Effect on Human Serum Lipids of Substituting Plant for Animal Fat in Diet.* (21260)

EDWARD H. AHRENS, JR., DAVID H. BLANKENHORN, AND THEODORE T. TSALTAS.
(Introduced by V. P. Dole.)
From the Hospital of The Rockefeller Institute for Medical Research, New York City.

Several workers(1-7) have reported marked reductions of serum cholesterol levels in patients ingesting foods containing little or no fat. When fat of vegetable origin was added to such diets, a rapid rebound in cholesterol levels was observed in 3 healthy subjects by Hildreth *et al.*(8), in one hypercholesterolemic patient by Keys *et al.*(9), in 7 hypercholesterolemic patients by Wilkinson *et al.*(10), and in one normal subject by Page(11). It has been concluded from such studies that serum cholesterol levels are controlled by fat intake, regardless of source, and not by the intake of cholesterol itself(7,11). Evidence to the contrary has been reported by Kinsell and coworkers(12-15), who have shown striking reductions in serum cholesterol and phospholipid levels in patients fed diets high in vegetable fat (60-100% of calories). Simultaneously, Groen *et al.*(16) in a 9-month out-patient study of 60 normal human volunteers showed that total cholesterol levels fell slowly but significantly when vegetable fat was substituted for animal fat. A statistical study by Hardinge and Stare(17) demonstrated that strict vegetarians have lower cholesterol levels than those vegetarians who eat dairy products, and that both groups have lower cholesterol levels than the general population. Dogs have recently been reported by Tsai *et al.*(18) to have lower serum cholesterol levels on a vegetable fat diet than on a commercial diet containing variable amounts of animal fat and cholesterol.

An experiment was designed to answer the question raised by the conflicting reports cited above: Does the substitution of plant for animal fat in isocaloric amounts lead to a significant change in the concentrations of serum lipids of human subjects? The results of a 4-month study of 6 subjects are presented.

Procedure. Subjects. The age and sex of the 6 subjects are listed in Table I. All patients were normal except for obesity of exogenous origin. Each patient's weight was held constant throughout the experiment in order to avoid effects of weight gain(19) or loss(20) on the concentration of the serum lipids. Patient 3 had had a mid-thigh amputation 15 years previously because of post-operative thrombosis after removal of an ovarian cyst. Patients 2 and 4 were Negroes, the others whites. All patients were hospitalized on a metabolic floor throughout their study; their activity was not restricted. They were permitted to leave the hospital for a few hours once per month. *Dietary regimen.* All patients were fed solid foods supplemented by fat- and protein-rich formulas. The solids permitted throughout the study included fruits, vegetables, spaghetti, macaroni, jams, jellies, and hard candies. Patient 1, for example, who consumed a total of 2775 cal. per day, derived about 1000 cal. (23 g protein, 11 g fat, 224 g carbohydrate) from solids. A certain freedom was permitted in the choice and quantity of these foods, provided enough was eaten to maintain constant weight. Table I summarizes the weight changes and dietary intakes of the 6 subjects. *Supplements* were fixed in composition and contained the major part of the protein and of the various fats under test. We insisted that the entire supplement be eaten each day. *Patients 1-4.* During the control period of animal fat feeding the supplement contained butter, salad oil, eggs, and a blended formula of milk, cream, and frozen egg yolk. During the test period of plant fat feeding the supplement contained milk-free oleomargarine (Mar-Parv), salad oil, peanut butter, avocado pear, and a blended formula of milk protein (Lesofac†), dextrose,

* The authors wish to acknowledge the technical assistance of Miss Barbara Polancer, Mrs. Helen Richards, and Miss Margaret Hildebrandt.

† Lesofac (Wyeth), manufacturer's analysis as follows: Protein 50.0%, carbohydrate 39.2%, fat 1.0%, cholesterol 25 mg%, ash 5.8% (including sodium 0.02, calcium 0.80, potassium 0.85, magnesium 0.10), moisture 4.0%, vitamin B_1 2 mg%, vitamin B_2 4 mg%, niacinamide 20 mg%.

TABLE I. Variations in Body Weight and in Food Intake (Means ± S. D.) of 6 Human Subjects when Animal and Plant Food Intakes Were Alternated Isocalorically, Other Dietary Intakes Remaining the Same. Proportion of total intake fed as an obligatory supplement listed separately. Differences in intakes between animal and plant regimens are not significant.

Patient	Fat feeding regimen	Duration, days†	Wt, k	Total food intake				% of total caloric intake			Intake as obligatory supplement			
				Cal./day	Fat, g/day	Protein, g/day	Carbohydrate, g/day	Fat	Protein	Carbohydrate	Cal./day	Fat, g/day	Protein, g/day	Carbohydrate, g/day
1. ♀, 45 yr	a*	28	111.8 ± .38	2777 ± 80	150 ± 2	80 ± 2	286 ± 36	49	12	40	1782	138	57	74
	p	70	111.4 ± .62	2871 ± 166	151 ± 2	85 ± 3	301 ± 27	47	12	41	1771	141	61	66
2. ♀, 33 yr	a	21	100.7 ± .25	3077 ± 115	151 ± 5	81 ± 4	346 ± 21	44	11	45	1782	138	57	74
	p	70	101.5 ± .34	3029 ± 160	152 ± 4	85 ± 4	332 ± 37	45	11	44	1771	141	61	66
3. ♀, 47 yr	a	25	78.5 ± .40	—	—	—	—	—	—	—	1782	138	57	74
	p‡	75	79.1 ± .58	2629 ± 108	151 ± 5	82 ± 2	243 ± 21	52	13	36	1771	141	61	66
4. ♀, 26 yr	a	35	124.0 ± .38	—	—	—	—	—	—	—	1782	138	57	74
	p§	49	123.9 ± .40	3036 ± 114	154 ± 6	84 ± 4	330 ± 26	46	11	43	1771	141	61	66
	a	21	122.9 ± .16	2809 ± 142	151 ± 4	77 ± 2	298 ± 47	48	11	41	1782	138	57	74
5. ♀, 22 yr	a	36	96.2 ± .39	2763 ± 210	153 ± 6	75 ± 8	268 ± 48	50	11	39	1773	140	59	65
	p	21	97.2 ± .25	2577 ± 161	151 ± 3	75 ± 4	230 ± 36	53	12	36	1761	140	60	64
	a	21	97.6 ± .45	2650 ± 127	151 ± 3	74 ± 3	245 ± 28	51	11	38	1773	140	59	65
	p	21	97.8 ± .30	2533 ± 112	152 ± 2	76 ± 2	224 ± 14	54	12	34	1761	140	60	64
6. ♂, 16 yr	a	30	114.7 ± .39	3983 ± 264	206 ±13	105 ± 6	428 ± 58	47	11	43	2350	192	74	79
	p	21	114.7 ± .28	4140 ± 83	206 ± 6	101 ± 4	471 ± 30	45	10	45	2410	189	70	106
	a	21	114.3 ± .33	4048 ± 40	207 ± 3	103 ± 3	426 ± 21	46	10	44	2350	192	74	79

* a = animal; p = plant.
† Exclusive of acclimatization and transition periods.
‡ Diet calculated only during last 41 days of plant fat regimen.
§ Diet calculated only during last 22 days of plant fat regimen.

corn oil (Mazola‡), and milk-free chocolate syrup. The total calories and caloric composition of the 2 supplements were almost identical (Table I). In the period of animal fat feeding 140 g of fat was of animal origin and 10 g was from plant sources; in the period of plant feeding all fat was of plant origin except for traces of animal fats (<1 g/day) in the milk protein product, Lesofac. The cholesterol intakes in the 2 periods, estimated from Lange's tables(21), were 1800 mg and 23 mg/day, respectively. *Patients 5 and 6.* The only solids were butter or oleomargarine; formulas differed only in the source of fat. The animal fat supplement consisted of butter and a blended formula of milk protein (Lesofac), frozen egg yolk, cream and chocolate syrup. The plant fat supplement contained milk-free oleomargarine and a blended formula of milk protein (Lesofac), dextrose, corn oil, and chocolate syrup. Patient 6, who required more calories than the other patients, received larger supplements (Table I). The cholesterol intakes of patients 5 and 6 were 1023 and 1457 mg/day in the animal fat period, 29 and 34 mg/day in the plant fat period, respectively. The supplements fed to Patients 5 and 6, which were mainly formula feedings, appeared to affect the serum lipids in the same manner as the earlier supplements fed to Patients 1-4 which contained mixed natural foods as well as formulas. All patients except 3 and 4 were fed throughout their study by trained dietitians in a metabolic kitchen. Rejected food was weighed and subtracted in calculations of daily intake from standard tables. Patients 3 and 4 were fed for the first 8-9 weeks by the general hospital kitchen, after which time it became possible to supervise their diets in the metabolic kitchen. The foods and supplements offered these 2 patients were as similar as possible to the diets served by the metabolic kitchen, and the patients were instructed to eat only enough of the solid foods to maintain constant weight. Their weight changes and serum lipid changes were similar in kind and degrees to those of the patients fed entirely by the metabolic kitchen. All patients were given added vitamins and ferrous sulfate daily, Unicaps (Upjohn), 2/day, and Feosol (Smith, Kline and French), 0.2 g/day. They were weighed daily before breakfast with a precision of ±0.1 k. Fluid intake and output were measured daily. Bleedings of 35 ml were made once or twice weekly before breakfast. Two ml of blood was collected in tubes with EDTA (Versene) for hematological studies, and the remainder in plain tubes. Separate aliquots of serum were stored at 4°C and −15°C.

Methods. Total lipids were measured microgravimetrically on alcohol-ether (3:1) extracts of 2 ml of $Zn(OH)_2$-precipitated serum, using the Somogyi reagents(22). The same extract was used for measurements of cholesterol fractions by the Sperry-Webb method(23), and for analysis of lipid phosphorus. All measurements were made in duplicate on single extracts of serum. The

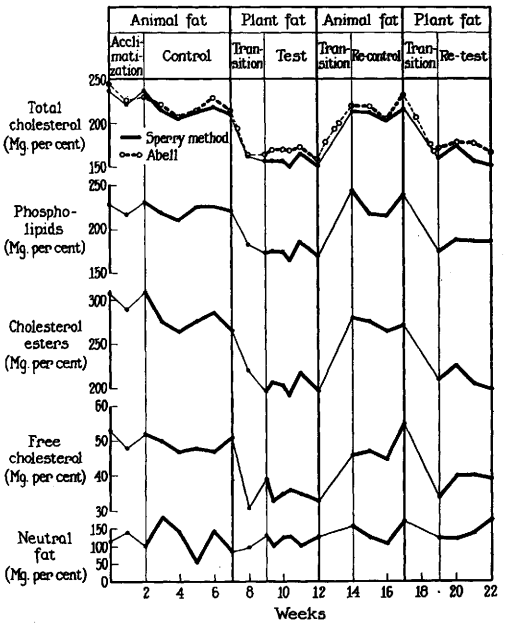

FIG. 1. Changes in concentrations of serum lipids in Patient 5 when animal and plant fat intakes were alternated isocalorically, other dietary intakes remaining the same.

‡ Mazola (Corn Products Refining Co.), manufacturer's analysis as follows: Glycerides 98.1%, non-saponifiable material 1.9%, free fatty acids 0.03%, phospholipids—trace; iodine number 125; component fatty acids—linoleic 56.2%, oleic 30.1%, palmitic 9.9%, stearic 2.9%, hexadecenoic 0.5%, myristic 0.2%, above C_{18} 0.2%; component glycerides—mono-oleo-dilinolein 49.2%, mono-saturated-dilinolein 34.2%.

TABLE II. Concentration of Serum Lipids (Mean ± S. D.) as Affected by Diets Containing Isocaloric Amounts of Animal or Plant Fats, Other Dietary Intakes Remaining the Same.

Patient	Fat feeding regimen	No. of weekly determinations	Total cholesterol (Sperry)	Total Ch* (Abell)	Free Ch (Sperry)	Ch esters (Sperry)	Total phospholipids	Neutral fat	Free Ch	Total Ch	Free Ch
			mg/100 ml serum						Total	Phospholipids × 100	Phospholipids
1	a†	5	230 ± 7.2	242 ± 5.2	52 ± 2.4	297 ± 10.2	237 ± 9.0	141 ± 22.9	22.6 ± 0.8	97 ± 1.6	22.1 ± 1.4
	p	11	177 ± 10.8	188 ± 8.2	41 ± 3.6	226 ± 14.7	194 ± 9.9	169 ± 29.3	23.4 ± 1.5	91 ± 0.5	21.3 ± 2.0
2	a	4	70 ± 1.7	80 ± 2.3			117 ± 5.7	122 ± 7.9		60 ± 3.7	
	p	11	52 ± 5.8	61 ± 2.4			93 ± 4.1	100 ± 14.5		56 ± 5.1	
3	a	4	224 ± 6.4	222 ± 7.9	56 ± 4.7	276 ± 9.3	230 ± 5.0	197 ± 24.7	25.2 ± 1.4	97 ± 2.2	24.4 ± 1.6
	p	12	163 ± 9.2	170 ± 7.6	37 ± 4.2	211 ± 12.7	186 ± 7.6	187 ± 25.1	22.4 ± 2.1	88 ± 1.4	19.6 ± 1.9
4	a	6	208 ± 7.8	219 ± 8.6	44 ± 3.8	274 ± 9.3	203 ± 9.2	198 ± 19.1	21.2 ± 1.3	101 ± 5.5	21.8 ± 2.0
	p	8	172 ± 8.1	182 ± 5.8	41 ± 3.0	217 ± 11.4	186 ± 5.9	190 ± 26.2	23.6 ± 1.2	92 ± 4.8	21.8 ± 1.6
	a	4	205 ± 4.0	212 ± 3.8	47 ± 1.0	264 ± 6.5	210 ± 3.3	182 ± 11.9	22.9 ± 0.6	98 ± 2.5	22.4 ± 0.2
5	a	6	216 ± 11.1	219 ± 9.4	49 ± 2.2	279 ± 16.7	222 ± 6.9	119 ± 46.0	22.7 ± 1.0	97 ± 2.0	22.1 ± 1.0
	p	4	156 ± 5.2	168 ± 5.0	35 ± 2.2	202 ± 9.0	174 ± 7.0	120 ± 13.7	22.4 ± 1.5	90 ± 1.1	20.2 ± 1.6
	a	4	211 ± 5.9	219 ± 11.1	48 ± 4.6	272 ± 6.4	229 ± 14.9	140 ± 28.6	22.7 ± 1.6	93 ± 4.5	21.0 ± 1.7
	p	4	161 ± 9.9	174 ± 5.3	38 ± 2.9	205 ± 16.2	184 ± 6.1	139 ± 25.1	23.8 ± 2.1	88 ± 5.6	20.8 ± 1.0
6	a	5	194 ± 11.9	203 ± 10.4	46 ± 5.0	247 ± 12.2	203 ± 2.1	199 ± 27.3	23.7 ± 1.4	96 ± 5.2	22.8 ± 2.3
	p	4	145 ± 8.1	157 ± 8.9	35 ± 3.8	185 ± 10.1	161 ± 7.5	191 ± 20.0	23.6 ± 1.9	90 ± 2.8	21.5 ± 2.2
	a	4	197 ± 6.9	198 ± 4.9	47 ± 2.2	251 ± 10.6	193 ± 6.4	194 ± 27.4	23.9 ± 1.2	102 ± 4.4	24.6 ± 1.8

* Ch = cholesterol. † a = animal; p = plant.

cholesterol ester fraction was calculated as 1.67 x (total—free cholesterol), on the assumption that cholesterol oleate is the typical ester. Lipid phosphorus was measured by a modification of the Stewart and Hendry method(24); values were converted to total phospholipids by multiplying by the factor of 25. Neutral fat was calculated as the difference between total lipids and the sum of (free cholesterol + cholesterol esters + total phospholipids). Total cholesterol was measured independently in the same sera by the method of Abell *et al.*(25). All reported data were determined on sera stored at 4°C less than one month.

Results. The hospital study of Patients 1-3 can be divided into 4 periods: acclimatization (2 weeks), control (3-6 weeks), transition (2 weeks), and test (10-11 weeks). Patients 4-6 were studied in the same manner except that their test periods were of 3-7 weeks duration, followed by a transition period of 2 weeks and a re-control period of 3-4 weeks; Patient 5 had a third transition period of 2 weeks and a second test period of 3 weeks. In the acclimatization and control periods the dietary fat was 93% of animal origin; in test periods the dietary fat was >99% of plant origin. A feeding plan is shown in Fig. 1; the duration of the experimental periods of all patients is given in Table I.

During acclimatization, patients' weights varied until maintenance caloric intakes were established. During this period there were significant rises or falls in serum lipids in 4 of 6 patients. These data reflect variations in caloric intake and also differences between home and hospital diets; they have been omitted from this report. Data collected during the 2-week transitional periods are also omitted. Data accumulated during control and test periods are summarized in Table II.

After acclimatization was accomplished, the concentrations of the various lipid fractions of the serum varied significantly only when the dietary fat was changed from one type to the other. Once a new level was reached, there was remarkably little fluctuation from week to week. A measure of this constancy is given by the standard deviations of the mean

TABLE III. Percentage Deviations of Mean Test from Mean Control Levels of Serum Lipids—Statistical Significance of Variations.

Patient	Total cholesterol—Sperry-Webb	Total cholesterol—Abell, *et al.*	Free cholesterol	Cholesterol esters	Phospholipids	Neutral fat	F/T	TC/PL	FC/PL
1	−23%†	−22%†	−17%†	−24%†	−18%†	+20%	+4%	−6%	− 4%
2	−26†	−24†			−21†	−22†		−7	
3	−27†	−23†	−34†	−24†	−19†	− 5	−11*	−9†	−20†
4	−17†	−17†	− 7†	−21†	− 8†	− 4	+9†	−9†	0
5	−26†	−22†	−25†	−27†	−21†	0	+2	−6†	− 6*
6	−25†	−23†	−24†	−25†	−21†	− 4	0	−8†	− 9*

* $p = .01$-$.05$. † $p = <.01$.
F/T = Ratio of free to total cholesterol. TC/PL = Ratio of total cholesterol to phospholipids. FC/PL = Ratio of free cholesterol to phospholipids.

values for each period (Table II). To illustrate the changes found when one type of dietary fat was substituted for the other, the serum lipid concentrations of Patient 5 are shown in Fig. 1. It is seen that cholesterol and phospholipid fractions fell abruptly when plant fat was substituted isocalorically for animal fat. These values returned to their original levels when the patient was placed again on animal fat, and fell to the previous low levels when plant fat was fed a second time. Patients 1-3 were maintained on the plant fat regimen for 10-11 weeks without interruption; the low levels of cholesterol and phospholipids reached at 2 weeks on this regimen showed no escape as long as plant fats were fed. Patients 4 and 6 were returned to the animal fat regimen after 7 and 5 weeks, respectively, on plant fats; their lipid levels during the re-control period were almost identical to those of the initial control period.

Percentage deviations of mean test from mean control levels are shown in Table III. Changes which were considered significant ($p = 0.01$ or less) and which occurred in more than half the patients may be listed: 1) levels of free and esterified cholesterol and of phospholipids were lower during plant fat feeding in 6 of 6 subjects; 2) total cholesterol/phospholipid ratios were lower in 4 of 6 subjects during the plant fat regimen.

The clinical status of Patient 2, a negro female in whom there was an unexplained hypolipemia, deserves special comment. She was in excellent general health. There was no clinical evidence of hyperthyroidism; basal metabolic rate and radioactive iodine uptake were normal. Although she had had pelvic inflammatory disease many years before and showed residual tenderness and some swelling of the right Fallopian tube, there was neither leucocytosis, elevation of sedimentation rate, nor elevation of gamma globulin at any time during her study period. Substitution of plant fat for animal fat resulted in a decrease in total cholesterol concentration from 70 to 52 mg per 100 ml serum (averaged Sperry-Webb values), and a fall in phospholipids from 117 to 93 mg. These differences were significant at a level of $p = <0.01$. Because of technical difficulties presented by its small concentration, free cholesterol was determined on only 2 sera during the control period and 2 during the test period. In the animal fat period, free cholesterols of 13.8 and 15.2 mg per 100 ml serum were found, in the plant fat period, 10.8 and 12.3 mg, with mean free/total cholesterol ratios of 21.7 and 25.4%, respectively. Calculations of neutral fat values recorded in Table II were based on the mean of these figures.

All patients felt well throughout their study. During the plant fat period there were no symptoms of increased irritability, inability to concentrate, or lack of vigor or endurance, as described by Groen *et al.*(16). Most patients passed larger stools during plant fat feeding, but there was no steatorrhea on microscopic examination. Patient 1 developed a peridental abscess in the 5th week of her test period with a temporary rise in all lipid levels; penicillin therapy and tooth extraction were followed by a return of values to the test period baseline. With this exception there were no illnesses during the study.

Discussion. Results of other studies(6, 8-11) have given rise to the widely held belief that the ingestion of fat of any type causes a

rise in serum cholesterol levels. However, in each of these experiments a test period of fat feeding was preceded by one in which little or no fat was fed. A fat-free diet imposes an unusual demand upon the body: unless the subject burns his own fat, all newly-formed lipids must be synthesized from exogenous protein and carbohydrate. Under these conditions lipids are synthesized more slowly (or utilized more rapidly), and concentrations of serum lipids fall. When exogenous fat of plant or animal origin is again made available, a rise in concentration of serum lipids can be expected to occur until a new equilibrium state is reached. Very few observations have been made in human subjects maintaining weight during prolonged periods of complete fat deprivation; these have indicated that unsaturated fatty acids may be essential dietary components for human beings(26,27). Watkin *et al.*(3) cited observations which suggested that such a deficiency may occur in some patients on Kempner's rice diet.

In contrast to those experiments in which diets containing more or less fat were tested, the present experiment aimed at comparing the effect of 2 types of fat fed at isocaloric levels, other nutrients remaining unchanged. The proportion of fat in our diets was 45-52% of calories, which contrasts with an average American intake of 40%(7). Our results in 6 subjects whose only known abnormality was obesity of exogenous origin confirm the findings of Kinsell *et al.*(12-15), who fed much larger proportions of fat to patients with various metabolic disorders. Our findings differ in 3 respects from theirs. Our data show that 1) free and total cholesterol levels decrease proportionately when plant fat replaces animal fat in the diet; 2) cholesterol levels decrease more than phospholipids; and 3) neutral fat levels are unaffected by the dietary change. The mechanism of the dietary effect and its practical value in the management of hypercholesterolemic disorders remain subjects for future investigation.

Summary. Six subjects with obesity of exogenous origin showed significant reductions in serum concentrations of free and esterified cholesterol and of phospholipids when plant fats were substituted isocalorically for animal fats during a 4-month metabolic ward study. The approximate magnitude of the change was a 20% decrease. Neutral fat levels showed no significant change. Body weights and caloric intakes were held constant throughout the experiment.

1. Kempner, W., *Ann. Int. Med.,* 1949, v31, 821.
2. Mellinkoff, S. M., Machella, T. E., and Reinhold, J. G., *Am. J. Med. Sci.,* 1950, v220, 203.
3. Watkin, D. M., Froeb, H. F., Hatch, F. T., and Gutman, A. B., *Am. J. Med.,* 1950, v9, 441.
4. Starke, H., *ibid.,* 1950, v9, 494.
5. Wilmot, V. C., and Swank, R. L., *Am. J. Med. Sci.,* 1952, v223, 25.
6. Wollaeger, E. E., Lundberg, W. O., Chipault, J. R., and Mason, H. L., *Gastroenterol.,* 1953, v24, 422.
7. Keys, A., *J. Mt. Sinai Hosp.,* 1953, v20, 118.
8. Hildreth, E. A., Hildreth, D. M., and Mellinkoff, S. M., *Circulation,* 1951, v4, 899.
9. Keys, A., Science, 1950, v112, 79.
10. Wilkinson, C. F., Jr., Boyle, E., Jackson, R. S., and Benjamin, M. R., *Program of Assn. Study Arteriosclerosis,* November 2, 1953.
11. Page, I. H., *Circulation,* 1954, v10, 1.
12. Kinsell, L. W., Partridge, J., Boling, L., Margen, S., and Michaels, G., *J. Clin. Endocrin. and Metab.,* 1952, v12, 909.
13. Kinsell, L. W., Michaels, G., DeWind, L., Partridge, J., and Boling, L., *Calif. Med.,* 1953, v78, 5.
14. Kinsell, L. W., Michaels, G. D., Partridge, J. W., Boling, L. A., Balch, H. E., and Cochrane, G. C., *J. Clin. Nutrition,* 1953, v1, 224.
15. Cochrane, G. C., Michaels, G. D., and Kinsell, L. W., *ibid.,* 1953, v1, 295.
16. Groen, J., Tjiong, B. K., Kamminga, C. E., and Willebrands, A. F., *Voeding,* 1952, v13, 556.
17. Hardinge, M. G., and Stare, F. J., *J. Clin. Nutrition,* 1954, v2, 83.
18. Tsai, S. Y., Futch, E. D., III, Kobayashi, M., May, L. G., and Gregory, R. L., *Texas Reports Biol. Med.,* 1954, v12, 423.
19. Walker, W. J., Lawry, E. Y., Love, D. E., Mann, G. V., Levine, S. A., and Stare, F. J., *Am. J. Med.,* 1953, v14, 654.
20. Keys, A., Brozek, J., Henschel, A., Mickelson, O., and Taylor, H. L., *The Biology of Human Starvation,* U. of Minn. Press, Minneapolis, 1950, p498.
21. Lange, W., *J. Am. Oil Chem. Soc.,* 1950, v27, 414.
22. Somogyi, M., *J. Biol. Chem.,* 1930, v86, 658.
23. Sperry, W. M., and Webb, M., *ibid.,* 1950, v187, 97.
24. Stewart, C. P., and Hendry, E. B., *Biochem. J.,*

1935, v29, 1683.
25. Abell, L. L., Levy, B. B., Brodie, B. B., and Kendall, F. E., *J. Biol. Chem.* 1952, v195, 357.
26. Brown, W. R., Hansen, A. E., Burr, G. O., and McQuarrie, I., *J. Nutrition,* 1938, v16, 511.
27. Hansen, A. E., and Wiese, H. F., *Fed. Proc.,* 1946, v5, 233.

Received July 19, 1954. P.S.E.B.M., 1954, v86.

Technic for Complete Pancreatectomy in the Rat. (21261)

C. R. TREADWELL AND JOSEPH H. ROE, JR.
From the Department of Biochemistry, George Washington University School of Medicine, Washington, D. C.

In the rat the pancreas is a diffuse, noncapsulated, discontinuous organ lying in the gastro-duodenal and the gastro-splenic mesenteries. The bile duct for a considerable portion of its length is embedded in the pancreatic tissue. The size of the animal, the anatomical distribution of the pancreas, as well as its relation to the bile duct have been obstacles to complete removal of the pancreas. Several technics for partial pancreatectomy in rats have been published. The procedures of Ingle and Griffith(1) and of Foglia (2) have been widely used experimentally. In rats subjected to partial pancreatectomy the diabetes is of a relatively mild type (unless the animal is subjected to additional stress) and requires weeks or months to develop.

In studies in this laboratory on the origin and function of serum amylase, it became desirable to make observations on completely depancreatized rats. These are reported in a following paper(3). The procedure of Foglia(2) for 95% pancreatectomy had been employed previously and because of this experience it was decided to attempt to remove the additional 5% of pancreas which lies in the narrow band of mesentery between the bile duct and upper duodenum. By using adult animals, introducing some modifications in procedure, and with standardization of the pre- and post-operative care, a high percentage (70 to 90%) of survival of completely depancreatized rats could be obtained. The present communication describes the operative procedure and the diabetic state which ensues. Data which show that the gland is essentially completely removed are also included.

Materials and methods. Several strains of white rats, male and female, were used. Body weight ranged from 150 to 400 g. The animals were kept in individual metabolism cages and received the synthetic low-residue diet described below. Qualitative tests for glucose and acetone bodies were run on 24 hour urine samples. Glucose tolerance was determined as follows: after an overnight fast, an 0.1 ml sample of tail blood was taken and 3.5 g of glucose per kg of body weight injected intraperitoneally as a 10% solution. Subsequent blood samples were taken at ½, 1, 2, 3, and 5 hours. Blood sugar was determined by a combination of the methods of Somogyi(4) and Nelson(5). Tissue amylase was determined by a modification of the method of Smith and Roe(6). The low-residue diet had the following composition: 30% casein, 25% lard, 14% starch, 14% sucrose, 5% salt mixture, 10% brewer's yeast, and 2% cod liver oil.

Operative procedure. The rats were placed on the low-residue diet at least 3 days prior to operation and fasted 18 to 24 hours immediately preceding it. The low-residue diet and the fasting period insured absence of solid material in the stomach and intestine. If there was an appreciable amount of residue in the stomach or intestine there was considerably more hemorrhage during and after operation. The animal is given an intraperitoneal injection of 0.05 ml of nembutal (60 mg/ml)/100 g body weight. The abdomen is moistened with 70% alcohol and opened by a mid-line incision extending about 3.5 cm caudal-ward from the xiphoid process. The stomach is drawn out and turned upward so that the