Metabolic Effects of the Pancreatic Hyperglycemic Factor. (21180)

N. KALANT.* (Introduced by J. S. L. Browne.)

From McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

Following the demonstration by Thorogood and Zimmerman(1) that pancreatectomy decreased the insulin requirement but increased the ketonuria of alloxan-diabetic dogs, attention has turned to the metabolic significance of glucagon, the pancreatic hyperglycemic glycogenolytic factor (HGF). There is general agreement as to its effect on blood sugar and liver glycogen, but recent work on its role in fat metabolism has given conflicting results. Indirect evidence such as that derived from the experiment of Thorogood and Zimmerman indicated that HGF was an antiketogenic substance. Stewart and Roitman(2) found that pancreatic extracts similar to those containing HGF did inhibit the increase in ketogenesis usually produced in liver slices by the addition of anterior pituitary extract. Haugaard found that HGF increased the ketogenesis by liver slices from glucose, but interpreted this as an effect secondary to an inhibition of lipogenesis from the glucose(3). *In vivo* experiments have been similarly equivocal. Foa(4) reported that insulin-free pancreatic extracts decreased the ketonemia of depancreatized dogs, while purified HGF produced an increase; it was concluded that the pancreatic extract contained an antiketogenic material other than insulin or HGF. Unfortunately this report does not make the experimental data available so that their quantitative significance cannot be judged. Pincus(5) found no effect of HGF on ketonemia in depancreatized dogs, while Zimmerman and Donovan (6) observed a slight increase followed by a more marked decrease when inactivated commercial insulin was given to such animals.

HGF has been reported to increase nitrogen excretion in acute experiments on rabbits (7); unfortunately the data as presented are not subject to statistical analysis.

The following work was undertaken in an attempt to assess the effect of HGF on fasting metabolism, and more specifically on fasting ketosis.

Method. Male hooded rats weighing 210-230 g were fasted for 24 hours. Blood was taken from the jugular vein, and the animals were then given an intraperitoneal injection of 33 γ of HGF in dilute NaOH solution (pH 9.5). Two more such injections were given at intervals of one hour. Four hours after the initial injection a second sample of blood was obtained from the jugular vein. All blood specimens were analyzed for total ketone content by the method of Lester and Greenberg(8).

A second group of rats received HGF injections of 50 γ each at 6, 10, 14 and 22 hours after the onset of fasting. Blood was taken after 24 hours of fasting.

A third group of animals was treated in the same manner as group two, but the total dose of HGF was 600 γ/day. Blood was then taken for sugar determinations(9) and samples of liver for glycogen content(10).

Control animals for these 3 groups received injections of the NaOH solution used as solvent for the HGF.

A fourth group of animals was placed in metabolic cages, and fasted for 24 hours, receiving only injections of the NaOH solution. The urine was analyzed for total nitrogen. A week later, when the rats had regained their initial weight and shown some increase as well, they were again fasted, this time getting injections of 600 γ of HGF in divided doses at 4, 10, and 15 hours after the start of the fast. The urine was again analyzed for total nitro-

TABLE I. Acute Effects of HGF on Ketonemia and Glycemia.

	4-hr change in ketonemia, mg%	4-hr change in glycemia, mg%
Control	(8) $+1.0 \pm .6$	(8) $+18.0 \pm 4.3$
HGF	(8) $-1.5 \pm .25$	(9) $+18.6 \pm 3.7$
	$p < .01$	$p > .05$

Figures in parentheses indicate the No. of animals used. Values shown are Mean $\pm$ S.E.$_M$.

* Medical Research Fellow, National Research Council, Canada.

TABLE II. Effects of HGF on Fasting Metabolism.

	Ketonemia,* mg%	Glycemia, mg%	Urine N, mg	Liver glycogen, %	Mouse liver fat, %
Control	(6) 7.3 ± .21	(21) 108 ± 2.2	(7) 212 ± 16.8	(8) .05 ± .02	(4) 7.2 ± .28
HGF	(8) 3.9 ± .45	(7) 102 ± 4.0	(7) 295 ± 9.6	(8) .12 ± .03	(4) 6.3 ± .17
	$p < .01$	$p > .05$	$p < .02$	$p > .05$	$p < .05$

* Ketonemia in fed state is 2.5 ± .2 mg%.
Figures in parentheses indicate the No. of animals used.

gen. The use of each animal as its own control overcomes the great individual variations in nitrogen excretion known to occur in these animals.

The effect of HGF on liver fat was measured in mice according to the assay procedure of Campbell(11). The dose used was 125 γ given subcutaneously.

The HGF preparation used was Lot No. 208-158B-197 of the Lilly Research Laboratories, supplied through the courtesy of Dr. W. R. Kirtley. This material contains only traces of insulin or none at all; 0.2 γ per kilo given intravenously to an anesthetized cat produces an optimal rise in blood sugar(12).

Results and discussion. The results of these experiments are shown in Tables I and II.

It is seen that HGF caused a reduction in ketonemia over a 4-hour period, and greatly inhibited the ketosis developed by a day of fasting. The acute effect on ketonemia is in agreement with results obtained by Zimmerman and Donovan(6) and dismissed by these workers as an effect of residual "hypoglycemic activity"; this interpretation appears unjustified both from their own data, since the fall in ketonemia occurred often with no accompanying fall in glycemia, and from the present results obtained with a highly purified material. At the same time there was an increase in nitrogen excretion indicating an increase in protein catabolism. These changes together with the slight decrease in liver fat indicate that HGF caused an alteration in energy metabolism so that less fat and more protein were used, in comparison with the control rats. This interpretation is supported by a preliminary report of Waters(13) that insulin contaminated by HGF produced an increase in gluconeogenesis by liver slices.

It is interesting that the liver glycogen and blood sugar were not increased above the control levels. This is in contrast to the changes brought about by cortisone, which in a dose of 10 mg given in divided doses during a day of fasting, caused a less marked inhibition of ketonemia, but a definite rise in liver glycogen. These effects are shown by the results obtained in groups of 8 animals (Table III). The difference in the effects on liver glycogen may be a quantitative one, due to a greater protein catabolism by cortisone with comparably increased gluconeogenesis; this is improbable in view of the weaker antiketogenic effect. Thus it is likely that the changes brought about by HGF are not mediated by the adrenal hormones and those brought about by cortisone are not mediated by HGF. In any case HGF appears as still another hormonal factor to be considered in problems of protein (and fat) metabolism.

These results do not prove that the action of HGF is responsible for the failure of alloxanized animals to develop severe ketosis, but they are compatible with this concept.

An incidental implication of the results described above is that growth hormone does not produce its metabolic effects by acting through HGF, as has been suggested(14,15); growth hormone given to fasted animals causes a decrease in nitrogen excretion while HGF caused an increase. However it is conceivable that growth hormone exerts 2 opposing influences on protein catabolism, one via HGF,

TABLE III. Effect of Cortisone on Fasting Metabolism.

	Liver glycogen, %	Ketonemia, mg%
Cortisone	1.46 ± .29	5.0 ± .86
Control	.05 ± .02	6.7 ± .55

and that a preponderance of the latter effect in man accounts for the failure to obtain nitrogen retention with growth hormone in this species. This is entirely speculative since there is no direct evidence that growth hormone does stimulate the production of HGF.

Summary. Purified pancreatic hyperglycemic factor has been given to normal rats during a period of fasting or at the end of such a period. In the latter case HGF caused a fall in the level of ketonemia which had developed as a result of the fast, but did not affect the blood sugar. When given during the fast, HGF greatly inhibited the development of ketonemia, increased the nitrogen excretion, and inhibited to some degree the rise in liver fat; it did not affect the liver glycogen or the blood sugar. These effects differ from those of adrenal hormones and of growth hormone. It appears that HGF is capable of playing an active part in fasting metabolism.

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Influence of Smoking on Urinary Pepsinogen Excretion.* (21181)

S. BORNSTEIN AND S. EICHEN.

From the Laboratory Service, Veterans Administration Hospital, Montrose, N. Y.

Whether smoking stimulates gastric secretion has not been definitely ascertained. The after-dinner smoke is believed to aid digestion and earlier workers reported that smoking stimulated gastric secretion and acidity. However, Schnedorf and Ivy(1) found that smoking produced no immediate effect on acid production but smokers had a higher gastric acidity (not statistically significant) than non-smokers. They reported that smoking had no effect on gastric evacuation but Dickson and Wilson(2) noticed a slight delay.

We have studied the effects of smoking on urinary pepsinogen excretion.

Method. To determine the excretion rate of urinary pepsinogen, the method of West, Ellis, and Scott(3) employing a casein substrate(4) was used. Twenty white male office and professional hospital workers between 18 and 45 years of age, who smoke 20 or more cigarettes per day, were examined on 5 not necessarily successive days. The results were compared with those of a control group of 20 non-smokers.

Results. The results are shown in Fig. 1. Aside from the diurnal variations(4) we found considerable day to day variation in healthy control individuals. These variations were not unexpected. They are usually within a limited range (though in rare instances they may exceed 100% of the mean value). These variations must be ascribed to the day to day

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