intestinal tract frequently occurred in the mice which died following irradiation and saline injection.

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Effects of the Hyperglycemic-Glycogenolytic Factor (HGF), Epinephrine and Insulin in Normal and Depancreatized Dogs.* (19715)

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During the course of experiments designed to study the action of certain insulin-free pancreatic extracts on the ketonemia and ketonuria of depancreatized dogs, it was noticed that the extracts had a marked hyperglycemic effect(1), probably because they contained the hyperglycemic-glycogenolytic factor (HGF). The extent of the hyperglycemia and its duration in different animals were extremely variable, however, even when the extracts were derived from the same batch of pancreas, were treated in the same manner and were injected under apparently identical conditions. Other experiments showed that the injection of epinephrine into depancreatized dogs with ketosis was also followed by a variable hyperglycemic response. The work described in this paper was undertaken to investigate the reasons for this variability.

Materials and methods. Well-fed mongrel dogs were depancreatized aseptically under sodium pentobarbital anesthesia. Following the operation, 100 ml of 0.9% saline, 100 ml of 10% glucose, 10 units of regular insulin (Lilly) and 30,000 of slow-acting penicillin were injected subcutaneously. This treatment was repeated on the first and, occasionally, on the second postoperative day. Penicillin injections were continued for 7 to 10 days. As soon as the animals started to eat, protamine zinc insulin (Lilly) was given instead of regular insulin. On the 7th postoperative day, the abdominal incision showed satisfactory healing and the stitches were removed. The dogs were kept in metabolism cages and fed the following daily ration: raw pancreas 100 g, sucrose 40 g, raw horse meat 500 to 700 g, depending upon the size of the animal. The urine was collected daily and the 24-hour glucose excretion was determined quantitatively; urinary ketone bodies were estimated according to an arbitrary scale (1+ to 4+) using Acetest§ tablets. The dogs were weighed once a week until the dietary and insulin requirements had been determined and

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§ Acetest Tablets were donated by the Ames Co., Elkhart, Ind.

TABLE I. Maximum Blood Sugar Rise Following Intravenous Injection of "Crude" HGF Extract in Depancreatized Dogs with Ketosis.

Group 1—"Mild" ketosis				Group 2—"Severe" ketosis			
		Max blood sugar rise				Max blood sugar rise	
No.	Initial blood ketones, mg %	mg	% of initial value	No.	Initial blood ketones, mg %	mg	% of initial value
1	0	71.8	46.7	20	30.2	62.3	18.7
2	3.9	32.6	46.7	21	38.1	7.8	20
3	4.5	151.3	73.7	22	38.3	27.1	26.4
4	5.2	33.3	39	23	41.5	83.1	22.2
5	5.9	200.9	55	24	42.6	45.4	25.2
6	6.5	40	44.6	25	44.8	114.3	40
7	7	89.1	55.8	26	61.6	65.9	17.1
8	9.5	76.6	22.5	27	63.8	117.3	61.3
9	13.2	49.5	89.6	28	69.4	86.3	44.4
10	13.4	134.7	38.5	29	75.1	33	11.9
11	14.4	34.2	40.5	30	82.3	22.3	12.5
12	15.6	64.5	74.3	31	84	26	19.6
13	18.9	36.8	62.3	32	94.5	26.1	7.5
14	19	62.1	31	33	96.3	32	6.5
15	19.7	73.9	56.8	34	106.4	180.3	52.3
16	22.4	44	38.6	35	187.5	33.4	35.6
17	24.6	151.3	30.6				
18	26	48.8	18.9				
19	29.1	191.5	47.2				
Avg	13.6	81.5	48	Avg	72.2	60.9	26.3

$P < .01$

$P < .01$

the animals had regained their preoperative weight, then at longer intervals. Protamine zinc insulin was administered at feeding time in doses sufficient to keep the urine acetone-free, the 24-hour glucose excretion below 10 g and the weight of the animal approximately constant. When an animal was needed for experiments, insulin was withdrawn until a strong (4+) ketonuria developed, usually 3 to 5 days. Quiet, well-fed mongrel dogs were used as controls. On the morning of an experiment the animals were not fed, control blood samples were taken, the extract containing HGF was injected intravenously and samples of blood were taken at various intervals of time for 4 hours. Blood ketone bodies were determined in duplicate following a modification of the method of Greenberg and Lester(2,3); blood glucose was determined in duplicate following the method of Folin and Malmrose(4). Urinary glucose was determined by means of Benedict's quantitative reagent. Two types of extracts containing HGF were used. The first one, which will be referred to as the "crude" HGF extract, was prepared as follows: 5.0 g of "12.5% salt cake," obtained by Eli Lilly and Company during the processs of insulin manufacture, were dissolved in 100 ml of 0.08 N NaOH; the solution was incubated at 39°C for 2 hours and the pH adjusted to 7.4 with dilute HCl. After filtration or centrifugation, the clear "crude" extract was injected intravenously in doses equivalent to 10 g of fresh pancreas per kilo of body weight. The second type of preparation was obtained by extracting lyophilized whole pancreas powder with liquid ammonia and purifying it partially by precipitation with 80% ethyl alcohol, according to a method described elsewhere(5). This preparation, dissolved in 10 ml of 0.9% saline, was injected in doses of 1 mg/kg and will be referred to as the "semi-purified" HGF. When indicated, the statistical significance of the results was calculated according to the method of Fisher(6) and expressed as "P", the probability that the results obtained might have been due to chance.

Results. The animals were listed in order of increasing concentration of ketones in the

TABLE II. Maximum Blood Sugar Rise Following Intravenous Injection of Epinephrine (.02 mg/kg) in Depancreatized Dogs with Ketosis.

Group 1—"Mild" ketosis				Group 2—"Severe" ketosis			
		Max blood sugar rise				Max blood sugar rise	
No.	Initial blood ketones, mg %	mg	% of initial value	No.	Initial blood ketones, mg %	mg	% of initial value
1	5.4	65.1	25	6	31.4	22.2	14.3
2	10.7	95.3	58.3	7	71.7	8	2.7
3	13.4	76.2	27.6	8	88.5	11.6	3.4
4	17.1	65.9	53.8				
5	18.2	46.5	25.6				
Avg	12.9	68.9	38.1	Avg	63.9	13.9	6.7

$P < .01$

$P < .01$

fasting blood and divided arbitrarily into 2 groups. Group 1 consisted of the animals with a ketonemia of less than 30 mg %, or with "mild" ketosis, Group 2 of the animals with a ketonemia of more than 30 mg %, or with "severe" ketosis. Table I shows that the average concentration of ketone bodies in the blood was 13.6 mg % in Group 1 and 72.2 mg % in Group 2. The response to the injection of the "crude" HGF extract was expressed as the maximal blood sugar rise reached during the 4-hour period of observation. It will be noted that the average hyperglycemic response was much greater in the animals with mild ketosis than in the animals with severe ketosis. The difference between these 2 average values is statistically highly significant ($P < 0.01$). Table II shows that similar results were obtained following the intravenous injection of epinephrine in doses of 0.02 mg/kg dissolved in 10 ml of 0.9% saline. Again the hyperglycemic response was inversely proportional to the initial concentration of blood ketones.

In another group of experiments, "semi-purified" HGF was injected intravenously into 10 normal dogs and 10 well controlled depancreatized dogs. These animals had received their last injection of insulin 24 hours before the experiment and had no ketonuria. Fig. 1 shows that the rise in blood sugar concentration produced by HGF was much greater in the controlled depancreatized dogs than in the normal ones. To determine whether or not, under the conditions of our experiments, depancreatized and normal dogs responded differently to the injection of insulin as well, regular insulin (0.1 unit/kg) was injected subcutaneously into 2 normal and 2 well controlled depancreatized dogs. Fig. 2 shows that insulin produced a more prolonged and pronounced hypoglycemia in the depancreatized than in the normal dogs. Thus the diabetic dog is more sensitive than the normal dog to the action of both HGF and insulin.

Discussion. The chemical and physiological properties of HGF have been reviewed(7,8). Like epinephrine, HGF stimulates the liver phosphorylase system and thus causes glycogenolysis and hyperglycemia(8,9). This effect depends upon an adequate supply of liver glycogen, and does not manifest itself fully when the glycogen stores have been depleted by fasting(9,10), uncontrolled experimental diabetes(10) and, possibly, certain forms of labile diabetes in humans(11). When insulin is withdrawn from a depancreatized animal, as the store of liver glycogen becomes progressively depleted, ketosis appears. This decreasing supply of liver glycogen is probably the cause of the inverse relationship between the concentration of ketone bodies in the blood and the rise in blood sugar produced by HGF and epinephrine. Glycogen depletion, however, cannot explain why the hyperglycemic response to the injection of HGF is smaller in the normal than in the depancreatized dog well controlled with insulin, as there is no reason to believe that these 2 types of animals have significantly different amounts of glycogen in

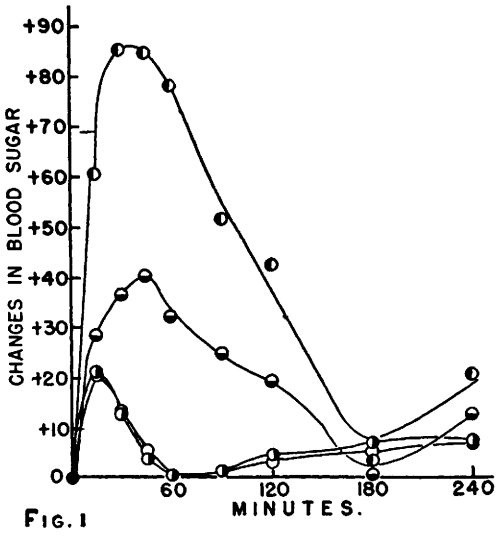

FIG. 1

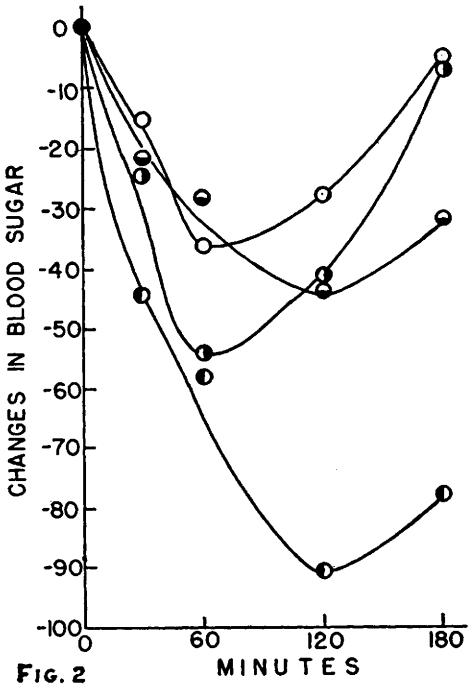

FIG. 2

FIG. 1. Hyperglycemia produced by intravenous injection of "semi-purified" HGF (1 mg/kg). Average of 10 normal and 10 depancreatized dogs without ketosis.

FIG. 2. Hypoglycemia produced by subcutaneous injection of regular insulin (.1 unit/kg). Average of 2 normal and 2 depancreatized dogs without ketosis.

their livers. A possible explanation is that, just as hyperglycemia produced by glucose administration stimulates the secretion of insulin by the pancreas of the normal animal (12-14), so the hyperglycemia produced by the injection of HGF might stimulate insulin secretion and thus, where a functioning pancreas is present, limit its own effect. Only in the depancreatized animal could HGF exert its full action. The observation that insulin has a greater effect in the diabetic than in the intact animal is in agreement with the results of others(15-17) and suggests that in the normal animal the reverse relationship between insulin and HGF may also exist: insulin hypoglycemia might cause the secretion of HGF and in turn be limited by it. In the depancreatized animal, on the other hand, HGF could not be readily secreted and insulin would be free to show its full effect. Thus, as several investigators have suggested (18-21), HGF would be a true anti-insulin hormone secreted by the pancreas itself and would have a function similar to that of other anti-insulin mechanisms(22). The secretion of the 2 pancreatic hormones would be mutually regulated by means of their opposing actions on the blood sugar. This hypothesis is being investigated further using cross-circulation experiments.

Conclusions. 1. The hyperglycemia produced by HGF and by epinephrine in the depancreatized dog with ketosis is greater in animals with mild ketosis than in animals with severe ketosis. 2. Since ketosis is believed to increase as liver glycogen decreases these results suggest that the hyperglycemic response to HGF and epinephrine depends upon the amount of liver glycogen present. 3. In the well controlled depancreatized dog without ketosis, on the other hand, the hyperglycemic effect of HGF is greater than in the normal animal, suggesting that in the presence of the pancreas, the action of HGF is limited by the secretion of insulin. 4. Similarly, in the well controlled depancreatized dog without ketosis, the hypoglycemic effect of insulin is greater than in the normal animal, suggesting that in

the presence of the pancreas the action of insulin is limited by the secretion of HGF. 5. It is suggested that HGF and insulin might be 2 mutually regulated pancreatic hormones and that their balanced secretion might be an important factor in the maintenance of a normal blood sugar concentration.

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Measurements of Radioisotopes in Blood Applied to Determinations of the True Hematocrit.* (19716)

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A convenient and accurate technic for sample preparation for use in measurement of concentrations of soft beta-ray emitting radioisotopes in blood is required in clinical and experimental investigations. Solid samples, especially at or near "infinite thickness," prepared by the isolation of a precipitate of the radioactive ion give reproducible results(1,2). However, their preparation requires considerable time and the counting rate of the sample is lowered by absorption of beta-rays by the sample. Measurement of radioisotopes in liquid samples(3,4) exposed to a thin-window counter has been recommended. Such liquid samples, like thick solid samples, reduce the counting rate by self-absorption, thus necessitating the administration of large amounts of labeled isotopes. Liquid samples suffer the additional disadvantage of possible evaporation, unless covered during counting, and are easily spilled.

Rapid and simple methods for sample preparation, particularly applicable to blood containing beta-ray emitters of low energy, have been developed and tested. These procedures

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