TABLE II. Effect of Dihydrocholesterol upon Degree of Atherosclerosis in the Rabbit.

No. of rabbits	Diet	Gross atherosclerosis (mm^2)	
8	No cholesterol (Group I)	0	
8	1% cholesterol (Group II)	140	
		18	
		0	
		16	Avg
		487	271 mm^2
		318	
		1176	
		10	
7	1% cholesterol + 2% dihydrocholesterol (Group III)	25	
		0	
		0	Avg
		0	4.9 mm^2
		9	
		0	
		0	
8	2% dihydrocholesterol (Group IV)	8	
		0	
		0	
		0	Avg
		150	19.8 mm^2
		0	
		0	
		0	

In the bird, an increase in the lipid content of the liver was observed after prolonged feeding of a diet containing 2% DHC.‡ In the rabbit, however, the feeding of a 2% DHC diet for as long as 2 months failed to affect the lipid content of the liver. The tissues of rats fed a diet containing 10% DHC for 8 weeks were examined histologically by Dr. Stuart Lindsay of the Pathology Department; no abnormalities were observed.

Summary. 1. Effects of dihydrocholesterol on cholesterol content of plasma and on development of atherosclerosis were investigated in the rabbit. 2. Rabbits fed a diet containing 1% cholesterol exhibited typical hypercholesterolemia (average, 1041 mg/100 cc of plasma) and atherosclerosis (average estimated plaque area was 271 mm^2). Rabbits fed 1% cholesterol plus 2% dihydrocholesterol, the average plasma cholesterol value was 120 mg % and the average atherosclerotic plaque area was 4.9 mm^2. 3. Addition of dihydrocholesterol to a normal diet had no effect on the levels of plasma cholesterol, but did result in an increase in the average atherosclerotic plaque area.

The technical assistance of J. Sherokoff is gratefully acknowledged.

‡ The lipid content of the livers of birds fed a diet containing 2% DHC for 12 weeks ranged from 6.5 to 9.7 (average, 7.8%). The range for control birds was 2.9 to 5.7 (average 4.0). (Unpublished observations.)

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Anterior Pituitary Growth Hormone (STH) and Pancreatic Secretion of Glucagon (HGF).* (20483)

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Houssay and Biasotti(1) were the first to demonstrate that the removal of the pituitary gland ameliorates pancreatic diabetes. This discovery was followed by the observation that injections of crude anterior pituitary extracts(2), or purified growth hormone (somatotrophic hormone or STH(3)) cause temporary hyperglycemia and glycosuria, followed by permanent "metahypophyseal" diabetes. It is believed(4,5) that anterior pituitary hormone(s) first increase the demand for insulin and overstimulate the Islets of Langerhans and, if the injections are continued, cause a progressive degranulation, exhaustion, and degeneration of the beta cells, and a decreased secretion of insulin. In contrast to the beta cells, the alpha cells of animals with metahypophyseal diabetes appear to be normal (4-6). On the basis of evidence recently

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reviewed(7,8), the alpha cells are believed to be the site of formation of the hyperglycemic-glycogenolytic factor of the pancreas (HGF or glucagon), probably a second pancreatic hormone secreted in response to hypoglycemia (9-12). For these reasons, two possible explanations for metahypophyseal diabetes are suggested: 1) decreased insulin production leaving the anti-insulin hormones (*e.g.*, glucagon) unopposed, and 2) stimulation of the alpha cells by anterior pituitary hormones with increased glucagon secretion. The experiments described in this paper were designed to study the second hypothesis.

Materials and methods. The problem was studied by means of 20 cross-circulation experiments. Mongrel dogs of both sexes were used. All animals were healthy, well fed, and received the last *ad libitum* meal about 16 hours preceding the experiment. The dogs were anesthetized with sodium pentobarbital (35 mg/kg), heparinized, and prepared as described previously(12). Due to the heparinization, the importance of painstaking surgical hemostasis cannot be overemphasized.

In 14 experiments, the pancreato-duodenal vein of a donor *dog D* was connected with a femoral vein of a recipient *dog R* by means of glass cannulae and polyethylene tubing. These 14 experiments were of 3 types: in 5 experiments, the donor dogs received an intravenous injection of STH at the time of the experiment; in 6 experiments, the donor dogs received a similar injection at the time of the experiment, but in addition, were pretreated with 6 daily intramuscular injections of STH; in 3 experiments, the donor dogs received a control intravenous injection of 10 cc of a dilute NaOH solution at the same pH (10) of the STH solution. In 6 additional control experiments, a mesenteric vein of *dog D* was anastomosed to a femoral vein of *dog R*, and *dog D* received an intravenous injection of STH at the time of the experiment.

Highly purified ACTH-free beef anterior pituitary STH (Armour batch No. R-285-183) was dissolved in 10 cc of dilute NaOH (pH 10). All intravenous and intramuscular doses were of 3,5 mg/kg. This dose caused a rapid and transient hyperglycemia in a few

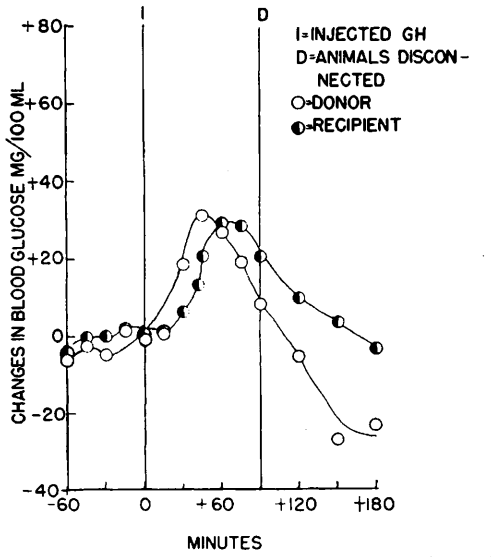

FIG. 1. Pancreatic-femoral anastomosis: Avg of 11 exp.

preliminary experiments in dogs and was of the same order of magnitude as that dose having a similar effect in normal men(13) and in mice with hereditary obesity(14). Blood glucose was determined according to Nelson's photometric adaptation of Somogyi's method(15).

Results. The results are presented as curves showing average changes in blood sugar concentration. Fig. 1 represents the results obtained in 11 pancreatic-femoral cross-circulation experiments in which the donor *dog D* received an injection of STH at time 0. Of these 11 donor dogs, 5 had received no treatment previous to the experiment and 6 had received 6 daily injections of STH as mentioned above. Pre-treatment with STH did not modify the results significantly; the data, therefore, were averaged. During the control period preceding the injection, there were no significant changes in the blood sugar of either the donor *dog D* or the recipient *dog R*. Following the injection of STH into *dog D*, the blood sugar of both dogs began to rise, reaching significant hyperglycemic levels in 30 to 90 minutes and returning to the initial values in about 120 minutes. The average maximum rise was approximately 30 mg/100 cc of blood in both donor and recipient, corresponding to a 32% and 36%

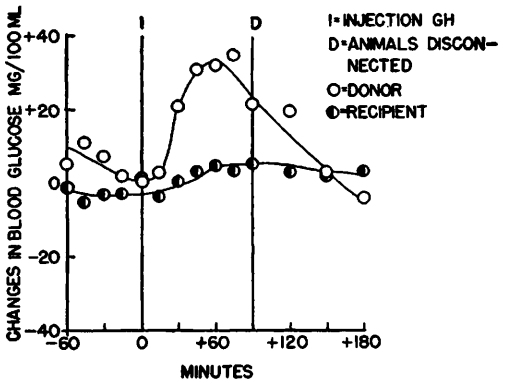

FIG. 2. Mesenteric-femoral anastomosis: Avg of 6 exp.

increase over the average blood sugar concentration at 0 time, respectively. This hyperglycemia was followed by hypoglycemia in 8 of the 11 donors and in 6 of the 11 recipients.

Fig. 2 represents 6 cross-circulation experiments in which a mesenteric vein of *dog D* and a femoral vein of *dog R* were connected. During the control period the blood sugar of both *dog D* and *dog R* remained essentially constant. Following the injections of STH into *dog D*, its blood sugar increased, reaching hyperglycemic levels in 60 to 90 minutes. The average maximum rise was again about 35 mg/100 cc of blood, or a 28% increase over the average blood sugar concentration at 0 time. The blood sugar returned to its original level in 120 to 150 minutes. This was followed by a hypoglycemia in 3 of the 6 donor dogs averaging 22% below the control level. This hypoglycemia occurred 120 to 180 minutes after the injection of STH and does not appear in the average curve because it occurred in only 3 out of 6 dogs, and not always at the same time. The blood sugar of the recipient dogs did not change significantly.

In 3 pancreatic-femoral cross-circulation experiments in which *dog D* received an injection of 10 cc of solution of NaOH at pH 10, instead of the solution of growth hormone, no significant changes in the blood sugar of either *dog D* or *dog R* were noted.

Discussion. The hyperglycemia observed in the recipient dogs following the injection of STH into the donors could be due to one or more of the following causes: 1) nonspecific causes, such as anesthesia or the general experimental procedure. This possibility is ruled out by the fact that the blood sugar remained reasonably constant during the preinjection control period and by the fact that when the donor dogs received alkaline water instead of STH, there was no rise in blood sugar in either *dog D* or *dog R;* 2) transfer of hyperglycemic blood from *dog D* to *dog R* through the anastomosis. This possibility is ruled out by the fact that the mesenteric blood of *dog D,* although containing the same amount of glucose as its pancreatic blood, did not cause hyperglycemia in *dog R;* 3) transfer of a significant fraction of the injected STH from *dog D* to *dog R.* This possibility similarly is ruled out by the fact that the mesenteric blood of *dog D* causes no hyperglycemia in *dog R;* 4) stimulation of the pancreas of *dog D* by the injected STH with secretion of a hyperglycemic factor (glucagon) and transfer of the material to *dog R* through the pancreatic-femoral anastomosis. This hypothesis, supported by the experimental evidence presented in this paper, is strengthened by the fact that the intensity and duration of the hyperglycemic response of *dog R* is similar to the intensity and duration of the response obtained in normal, non-anesthetized animals receiving intravenous injections of glucagon(10,11).

An increased secretion of glucagon can also explain the hyperglycemia observed in *dog D.* Although this dog loses part of its pancreatic blood, enough glucagon-containing blood, such as the blood coming from the head of the pancreas or from the gastric mucosa, does not leave the animal. Thus part of the glucagon produced by *dog D* remains in *dog D,* and part passes to *dog R,* causing hyperglycemia in both animals. The experimental data do not allow even an approximate estimate of the relative quantities of glucagon available to the 2 animals, since the intensity of the hyperglycemic response depends not only upon the amount of glucagon injected, but also upon the amount of liver glycogen present(16). Glucagon hyperglycemia has been shown to stimulate insulin secretion(12). and this is probably the cause of the hypoglycemia noted

at the end of the experiments described here (Fig. 1).

We believe these results confirm and extend the findings of Bornstein, Reid, and Young(17), and give further support to the hypothesis that glucagon is a pancreatic anti-insulin hormone secreted not only in response to hypoglycemia, but also to the administration of anterior pituitary STH.

Summary and conclusions. Fourteen cross-circulation experiments were performed by anastomosing the pancreato-duodenal or a mesenteric vein of a donor *dog D* with a femoral vein of a recipient *dog R*. The intravenous injection of purified anterior pituitary STH into a normal donor or into a donor previously treated with the same STH causes the appearance of a hyperglycemic material (glucagon) in the blood of the pancreatic but not of the mesenteric vein. This glucagon causes hyperglycemia in a normal recipient dog. In addition to decreasing insulin secretion(5) and inhibiting peripheral utilization of glucose (18), STH appears to cause hyperglycemia by stimulating the secretion of glucagon by the pancreas. It is believed that these results further support the hypothesis that glucagon is a pancreatic anti-insulin hormone contributing to the maintenance of a normal blood sugar concentration.

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