INTERRELATION OF PARATHYROIDS, SUPRARENALS AND PANCREAS.

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THAT the parathyroid glands have an influence on carbohydrate metabolism has been indicated by the experimental results of numerous workers, notably by Underhill and his associates (1), who showed that removal of the parathyroids brought about a hypoglycæmia with disappearance of liver glycogen. They consider that these conditions are neither the cause nor the effect of the tetany resulting from parathyroidectomy. It is well known that the guanidine content of the blood is increased after parathyroidectomy and also that administration of guanidine to the normal animal can produce symptoms similar to those of tetany (2), including profound changes in the blood-sugar. These blood-sugar changes have been investigated by Watanabe (3) who, using doses of guanidine sufficient to cause symptoms of tetany, showed that a single injection produced a hyperglycæmia, followed in about 7 hours by a hypoglycæmia. A second dose several days after the first resulted in a hyperglycæmia without any subsequent lowering of blood-sugar, unless a lethal dose were administered, in which case hypoglycæmia was observed just before death. Glycosuria was not found. That guanidine may have a direct action on blood-sugar is suggested by the fact that guanidine salts added to dilute glucose solutions alter the ratio of the polarimeter value to the copper-reducing value as determined by Bang's method (4).

The present investigation was begun to determine the effect of guanidine on the blood-sugar of rabbits, avoiding as far as possible the production of gross symptoms of tetany. The dose was therefore limited to ·1 gm. per kilo. of body-weight. It should be noted, however, that Paton and Findlay have in some cases found this amount sufficient to cause an increase in electrical excitability in rabbits (2). Guanidine hydrochloride was injected in concentrated aqueous solution into the marginal vein of one ear, while blood for sugar estimation was obtained by venesection from the opposite ear every hour or half-hour. The majority of animals showed no abnormal signs after an injection but

in a few cases shaking of the forepaws, movements indicating irritation of the mouth or nose and increased salivation were observed; on two occasions dyspnœa was present. In all cases the rabbits appeared normal at the end of an hour. None of the animals had food for 18 hours before an experiment. For the estimation of blood-sugar Bang's old micro-method was used, a small platinum capsule, as in Calvert's method (5), being utilised in which to collect and weigh the blood in place of the usual absorbent paper. This procedure shortened considerably the time required for each estimation. Before every experiment two and often three samples of blood were taken, at intervals of half-an-hour, to determine the normal sugar content. Throughout the paper, unless otherwise stated, sugar values are given in mgms. per 100 mgms. of blood. In Fig. 1 is given one typical result from each of the first four series of experiments; a summary of other results is given at the end of the paper.

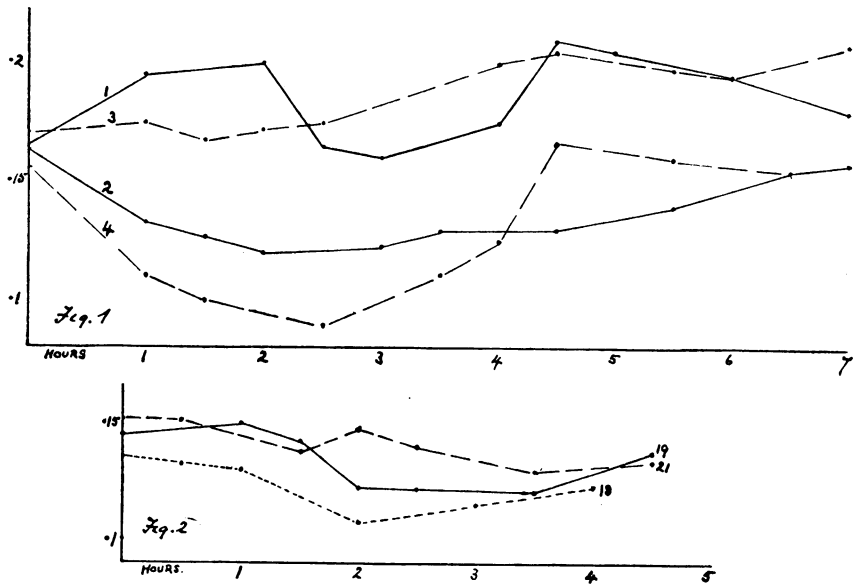
Effect of a single dose of guanidine hydrochloride. A first injection of guanidine causes the blood-sugar to vary in an apparently erratic manner, but the curves depicting these variations show a certain similarity in that a rise occurs during the first hour, followed by a fall of varying duration. In two cases this fall is succeeded by a second rise; in all there is a tendency to revert to the original level after 6 or 7 hours (Curve 1, Fig. 1). That conflicting factors were at work to produce these results is obvious and, as it is known that guanidine has a stimulating action on the sympathetic nervous system (6), it was reasonable to suppose that the hyperglycæmia was of the adrenaline type. Miculicich (7) and Burn (8) showed that adrenaline hyperglycæmia is inhibited by ergotoxine. In a second series of experiments, therefore, each rabbit was given a preliminary dose of 3 mgms. ergotamine tartrate, which has been shown by Dale and Spiro to have the same pharmacological action as ergotoxine (9). One hour later guanidine was injected and blood-sugar estimations carried out as before. An immediate fall in blood-sugar occurred and persisted in two animals for at least 7 hours (Curve 2, Fig. 1). In the third case the normal level was regained after 3½ hours.

Effect of a second dose. Two rabbits received a second injection of guanidine alone, 10 weeks and 12 weeks respectively after the first. In both cases a hyperglycæmia resulted without any fall of the blood-sugar below normal and in one animal the original level was not regained for 25 hours (Curve 3, Fig. 1). That this hyperglycæmia is due to hyperactivity of the suprarenals or to a lowered threshold to stimuli of the sympathetic system, brought about by the first dose, is suggested by the following experiments: Three rabbits received a dose of ergotamine

tartrate an hour before a second injection of guanidine, which now produced an immediate fall in blood-sugar, more abrupt than that seen after a first dose, but the normal level was regained in all cases after 4½ hours (Curve 4, Fig. 1). Ergotamine alone was not found to have any marked influence on blood-sugar.

That this return to normal was not due to waning of the ergotamine action on the sympathetic was shown by the fact that in one rabbit a second dose of ergotamine was given when the blood-sugar was at a minimum; no prolongation of hypoglycæmia followed. Thus, if the normal supply of glucose from the liver is unavailable, guanidine produces an immediate fall in blood-sugar. This is further illustrated by two experiments in which guanidine was administered after the supply of glycogen had been depleted by starvation and the injection of strychnine on the day previous to that of the experiment. As will be seen from the following results, the hypoglycæmia is less than that after ergotamine, probably because of the difficulty of ensuring complete exhaustion of the glycogen supply:

Hours after injection	0	1	2	3	4	5	6
No. 3	·150	·141	·133	·141	·140	·138	·139
No. 15	·155	·122	·116	·125	·135	·133	·130



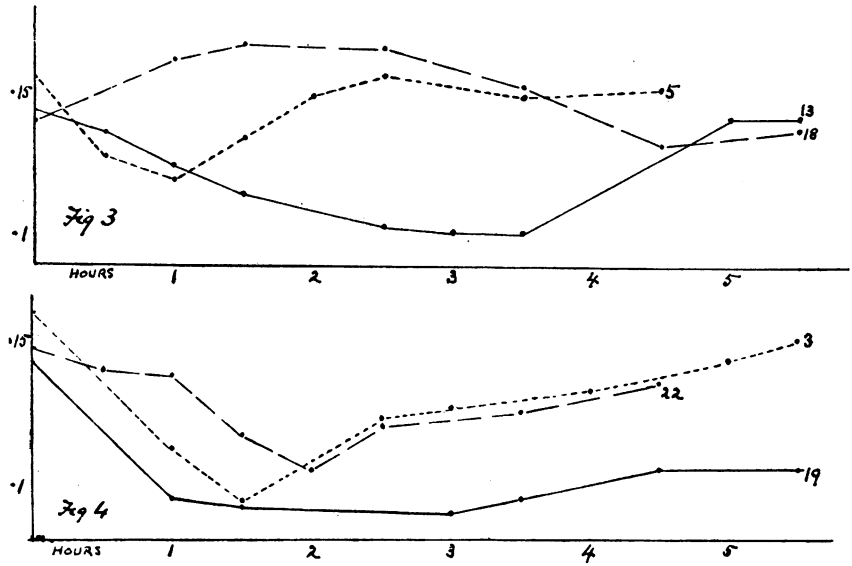
On the other hand, as long as glycogen is available, guanidine will cause its liberation from the liver by sympathetic stimulation. It appears

from Watanabe's work (3) and from the above results that this glycogenolytic action is stronger than the hypoglycæmic action, so that, if guanidine is the cause of the low blood-sugar observed after parathyroidectomy, it can only be so when the liver no longer contains glycogen.

Burns and Watson produced evidence to show that guanidine salts have a nicotine-like action on the vagus, first stimulating and later paralysing the fibres to the heart (10). It is therefore reasonable to suppose that other branches of the vagus are similarly affected. McCormick and O'Brien showed that it is possible to produce a lowering of blood-sugar in animals by stimulation of the right vagus, taking precautions to avoid cardio-inhibitory effects (11). The nerve supply to the islets of Langerhans is believed to come in large part from this nerve and the hypoglycæmia resulting from its stimulation can be ascribed to liberation of insulin. It is possible to explain the hypoglycæmic action of guanidine in this way, and three experiments were carried out to determine this point. The rabbits were given 3 mgms. of ergotamine tartrate one hour before, and ·15 mgm. of atropine sulphate intravenously 15 minutes before an injection of guanidine hydrochloride; ·05 mgm. of atropine was also given at the same time as the guanidine. From Fig. 2 it is seen that No. 19 showed no hypoglycæmia for 1½ hours, and that the fall in blood-sugar occurring then is considerably less than that observed in parallel experiments without atropine (Curve 4, Fig. 1). A similar result was obtained in No. 18. No. 21 was given a further injection of atropine sulphate (·15 mgm.) 1½ hours after the guanidine, producing a slight rise in blood-sugar half-an-hour later, which, however, does not reach above the normal level.

Effect of calcium on guanidine hypoglycæmia. The administration of calcium relieves the symptoms of tetany after parathyroidectomy and Underhill and Blatherwick found that in dogs the accompanying hypoglycæmia was also diminished (12). Watanabe on the other hand was unable to restore the blood-sugar to normal by injections of calcium lactate during guanidine hypoglycæmia (13). In the present investigation calcium chloride was given intravenously, the animals having had ergotamine. Rabbit 13 received 1 c.c. of a 1·5 p.c. solution at the same time as the guanidine; the resulting fall in blood-sugar (Fig. 3) is neither so abrupt nor so great as that seen without calcium (Curve 4, Fig. 1). Rabbit 18 was given 2 c.c. with guanidine and the result is in this case a transient hyperglycæmia. In a third animal a similar dose of calcium was given during the guanidine hypoglycæmia, with the result that the

blood-sugar regained its normal level within 1½ hours. Calcium alone after ergotamine was not found to have any effect on blood-sugar.



Effect of parathyroid extract. The preparation used was the Parathyroid Tablets 1/10 gr. (Armour). These were triturated with normal saline and the fluid obtained after filtering or lightly centrifuging was given intravenously. Three rabbits were employed, ergotamine having been injected one hour before guanidine. No. 19 was given the extract from three tablets at the same time as a first dose of guanidine. The resulting hypoglycæmia appears to be accentuated rather than relieved (Fig. 4). In No. 3 the extract of four tablets with guanidine caused a more rapid fall in blood-sugar and a more rapid initial recovery, but complete recovery was somewhat delayed. The remaining rabbit was given two parathyroid tablets by mouth on the morning and evening of the day before the experiment, and also an intravenous injection of the extract of three tablets with the guanidine. In this case the fall in blood-sugar was delayed, and the recovery was similar in type to that of No. 3. The parathyroid extract employed was found to have no influence on blood-sugar when given alone after ergotamine.

Discussion. From the foregoing experiments it is seen that guanidine produces two effects, the first and more powerful being a stimulation of the sympathetic system causing a hyperglycæmia, which is probably augmented by an increased production of adrenaline, and the second a

hypoglycæmia which can be explained by an increased liberation of insulin due to vagal stimulation. The action of a single injection of guanidine seems to render the sympathetic nerves more susceptible to further doses of guanidine. A similar sensitising action on the vagus is suggested by a comparison of Curves 2 and 4, Fig. 1. The fall in blood-sugar after a second injection of guanidine following ergotamine is greater and more rapid than that after a first injection and the equally rapid recovery in the former case may be due either to the supervention of vagal paralysis or to the fact that the whole of the available insulin has been discharged from the islets. That guanidine causes an increase in the ratio of polarimeter value to copper value when added to dilute glucose solutions *in vitro* has already been described (4). If it is possible to cause exhaustion of the islets by guanidine, it is evident that, because of the hyperglycæmia simultaneously produced by stimulation of the sympathetic system, a blood condition similar to that of diabetes will occur. This possibility is being investigated and the following experiment is suggestive: A male rabbit was given five doses of guanidine over a period of 10 days. The animal's weight fell from 2·4 to 2·1 kilos. Three days after the last injection the blood-sugar was found to be ·183, the value at the beginning of the experiment being ·138. The rabbit was killed and the blood-sugar extracted according to the method used by Winter and Smith (14). The initial polarimeter value of the extract was equivalent to ·104 p.c. but fell to coincide with the Bang value at ·056 p.c. after 38 hours. No sugar was detected in the urine.

It is known that the sugar in diabetic blood is present in a form which cannot be readily utilised by the organism; the blood-sugar in adrenaline hyperglycæmia shows a similar ratio of polarimeter value to copper value (15). It may be inferred then that glucose as liberated from the liver must be altered in some way by insulin to render it readily assimilable by the tissues, so that an increased glycogenolysis will need an increased production of insulin. It is of value therefore that the same factor which liberates glucose from the liver should also control the supply of insulin.

The apparently contradictory results obtained with parathyroid extract may be explained in the light of recent experiments by Winter and Smith, showing that parathyroid extract augments the action of insulin (16). The effect of parathyroid extract on the blood-sugar in guanidine hypoglycæmia will be the resultant of its action on insulin and that on guanidine.

SUMMARY.

1. Further evidence of sympathetic and parasympathetic stimulation by guanidine is given.

2. Guanidine appears to exert an action on the organism lasting at least 10 to 12 weeks, which alters the balance between glycogenolysis and the production of insulin.

3. The antagonism between calcium and guanidine is illustrated.

4. The results of parathyroid administration appear to show that, when given with guanidine, the extract augments the hypoglycæmia, but, given some time before, delays its onset.

5. A possible factor in the etiology of diabetes is suggested.

I wish to express my thanks to Professor Burns for much valuable criticism and assistance.

The expenses of this research are covered by a grant from the Medical Research Council.

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RESULTS OF EXPERIMENTS NOT GIVEN IN FIG. 1.

First dose of guanidine.

Alone			After ergotamine		
Hours after injection	No. 3	No. 4	Hours after injection	No. 10	No. 12
0	·110	·125	0	·140	·130
1	·136	·184	1	·117	·090
1½	—	·151	2	·100	·095
2	·122	·143	3	·084	·105
3	·090	·240	4	·084	·125
4	·100	·215	5	·087	·120
5	·103	·185	6	·095	·120
6	·117	·160	7	·105	—
7	—	·157			

Second dose of guanidine.

	No. 6		No. 5	No. 13
0	·155	0	·158	·160
1	·175	1	·116	·095
2	·160	2	·093	·100
3	·170	3	·120	·105
4	·176	4	·140	·130
5	·188	5	·175	·155
6	·195	6	·150	·165
7	·180	7	·155	—

Out of 24 rabbits only one was found in which it was impossible to produce hypoglycæmia by injection of guanidine after ergotamine or after exhausting the glycogen supply by starvation and strychnine.