developed about the same aortic sensitivity as males. In comparison to females, castration did not have much effect on males, since little difference was noted when the aortae of operated animals were compared to normal ones. Likewise, the salt diet did not increase aortic responsiveness to epinephrine in castrated males beyond that found in normal males. These findings indicate that the presence of female sex hormones is required to maintain a low reactivity to epinephrine in the aorta. Removal of the male hormone, on the other hand, did not change the reactivity of the aorta, nor sensitize the animal to the salt diet. The mechanism whereby the salt diet increases the reactivity of vessel strips to epinephrine may be explainable by the theory of Raab(5) that increased intracellular sodium in smooth muscle of blood vessels potentiates the constrictor effects of epinephrine, possibly by raising the membrane electrical potentials of muscle cells upon which catecholamines act.

Summary. Strips were prepared from aortae of rats and their reactivity to epinephrine tested by means of a lever system that recorded shortening of the smooth muscle. A 2% salt diet increased the sensitivity of the vessel to l-epinephrine in both sexes. Male aortae in all instances had greater sensitivity to epinephrine than those of females under the same conditions. Castration markedly increased the sensitivity to epinephrine in females, especially when the castrates were placed on the salt diet. In contrast, male aortae were not particularly changed by castration.

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Insulin Secretion Following Carbutamide Injections in Normal Dogs.* (22811)

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The hypoglycemic action of orally active sulfonylureas may be due to one or more of the following reasons: a) destruction of the pancreatic A cells with decreased glucagon production(1), b) inhibition of one or more of the enzyme systems involved in liver glycogenolysis(2-6), c) decreased intestinal absorption of glucose(7), d) enhancement of insulin action(8), e) inhibition of the enzymes responsible for insulin degradation (9), and f) stimulation of insulin secretion (10-12). The possibility that these drugs may act through pituitary, adrenals, thyroid, parathyroid, testicles or the central nervous system appears unlikely(13) while the possibility that they may stimulate peripheral glucose utilization is uncertain(14,15). Purpose of this work was to investigate the hypothesis that carbutamide stimulates release of insulin into pancreatic blood.

Materials and methods. The problem was investigated by means of 7 pancreatic-femoral and 5 mesenteric-femoral cross-circulation ex-

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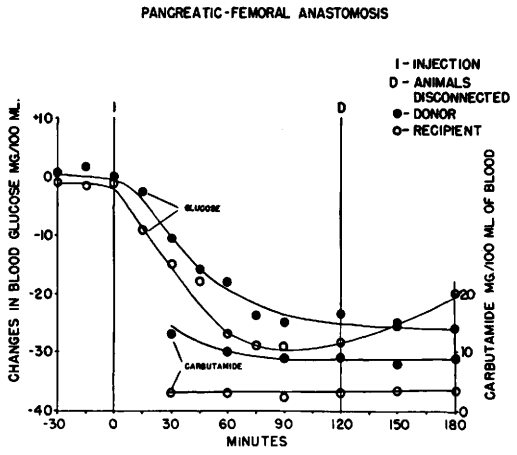

FIG. 1. Effect of carbutamide on blood glucose of dogs. Avg of 5 pancreatic-femoral cross-circulation experiments. ●, donor *dogs D*; ○, recipient *dogs R*. I = Inj. of carbutamide into *dogs D*; D = Dogs disconnected.

periments, using normal mongrel dogs of both sexes. Animals fasted for about 24 hours preceding the experiments were anesthetized with sodium pentobarbital (35 mg/kg, intraperitoneally) and heparinized.‡ The experiments were performed as described previously (16). Carbutamide was injected intravenously (50 mg/ml, 1 ml/kg, pH 8).§ Blood glucose was determined according to the method of Nelson(17), blood carbutamide according to the method of Bratton and Marshall(18). At the end of the experiment the animals were killed with excess anesthetic and portions of head, body and tail of pancreas were fixed in Bouin's solution for subsequent histological study, according to the method of Gomori(19).

Results. Results are presented in curves showing average changes in blood glucose concentration. Fig. 1 represents 5 pancreatic-femoral cross-circulation experiments in which donor *dog D* received an injection of carbutamide. During the control period preceding injection, there were no significant changes in blood sugar of either dog. Following carbutamide injection the blood sugar of dog D started to decline rapidly, reaching a minimum in about 1 hour and remaining low for the duration of the experiment. Maximum reduction varied between 25 and 60% in individual dogs D and was accompanied by similar hypoglycemia (25-68%)‖ in recipient *dogs R*. After interruption of the anastomosis, while the blood sugar of dog D remained low, that of dog R began to rise slowly toward preinjection levels. Fig. 2 represents 3 mesenteric-femoral cross-circulation experiments in which dog D received an injection of carbutamide. No significant changes in blood sugar of either dog D or Dog R were noted during the preinjection control period. Following the injection, blood glucose of dog D fell rapidly, while dog R showed a mild and not significant hyperglycemia. It should be pointed out that the maximum hypoglycemic effect of carbutamide did not occur at the same time in all animals and, therefore, the average curves are flatter than those representing individual experiments. Concentration of carbutamide in blood after initial mixing, reached values of about 8 mg % in dog D and about 2 mg % in dog R and remained essentially unchanged for the duration of the experiment. In 2 additional pancreatic-femoral and 2 additional mesenteric-femoral cross-circulation experiments, not included in the average curves, carbutamide did not cause hypoglycemia either in dog D or dog R, al-

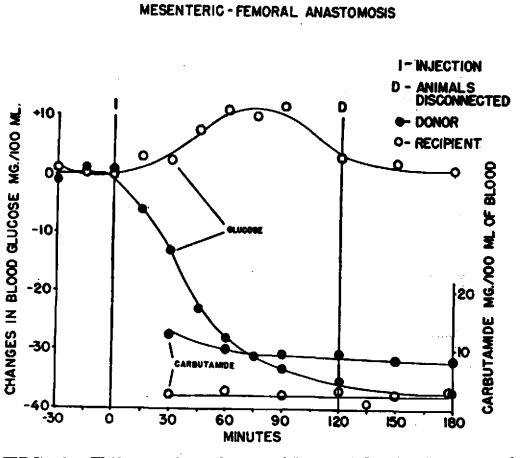

FIG. 2. Effect of carbutamide on blood glucose of dogs. Avg of 3 mesenteric-femoral cross-circulation experiments. Symbols as in Fig. 1.

‡ Heparin was gift of Dr. Stanley Hier of Wilson Laboratories.

§ Carbutamide was gift of Dr. W. R. Kirtley of Lilly Research Laboratories.

‖ $P < 0.01$.

though the same dose had been used in all experiments and the same blood concentrations had been obtained. No significant changes were noted in the general appearance, in granules and other staining characteristics of A and B cells of the islets of Langerhans.

Discussion. The results suggest that carbutamide hypoglycemia in dog D is accompanied by a release of insulin into the blood of the pancreatic vein and that this insulin is carried through the pancreatic-femoral anastomosis, causing hypoglycemia in dog R. Small amounts of carbutamide are carried through the anastomosis also, but carbutamide concentration in blood of dog R appears to be too low to cause significant changes in blood sugar. This is shown by the fact that when dog R receives mesenteric instead of pancreatic blood from dog D, no hypoglycemia occurs, even though concentration of carbutamide in blood of dog R is approximately the same in both types of experiment. Failure of blood sugar of dog R to decrease in those experiments in which dog D did not respond to carbutamide, the slow return toward normal of blood sugar in dog R after separation from its extra insulin supply and the sustained hypoglycemia in dog D under the lasting effect of the injected drug, lend further support to the stated hypothesis. These results are in agreement with those obtained by Loubatieres using p-amino-benzene-sulfamido-isopropyl thiodiazole(10) and with the fact that carbutamide has no hypoglycemic effect in the absence of pancreatic tissue(10) and in those diabetic patients most likely to have little or no insulin in their pancreas(20). The available data do not permit the differentiation between the release of preformed insulin and true insulin secretion, although the fact that the B cells did not appear degranulated suggests active secretion. This is suggested also by the observation that carbutamide stimulates the growth of islet tissue in normal(12) and alloxan-diabetic animals(10).

Summary and conclusions. The mechanism of the hypoglycemic action of carbutamide in normal dogs was studied by means of pancreatic-femoral and mesenteric-femoral cross-circulation experiments. The results of these acute experiments suggest that: 1) carbutamide hypoglycemia is accompanied by secretion of insulin; 2) carbutamide does not cause degranulation or other significant changes in appearance of pancreatic A and B cells; 3) clearance of carbutamide from blood is relatively slow, as reported by others(21); 4) some dogs are refractory to the hypoglycemic action of carbutamide in doses of 50 mg/kg I.V. The results are consistent with the hypothesis that insulin secretion is one of the results of carbutamide action. The continued administration of carbutamide in animals and men with reduced pancreatic reserve may stimulate the islets of Langerhans to regeneration or to exhaustion. Although some evidence suggests that islet growth does occur (10,12), the second alternative has not been ruled out and should be investigated before the sulfonylureas can be recommended for the prolonged treatment of diabetes mellitus.

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Plasma Phospholipide Synthesis in the Eviscerated Rabbit.* (22812)

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Fishler *et al.*(1) studied the synthesis of plasma phospholipides in fasting hepatectomized dogs with P^{32} and Goldman *et al.*(2) repeated this study with C^{14}-labelled palmitic acid. From this work it was concluded that the liver is the major source of plasma phospholipides in the fasting dog. Plasma phospholipides of rats in the post-absorptive state were also found to originate in the liver(3). On the other hand, Ranney *et al.*(4) demonstrated that, in the chicken, extrahepatic tissues contribute phospholipides to the plasma.

The following experiments were conducted to determine the site(s) of plasma phospholipide synthesis in the rabbit.

Methods. Normal white rabbits maintained on a diet of 100 g Purina rabbit chow per day, and fasted 24 hours prior to the experimental period, were sedated with intraperitoneally administered urethane (750 mg/kg) and anesthetized 30 minutes later by intravenously administered sodium pentobarbital (15 mg/kg). The gastrointestinal tract and spleen, and in some instances the kidneys, were removed through a median abdominal incision. The liver was left *in situ* isolated from the hepatic artery and portal vein. Before closing the incision, bandages wet with warm saline were placed in the abdominal cavity. Eviscerations were generally completed within 10 to 20 minutes, following which the rabbits were kept warm by application of external heat. Immediately after evisceration, rabbits received an intravenous dose of 100 mg glucose (10% in saline), followed by 100 mg/kg at 30 minute intervals through the remainder of the experiment(5). Control animals were sham operated, some receiving glucose, others not. Rabbits were injected intravenously with 0.5 to 1.0 mc of labelled phosphate and sacrificed four hours later by intravenous administration of sodium pentobarbital. Two series of experiments were performed. In series I plasma and tissues were extracted with alcohol-ether, petroleum ether and analyzed as previously described

TABLE I. Tissue Phospholipide Concentrations.

Tissue	Sham operated (mg P/g)		Eviscerated (mg P/g)	
Series I*				
Liver	1.36‖	± .02§	1.11‖	± .02
Kidney	1.11	± .03	1.12	± .01
Muscle	.283	± .010	.272	± .009
Lung	1.13	± .01	1.11	± .05
Plasma (4 hr)	.0359	± .0065	.0273	± .0050
Series II†				
Liver	1.20‖	± .06	.995‖	± .012
Kidney	1.02	± .07	1.03	± .11
Muscle	.310	± .022	.311	± .24
Lung	.990	± .008	.944	± .033
Plasma‡ (0 hr)	.0416	± .0052	.0494	± .0156
(4 hr)	.0481‖	± .0076	.0332‖	± .0089

* 5 sham-operated, 6 eviscerated rabbits.
† 4 " , 4 " " .
‡ 9 " , 9 " " .

§ Stand. error $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

‖ Diff. significant at 5% level.

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