muscular implantation adrenocortical grafts while progressively resuming their function, are unable to deliver enough cortical secretion to establish the pituitary-adrenal equilibrium which is thought to regulate size and performance of adrenocortical tissue(1). After 2 weeks, however, the equilibrium becomes established, imperfectly at 21 days but completely at 90 days, and the amount of cortical tissue which can regenerate becomes more a function of time than of the number of regenerating masses.

Summary. The total volume of adrenocortical tissue regenerating in intramuscular autotransplants in rats during the first 2 weeks after operation is proportional to number of transplants growing. Thereafter, the total volume is about the same irrespective of the number of implants made or transplants regenerating, so the volumes per transplant are inversely proportional to the number of grafts. Average volume of regenerating tissue and cell depth increases with increasing age of transplant. At 90 days after operation the cell depth is like that of intact adrenal glands but the volume is less. Apparently transplants cannot deliver the amount of cortical secretion necessary to establish a pituitary-adrenal equilibrium until after 2 weeks of growth.

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Received July 17, 1957. P.S.E.B.M., 1957, v96.

Effect of Insulin on Clearance of Emulsified Fat from the Blood in Depancreatized Dogs.* (23446)

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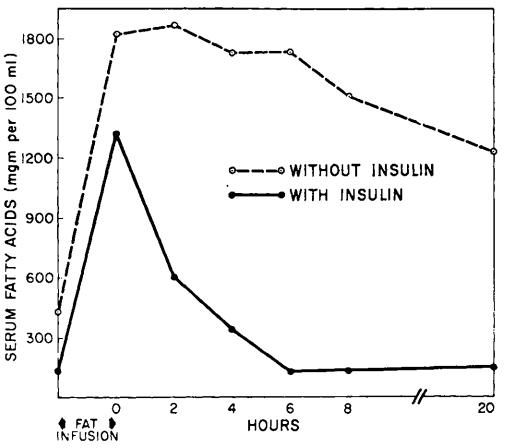
The metabolic abnormality that underlies development of lipemia has not been determined. The frequent occurrence of this condition in association with uncontrolled diabetes and its disappearance with treatment suggests that carbohydrate metabolism is in some way linked to development of lipemia. Similarly, the presence of extreme lipemia in patients with von Gierke's disease seems indicative that abnormal carbohydrate metabolism is in some manner related to lipemia. Since diabetes and von Gierke's disease represent different disorders it is apparent that lipemia may arise from various metabolic disturbances. The blood lipids in these diseases are generally considered to be of exogenous origin(1) although this has not been proven and is in fact, contradicted by numerous clinical examples of severe and worsening lipemia despite a diminished food intake. In the diabetic there is rough parallelism between fasting blood sugar and degree of lipemia(2). Inasmuch as fat utilization is increased in the diabetic state, there has been no reason to suspect that failure to oxidize fat by liver and extrahepatic tissues could cause high-fat levels in the blood. Neither is there any connection between impairment of fat synthesis in diabetes(3) and the lipemic state. Stanley and Thannhauser(4) and Lever and Waddell (5) have indicated that there is slowed passage of fat from the blood in patients with idiopathic hyperlipemia.

It has long been known that insulin lowers the fat and cholesterol levels of the blood(1) and that it will prevent elevation of blood lipids and hepatic fat accumulation in ani-

* These studies supported in part by contract with the Research and Development Division, Office of Surgeon General, Dept. of Army; The John A. Hartford Memorial Fund, and the Fund for Research and Teaching, Department of Nutrition.

mals with phlorizin diabetes(8). Insulin administration to diabetic patients also flattens and shortens alimentary lipemia curves(2). In an attempt to clarify this problem the clearance of injected fat emulsion from the blood has been studied in normal dogs and in pancreatectomized dogs with and without insulin. It has been shown that pancreatectomized animals maintained on insulin can clear injected fat from the blood normally whereas deprivation of insulin results in delayed clearance of all lipid components and a rise in cholesterol. These experiments confirm and extend the observations of Lombrosa(6) and Rony and Mortimer(7).

Methods and Material. Male mongrel dogs weighing 10 to 20 kg were fed a constant diet consisting of 1 lb of horse meat, 1 qt of milk and 50 g of sugar plus supplements of iron and vit. B complex. After 2 weeks of this diet a fat tolerance curve was run. For this the animals were fasted for 24 hours, then infused with a 10% cottonseed oil emulsion which contained soyabean phosphatide 1.2% and Pluronic F68 0.2% by weight(9). This was administered intravenously at a rate of 5 ml per minute. Twenty ml per kg of body weight were given. Throughout the next 20 hours the animals were fasted. Venous blood samples were drawn before infusion, at the end of infusion and at 2, 4, 6, 8, and 20 hours following the completion of infusion. On each of these samples the following determinations were made—turbidity(10), total fatty acids(11), phospholipid(12), total and esterified cholesterol(13). Neutral fat concentrations were calculated by the method of Peters and Man(14). Total pancreatectomy was then carried out on 5 dogs. After a 2- to 3-week period of convalescence and regulation of diabetes another fat clearance study was performed exactly as before the operation. Each dog lost weight during this period so the amount of fat injected was reduced accordingly. The animals received their regular dose of NPH insulin (8-15 units) on the day of this test. One week later another fat clearance test was carried out in exactly the same manner except that insulin was omitted the day preceding and day of this test.



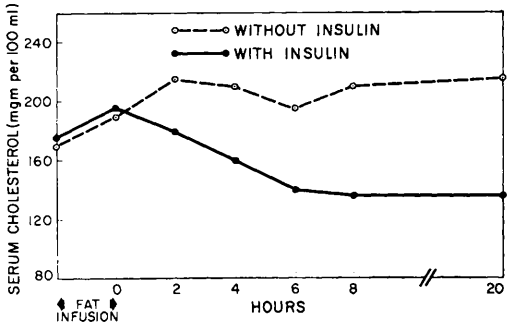


FIG. 1 (top). Serum fatty acid during and after fat infusion with and without insulin in a pancreatectomized dog.

FIG. 2 (bottom). Total serum cholesterol during and after fat infusion into a pancreatectomized dog.

Results. The passage of fat from the blood stream is impaired in the animals without insulin whereas in those receiving insulin the rate of removal of fat from the blood proceeds at a normal or elevated rate. (Tables I, II, III, Fig. 1, 2.) These results are not compatible with the concept that over-mobilization of endogenous fat is the primary cause of lipemia in uncontrolled diabetes. The amount of fat introduced into the blood was the same in each group of animals, therefore it seems clear that there was impairment in the removal of this fat from the blood.

Direct relationships between insulin and fat metabolism are not known, whereas a number of indirect ones mediated through the action of the hormone on carbohydrate metabolism are well established. It can be seen from the data in Fig. 1 and 2 and Table I

TABLE I. Normal. Turbidity and serum lipids in 5 normal dogs before and after emulsified fat infusions. Except for turbidity the numbers express conc. in mg/100 ml. Upper figure is the mean and lower figures give ranges.

	Pre	0 hr	2 hr	4 hr	6 hr	8 hr	20 hr
Turbidity	2.1 1.0–3.7	85.6 54.8–146	44.5 32.0–60.7	27.3 14.3–43.0	16.8 8.2–24.7	9.6 2.7–14.4	2.9 1.4–5.6
Fatty acids	328 223–337	1473 1230–1800	1036 973–1175	721 563–804	638 368–810	476 380–557	350 288–435
Neutral fat	3 0–8	1143 895–1390	684 635–738	360 224–470	282 48–400	124 65–267	9 0–28
Phospholipid	361 327–387	401 372–430	395 365–430	373 357–400	363 357–400	357 335–400	361 320–430
Total cholesterol	270 235–330	270 220–320	282 245–355	272 235–330	262 220–320	255 210–320	243 190–320
Ester cholesterol	188 150–245	200 165–235	193 165–245	185 160–225	177 150–220	170 140–220	162 115–235

TABLE II. Pancreatectomy with Insulin. Turbidity and serum lipids in 5 pancreatectomized dogs receiving insulin. Except for turbidity the numbers express conc. in mg/100 ml. Upper figure is the mean and lower figures give ranges.

	Pre	0 hr	2 hr	4 hr	6 hr	8 hr	20 hr
Turbidity	1.9 1.7–2.3	67.0 49.0–98.0	36.7 19.8–66.8	17.2 4.5–46.8	6.7 2.5–14.6	3.9 1.5–7.1	1.2 1.0–1.5
Fatty acids	299 226–450	1428 905–1725	845 610–1150	505 307–900	319 201–470	277 195–372	256 215–349
Neutral fat	68 9–122	1141 62–1390	575 350–845	263 71–688	90 0–267	64 0–173	34 6–65
Phospholipid	271 227–392	359 320–490	322 247–495	283 217–465	260 200–435	248 190–400	250 183–340
Total cholesterol	185 150–245	184 140–230	193 165–230	183 160–230	158 140–265	156 115–210	156 120–200
Ester cholesterol	85 40–140	117 90–160	109 80–140	99 75–140	87 65–130	92 60–150	86 60–135

TABLE III. Pancreatectomy without Insulin. Turbidity and serum lipids in 5 pancreatectomized dogs without insulin. Except for turbidity the numbers express conc. in mg/100 ml. Upper figure is the mean and lower figures give ranges.

	Pre	0 hr	2 hr	4 hr	6 hr	8 hr	20 hr
Turbidity	2.0 0–5.6	60.8 50.6–70.1	57.2 43.9–77.7	47.9 35.3–62.0	43.1 31.7–57.0	38.6 25.7–53.0	13.5 3.4–25.4
Fatty acids	332 255–428	1569 1125–2010	1785 1395–2470	1525 1190–2020	1152 995–1735	1279 710–1840	1003 460–1500
Neutral fat	110 40–238	1287 860–1730	1503 1145–2160	1260 945–1730	949 750–1495	1022 465–1575	740 258–1185
Phospholipid	288 240–320	379 333–410	367 305–400	344 302–365	345 295–367	332 288–350	354 243–440
Total cholesterol	188 165–245	180 165–210	216 195–280	230 195–300	212 195–265	212 195–265	197 165–255
Ester cholesterol	72 45–110	104 85–130	117 95–150	105 65–145	98 75–130	97 85–115	77 45–110

that all lipid components reflected the withholding of insulin from the depancreatized animals. Thus, there was no evidence to suggest that the primary defect was in any particular lipid fraction. One of the striking effects of diabetes on lipid metabolism is the almost complete inability to synthesize fatty acids. The same phenomenon is found in normal animals when fasted for 24 hours. If this block in fatty acid synthesis was the underlying reason for the delayed removal of fat from the blood, fasted normal animals should also display this retardation. Such was not the case, however. In contrast to retarded fatty acid synthesis is the accelerated formation of ketone bodies which occurs in diabetes(15). It is possible that excessive ketones may delay fat clearance because of an influence on cellular physiology in addition to those recognized and characterized chemically(16).

If this reasoning is correct then the problem becomes more clearly one of membrane permeability (in the liver) to hydrophobic lipid molecules, aggregates and particles. It seems possible that in the diabetic animal deprived of insulin at least 2 facets of abnormal carbohydrate metabolism might lead to impairment of the passage of negatively charged lipid particles into the liver. First, decreased energy production from Krebs cycle oxidations may alter membrane potential directly (17) and secondly the acidosis that is invariably present might have a similar effect. To test the latter possibility 3 pancreatectomized dogs receiving insulin were made acidotic by infusion of ammonium chloride solution prior to and during the fat infusion. This resulted in slowed fat clearance curves identical to the uncontrolled diabetic dogs. The possibility remained that ammonium ion toxicity itself was responsible since it has been shown *in vitro* that ammonium causes increased ketone body production due to interruption of the Krebs cycle and diversion of ketone body precursors in the direction of acetoacetate(18, 19). In normal dogs slow administration of ammonium chloride or more rapid administration of ammonium carbonate had no effect upon rate of fat clearance. It would thus appear that the effect of ammonium chloride is more likely due to acidosis. Furthermore in 2 pancreatectomized dogs administration of dilute hydrochloric acid caused slowing of fat clearance from the blood even though insulin was given.

In Table I and Fig. 1 and 2 it can be seen that infusion of fat emulsion into the group of pancreatectomized animals receiving insulin and also in normal animals resulted in a decrease in serum cholesterol concentrations which persisted through the 20-hour period of observation. The reverse was true in the pancreatectomized animals deprived of insulin. This cholesterol lowering effect has been observed in patients receiving similar fat emulsions intravenously(5). This phenomenon appears to be contingent upon normal carbohydrate utilization and it seems justifiable to suggest that in these experiments is the *in vivo* counterpart of the experiments of Hotta, Hill and Chaikoff(20,21), who observed that acetate-1-C^{14} was incorporated into cholesterol at an increased rate by the livers of diabetic rats as compared to normal, and that insulin treatment of diabetic animals resulted in diversion of acetate into metabolic paths other than cholesterol synthesis. It seems likely that the rise in cholesterol observed after infusion of fat into diabetic animals is a reflection of the same phenomenon.

Since no direct experimentation concerning the function of clearing factor in diabetic subjects has been reported there is insufficient evidence for evaluating the possible relationship to the above experimental results. It seems possible that enzyme synthesis or physiologic activity might be influenced by abnormal carbohydrate metabolism but from a quantitative point of view the likelihood of clearing factor activity being an important facet of the above described phenomena appears quite remote.

Summary. Emulsified fat infused into pancreatectomized dogs is cleared from the blood at a normal rate if the animal is receiving insulin whereas without insulin the injected fat is cleared slowly. It thus appears that the clearance of fat from the blood is dependent upon normal carbohydrate utilization. Serum cholesterol levels rise follow-

ing infusion of fat in diabetic animals deprived of insulin. Serum cholesterol concentration falls following infusion of fat into pancreatectomized animals receiving insulin. Observations on fat metabolism in which the fat is administered intravenously give data comparable to those obtained following oral administration of fat and the mechanisms of clearance seem identical by the two methods.

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Received July 17, 1957. P.S.E.B.M., 1957, v96.

Amperometric Determination of Sulfhydryl Changes in Mouse Epidermis and Dermis During Treatment with Certain Polycyclic Hydrocarbons.* (23447)

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Investigation of sulphur metabolism in biological systems is challenging because of its integration with cell activities. Currently, the relationship between sulfhydryl groups and the carcinogenic process is receiving much attention. Polycyclic carcinogenic hydrocarbons have been presumed to interfere with some phase of sulphur metabolism related to growth. Reimann and Hall(1) showed that parathiocresol inhibited induction of tumors by 1,2,5,6-dibenzanthracene. They concluded that the sulfhydryl group of parathiocresol had an anti-carcinogenic effect. Fieser(2) discovered a close relationship between carcinogenicity and certain types of substitution reactions at positions in the molecule. He suggested a reaction of the hydrocarbon *in vivo,* whereby an S-S linkage of a protein or peptide is broken, giving rise to a free sulfhydryl group and a hydrocarbon-peptide link. Later, Crabtree(3,4) suggested that during carcinogenesis, inhibition may occur by some interference with sulphur metabolism. Crabtree postulated that in the production of a skin tumor by a carcinogen, the initial step involved was the fixation of the carcinogen by combination with free sulfhydryl groups.

* This investigation was supported in part by a *research grant from the National Cancer Institute* of the N.I.H., Public Health Service.