed, chilled and homogenized in chilled, isotonic KCl solution containing, per liter, 8 ml of 0.04 M $KHCO_3$. Aliquots of heart homogenates were equilibrated in Warburg vessels containing rat serum, and subsequently incubated with 10 μc of uniformly labeled glucose-C^{14}. Carbon dioxide was collected on filter paper saturated with 5 N KOH and placed in the center well. Duplicate samples from each rat group were studied under the following conditions:

1) control homogenate in control serum; 2) control homogenate in starved-refed serum; 3) starved-refed homogenate in control serum; 4) starved-refed homogenate in starved-refed serum.

Oxygen uptake was observed during one hour of incubation after which lipids from

† Casein, purified, 18%; DL-methionine, 0.2%; sucrose, 34.0%; starch, 33.8%; mineral mixture, 4%; vegetable fat ("Crisco"), 10.0%; vitamin mixture and methyl linoleate, adequate.

TABLE I. Size and Composition of Organs in Starvation and Refeeding.

Item		Treatment: Non-starved	Starved	Starved 10 days, refed 4 days	Starved 10 days, refed 6 days
No. of animals		2	8*	2	2
Avg body wt†, g:		220	161	194	194
Body wt changes, %:					
	mean	—	−27	−35, +18‡	−38, +24‡
	(range)	—	−(16 to 41§)	−(33, 37), +(17, 19)	−(36, 41), +(23, 25)
Livers					
Size, g/100 g body wt,	mean	4.1	2.9	6.9	8.4
	(range)	(4.5, 3.7)	(1.8 to 3.8)	(6.7, 7.1)	(7.9, 9.0)
Nitrogen conc, mg/g liver,	mean	10.1	14.9	9.6	6.8
	(range)	(9.2, 10.9)	(13.3 to 20.7)	(9.0, 10.1)	(6.2, 7.3)
Lipid conc, mg/g liver,	mean	20	31	198	318
	(range)	(10, 30)	(16 to 57§)	(198, 198)	(304, 333)
Glycogen conc, mg/g liver,	mean	10.8	24.9	6.8	6.4
	(range)	(9.4, 12.2)	(16.2 to 35.0)	(6.7, 6.9)	(5.6, 7.1)
Hearts					
Size, g/100 g body wt,	mean	.43	.41	.40	.42
	(range)	(.42, .44)	(.33 to .48)	(.38, .41)	(.39, .44)
Nitrogen conc, mg/g heart,	mean	22	23	23	22
	(range)	(21, 23)	(11 to 26)	(22, 23)	(20, 23)
Lipid conc, mg/g heart,	mean	14	14	11	11
	(range)	(10, 18)	(8§ to 18)	(10, 12)	(8, 13)
Kidneys					
Size, g/100 g body wt,	mean	.94	.94	.88	.92
	(range)	(.90, .98)	(.77 to 1.05)	(.86, .89)	(.84, .97)
Nitrogen conc, mg/g kidney,	mean	25	26	26	26
	(range)	(24, 25)	(24 to 29)	(25, 26)	(22, 31)
Lipid conc, mg/g kidney,	mean	58	55	52	55
	(range)	(58, 58)	(40§ to 64)	(47, 56)	(53, 56)

* Values are for 2 rats each at 4, 6, 8, and 10 days of starvation.
† At time of slaughter.
‡ Loss in 10 days' starvation, gain in refeeding.
§ Values associated with longer starvation.

the reaction were extracted first with toluene and subsequently with toluene-ethanol (10:8 v/v). Radioactivity of these lipid fractions and of CO_2 evolved was assayed by liquid scintillation counting in absolute ethanol and toluene containing 0.3% diphenyloxazole.

Results and discussion. Experiment 1. Rats starved from 4 to 10 days lost from 16% to 41% of their prestarvation weights. Weight losses were not closely correlated with length of starvation period. Furthermore, data from starved rats were not necessarily progressive with either length of starvation or with extent of weight loss. For this reason, data from starved rats are presented and discussed collectively rather than in terms of progressive stages of starvation. There were notable differences in data from rats refed 4 *vs* 6 days, and some of these data are presented and discussed separately. One rat starved 10 days was moribund at time of glucose injection and responded differently from the other starved rats. Glucose metabolism data from this rat are shown individually.

Changes in body and organ weights of rats starved and refed in Exp. 1 are summarized in Table I. Rats starved 10 days and refed 4 days lost approximately 35% of prestarvation weights and regained in refeeding approximately 18% of their prestarvation weights. Rats starved 10 days and refed 6 days lost 38% and in refeeding regained 24% of initial weights. In starvation the relative liver size was diminished to approximately 70% of the prestarvation size and was approximately doubled over prestarvation size after refeeding for 4 days, and more than doubled after 6 days refeeding. The changes in liver composition reflect relative increases in concentration of TCA-precipit-

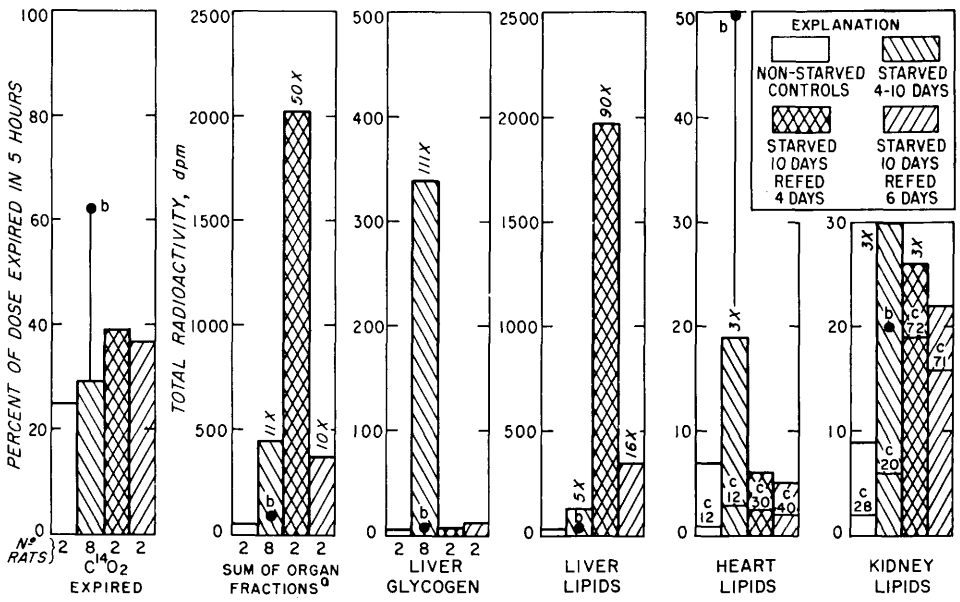

FIG. 1. Distribution of C^{14} from glucose-U-C^{14} 5 hr after injection (i.p.) into non-starved, starved (4-10 days), and starved-refed rats (dosage 10 μc in 400 mg/100 g body wt). (a) Sums of liver glycogen and lipids of livers, hearts and kidneys. (b) Single value for one rat starved 10 days and moribund at time of study. (c) Percentage of lipid-C^{14} in the acetone-soluble fraction.

able nitrogen, lipid and glycogen in starvation reflecting a change in moisture content. The data for refed rats reflect a marked increase in liver lipid upon refeeding, involving not only an increase in total content of liver lipid, but also a notable increase in lipid concentration of livers. The ratios of heart weight to body weight, and kidney weight to body weight remained relatively constant during starvation and refeeding. Nitrogen concentrations of hearts and kidneys were relatively constant during starvation and refeeding; however, hearts decreased slightly in concentration of lipids in refed rats, and kidney concentration of lipids decreased in starvation, especially late starvation, and failed to recover in early refeeding, but recovered in later refeeding. These observations appear to be in agreement generally with findings in the literature(1).

Some aspects of the overall metabolism of glucose in the non-starved, starved and starved-refed rats are shown in Fig. 1. In view of the dosage technique employed, the data reflect metabolism of glucose in hyperglycemia. The amount of glucose oxidized to CO_2 is reflected in the first graph which shows the percentage of injected glucose-C^{14} that was oxidized in the initial 5 hours after injection. The amount of C^{14} expired as CO_2 varied from approximately 25% of injected dose in non-starved rats to almost 40% in rats starved 10 days and refed 4 or 6 days. Rats starved from 4 to 10 days expired slightly more $C^{14}O_2$ than did the non-starved rats. The remaining graphs in Fig. 1 reflect the distribution of C^{14} in liver glycogen and in lipids of liver, heart and kidneys. The graph which reflects the sum of these fractions shows that rats starved 4 to 10 days incorporated into various fractions 11 times as much label as did the non-starved rats; whereas rats starved 10 days and refed 4 days incorporated 50 times as much label as did non-starved rats. Starved rats incorporated into liver glycogen approximately 111 times as much label as did the controls, while the composition data in Table I reflect that only approximately 2 times as much total glycogen was found in the livers. Both total content (Table I) and radioactivity (Fig. 1) of liver glycogen were near normal in the starved-refed rats. Starved rats incorporated only slightly more C^{14} into liver

TABLE II. Glucose Metabolism by Heart Homogenates from Control vs. Starved-Refed Rats.

Item	Incubation systems: Control heart Control serum	Control heart Refed serum	Refed heart Control serum	Refed heart Refed serum
Total radioactivity of CO_2:	8.0	7.0	10.4	8.9
(cpm/mg N in flask)	5.0	13.3	14.4	16.5
Avg	6.5	10.1	12.4	12.7
Total radioactivity of toluene-soluble lipid:	182	184	220	158
(cpm/mg N in flask)	249	324	230	367
Avg	215	254	225	262
Total radioactivity of toluene: alcohol soluble lipid	4200	5450	6000	5000
(cpm/mg N in flask)	6500	5400	6000	5100
Avg	5350	5425	6000	5050

lipid than did controls; however, rats starved and refed 4 days incorporated into liver lipids approximately 90 times as much glucose from the labeled dose as did controls. After 6 days of refeeding, the amount of label incorporated into liver had decreased to 16 times the value for non-starved rats; whereas fat concentration in the livers had increased from 198 mg/g in rats refed 4 days to 318 mg/g in rats refed 6 days. These relative changes in lipid content and lipid radioactivity reflect a drastic increase in specific radioactivity of liver lipids in early refeeding with a relative decrease in specific activity during subsequent refeeding associated with the extreme fatty liver.

The relative changes in radioactivity of kidney lipids of starved and refed rats reflect a marked increase in radioactivity of total extracted lipid. In starvation the proportion of radioactivity from acetone soluble lipids (primarily "neutral fat") to total lipids in hearts and kidneys remained relatively constant; however, in refeeding after starvation the proportion of radioactivity in "neutral fat" was markedly increased. It appears that a notable effect of starvation upon glucose metabolism in heart and kidney was a tremendous increase in radioactivity of the "phospholipid" fraction (indicative of increased phospholipid synthesis); whereas in starved-refed rats the data reflect increased synthesis of triglycerides only.

Single values plotted in Fig. 1 for a rat starved 10 days and moribund at the time of glucose injection reflect severe abnormalities in the patterns of glucose metabolism. Approximately 68% of the injected dose was oxidized to $C^{14}O_2$. Almost 75% of the $C^{14}O_2$ expired was expired in the first 2 hours after administration of the dose. Incorporation of the C^{14} into liver glycogen and liver and kidney lipids was remarkably low, as compared to other starved rats, but the incorporation of label into heart lipids was drastically increased. Only 6% of the total radioactivity in the heart lipids of this rat was present in the acetone-soluble fraction.

Only trace amounts of cholesterol were found in the livers of rats in any of the treatment groups, and the radioactivity of digitonide precipitates from extracts of livers, hearts and kidneys was negligible.

Experiment 2. The results from the study of glucose utilization by heart tissue from normal *vs* starved-refed rats are summarized in Table II. The data shown are values from duplicate assays within each treatment group and their respective means. These observations suggest that the repeated episodes of starvation-refeeding elicited no appreciable change in glucose oxidation or incorporation into lipids by homogenates of the heart tissues. These results suggest that neither changes in the heart tissues in refeeding nor factors released into the blood serum of refed animals exerted any apparent effects upon glucose utilization by heart homogenates that

were different from responses of non-starved rats.

Discussion. The data reported herein relate directly to glucose utilization by the rat in hyperglycemia. They leave little doubt that the metabolism of glucose is greatly altered in starvation and in refeeding after starvation. While the results do not express "glucose tolerance" as such, the aspects of glucose utilization considered reflect no impairment of overall glucose utilization in starvation. The data for starved rats (in Experiment 1) reflect only a slight increase in *net* content, but an increased specific radioactivity of liver glycogen. Total content of liver lipids was increased almost 2-fold in starvation, whereas total radioactivity was increased 5-fold. Heart lipids in starved rats showed a pronounced increase in specific activity, reflecting perhaps an increased turnover of lipids in the heart as an initial response to hyperglycemia following starvation. The failure of heart tissue from starved-refed rats in Exp. 2 to utilize glucose differently than did normal rats suggests that the altered metabolism of glucose by hearts of starved rats of Exp. 1 was probably due to changes in tissues other than the heart itself.

The observations of increased lipid concentration of livers in refed animals and of drastically increased incorporation of C^{14} into lipids of liver and kidneys in starved-refed animals (Fig. 1) confirm reports of "supernormal lipogenesis" in refeeding(3,6,10), and extend the knowledge regarding the nature of such lipogenesis. It is somewhat surprising that practically none of the glucose C^{14} was incorporated into liver cholesterol. Partial explanation may reside in the suggestion(14) that the rate of glycolysis is a selective determinant for synthesis of fatty acids *vs* cholesterol.

In refeeding following starvation the total lipid radioactivity in hearts and kidneys was decreased below starvation levels, and returned to prestarvation levels in the case of the heart but not the kidney. The proportion of radioactivity in the acetone-soluble fraction (neutral fat) was increased in refed rats over prestarvation levels in hearts and kidneys, indicating perhaps a deficiency in lipotrophic agents, although the refeeding diet contained supplemental methionine at 0.2 g and choline at 15 mg, respectively, per 100 g of diet.

The profound changes observed in the metabolism of glucose by the one severely starved rat are of particular interest. Do these changes, especially the phenomenon of extremely high incorporation of label into the phospholipid fraction of heart lipids, offer further explanation of the mechanisms involved in the "stresses" of hyperglycemia in the severe diabetic as well as the severely starved individual? The magnitude of the apparent lipogenesis in tissues of starved and/or starved-refed rats raises questions regarding the extent to which various tissues are so affected. If the intimal and medial tissues of the vascular system respond similarly to the tissues examined, it is conceivable that vascular damage could result from lipid accumulation(13) as well as from lipid infiltration during lipemia in the starved-refed individual. Tissue damage produced acutely during refeeding in the vascular system, and even in the kidneys or adrenals, could conceivably result eventually in atherosclerosis (7,10) and/or hypertensive(15,16) cardiovascular disease. Such relationships may partially explain the development of persistent, diastolic hypertension and the significant incidence of vascular lesions in young adult swine subjected to repeated episodes of starvation and refeeding involving severe responses particularly to glucose refeeding(16, 17).

Summary. Metabolism of glucose in starved and starved-refed rats was markedly different from pathways observed in non-starved, normal rats. "Supernormal lipogenesis" was observed in liver, heart and kidneys of starved rats, and the lipogenesis was increased some 90-fold in livers of starved-refed rats. The proportion of C^{14} in "neutral fat" to total fat was increased 2- to 3-fold in hearts and kidneys of starved-refed rats, although the total lipid contents in these organs were not appreciably affected. Neither glucose oxidation nor incorporation of C^{14} from glucose into lipids by homogenates of rat

hearts was affected by prior starvation and refeeding.

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Effect of Metrazol upon Membrane Potential and Rate of Locomotion in *Amoeba proteus.** (28936)

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Since the initial measurement of the membrane potential in amoeba(1), numerous reports upon such values in protozoa, and relations to environmental conditions and cellular behavior have appeared. Cation involvement in the membrane potential in paramecium has been indicated(2), and changes in potential have been related to function of locomotor organelles(3,4). More recently a theory has been proposed linking changing membrane potentials to amoeboid locomotion (5). The central stimulant, Metrazol (pentamethylenetetrazol) has been shown to alter membrane permeability(6,7) in such a manner that membrane potentials might be expected to change. The stability of Metrazol makes it a desirable test substance for cellular testing. Amoeba were exposed to that substance while cell membrane potentials were determined and cellular behavior was closely observed.

Materials and methods. Amoeba proteus were cultured in Chalkley's solution and fed *Tetrahymena pyriformis* and *Chilomonas paramecium.* Amoeba were transferred every 14 days to fresh medium and cells from cultures 7 to 12 days old were used in all experiments.

Glass capillary electrodes of 0.5 to 1.0 μ inner tip diameter were employed. They were filled with 2.7 M KCl, and coupled through Ag-AgCl electrodes to a Keithley 603 Electrometer.

Amoebae were placed in Chalkley's solution on a glass slide in a special leucite chamber with side injection ports. A cover glass and mineral oil seal prevented water evaporation. Cells were continuously observed under 100 $\times$ magnification during electrode penetration, membrane potential measurement and Metrazol addition.

Amoeboid locomotion was evaluated by de-

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