Several observations give support to the view that a hyperglycemic glycogenolytic factor, called glucagon in older literature, is normally produced and secreted by the alpha cells of the pancreatic islets and perhaps by the argentophyl cells of the gastrointestinal mucosa (Sutherland & de Duve, 1948; Cavallero & Malandra, 1951). It has been assumed that this factor could be of no little importance in some disturbances concerning carbohydrate metabolism, namely in the diabetic disease (Ferner, 1942, 1951). It was therefore of particular interest to investigate first whether this pancreatic factor can act in the experimental animal as a hyperglycemia and glycosuria inducing factor and secondly, whether it can potentiate the effects on carbohydrate metabolism of other known diabetogenic preparations.

Since in previous unpublished experiments neither a consistent glycosuria nor a lasting hyperglycemia were obtained giving glucagon to intact and partially depancreatized rats fed a standard diet ad libitum, it was decided to assay the activity of this principle on the rat force-fed a high carbohydrate diet, which is more sensitive in this kind of experiment. In the experiments here presented the effect of glucagon on the glycosuria and glycemia of the intact tube fed rat was compared with that of cortisone, adrenocorticotrophic hormone (ACTH) and somatotrophic hormone (STH), and a possible potentiation by glucagon of the diabetogenic activity of cortisone, ACTH and STH was investigated.
MATERIAL AND METHODS

Adult intact male rats, weighing approximately 300 gm. at the start of the experiment, were used. The animals were kept in metabolism cages in an air-conditioned room at 25° C. The animals were fed by stomach tube a high carbohydrate liquid diet, twice a day at 10 A.M. and 7 P.M. The diet contained 50 per cent carbohydrates, 38 per cent protein, 10 per cent fat and 2 per cent McCollum salt mixture; lipotropic factors and vitamins were added in sufficient amounts and water was allowed ad libitum.

The adaptation period to the tube feeding lasted from 10 to 30 days; during this time the amount of the diet, beginning at 20 ml., was gradually increased at a constant rate of increment, i.e. 1 ml. of diet per day, until the limit of the tolerance to carbohydrate was reached. The amount of diet at which a slight, but measurable glycosuria developed, varied from animal to animal, the lowest value being 30 ml. and the highest 50 ml. per day. From this time, the animals were kept at a constant diet intake; urine and blood sugars were determined during a pretreatment period for 5 days and successively from 5 to 15 days during the treatment with single or combined hormones. The data obtained from the different groups of animals before and during treatment were statistically compared; the standard errors and the 'P' values of the differences were calculated. 'P' values < .01 were considered statistically significant.

Blood samples were taken from the tail one hour after the morning feeding and were daily analyzed for their glucose content by the Hagedorn-Jensen method; urine was collected and analyzed every day for glucose by the quantitative method of Benedict. The animals were weighed daily before the morning feeding.

The following hormonal preparations were tested: Hormone somatrotrope Lab. Choay, 10 I. U. daily, Cortrophine N. V. Organon, 10 I. U. daily, and Cortone acetate Merck Inc., 10 mg. daily. Glucagon was prepared from alkali inactivated insulin according to the method of Sutherland et al. (1949); zinc insulin crystals Eli Lilly a. Co., lot. N. 491516) used for its preparation, when administered to a cat at 0.025 mg./kg., caused a rise in blood sugar of 30 mg. per cent, indicating that fairly high quantities of glucagon were present. The daily dose of glucagon, referred as mg. of zinc insulin crystals, was 10 mg. and contained 2.5 mg. of protein. Cortisone, ACTH and STH were injected subcutaneously, glucagon intraperitoneally, the total daily amount of the hormones being administered in two divided doses immediately before each tube feeding.

RESULTS

TREATMENT WITH SINGLE HORMONES

As shown by Table 1, all the hormonal preparations tested determined a consistent increase of the blood and urine sugars of the intact force-fed rat.

The glycosuria was enhanced in the cortisone group as well as, but to a lesser degree, in the glucagon and ACTH groups, whereas STH did not sharply increase the urine glucose. Glucagon showed comparatively a maximal activity on the blood glucose, while the other preparations were less effective in this respect. In all the groups studied the differences between the pretreatment and treatment levels of the urine and blood sugars were statistically significant ('P' < .001).

80
Table 1.
Mean changes of the urine and blood sugars and of the body weight in force-fed rats under hormonal treatment; standard errors and significance of the differences.

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Treatment</th>
<th>Glycosuria mg./day</th>
<th>Mean change</th>
<th>S. E.</th>
<th>Glycemia mg. per cent</th>
<th>Mean change</th>
<th>S. E.</th>
<th>Body weight gm./day</th>
<th>Mean change</th>
<th>S. E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>No</td>
<td>271</td>
<td>+45 ± 6.3</td>
<td></td>
<td>177</td>
<td>+42 ± 2.0</td>
<td></td>
<td>+4.11</td>
<td>+4.83</td>
<td>± 1.4</td>
</tr>
<tr>
<td></td>
<td>STH</td>
<td>316</td>
<td></td>
<td>('P' &lt; .001)</td>
<td>135</td>
<td>('P' &lt; .001)</td>
<td>+0.71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>No</td>
<td>138</td>
<td>+137 ± 18.2</td>
<td>('P' &lt; .001)</td>
<td>156</td>
<td>+30 ± 1.5</td>
<td>+0.30</td>
<td>('P' &lt; .001)</td>
<td>-1.01</td>
<td>± 0.5</td>
</tr>
<tr>
<td>15</td>
<td>ACTH</td>
<td>275</td>
<td>+256 ± 15.6</td>
<td>('P' &lt; .001)</td>
<td>170</td>
<td>+34 ± 3.1</td>
<td>-1.96</td>
<td>('P' &lt; .001)</td>
<td>-1.84</td>
<td>± 0.4</td>
</tr>
<tr>
<td>18</td>
<td>No</td>
<td>224</td>
<td></td>
<td></td>
<td>136</td>
<td></td>
<td></td>
<td>-0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Cortisone</td>
<td>480</td>
<td>+136 ± 10.9</td>
<td>('P' &lt; .001)</td>
<td>198</td>
<td>+67 ± 4.0</td>
<td>+0.88</td>
<td>('P' &lt; .001)</td>
<td>+0.80</td>
<td>± 0.6</td>
</tr>
<tr>
<td>15</td>
<td>No</td>
<td>166</td>
<td></td>
<td></td>
<td>131</td>
<td></td>
<td></td>
<td>+0.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The body weight was augmented by the STH treatment and a little stimulated by glucagon; it was clearly inhibited in both cortisone and ACTH treated groups. At the statistical analysis, the changes determined by cortisone and STH were well significant, while no significant changes could be demonstrated for ACTH and glucagon.

*Treatment with combined hormones*

Table 2 shows the results of the second experimental series: in this series intact force-fed rats received glucagon simultaneously with STH, ACTH or cortisone. As shown by the table, a potentiation by glucagon of the diabetogenic activity of the pituitary and adrenocortical preparations under consideration was well evident; during the combined treatment all the groups showed a significant increase of the urine and blood sugars.

The maximal synergic effect was observed when giving glucagon to cortisone treated rats; glucagon was also active, but to a lesser degree, as synergist in the STH and ACTH treated groups. The effect of combined treatment on the body weight varied greatly from animal to animal. In general a slight, but not constant, loss of weight was observed when adding glucagon; however, the changes were not statistically significant.

On stopping treatment the animals returned almost immediately to pre-treatment levels; no persistent glycosuria nor hyperglycemia was observed.

Figures 1 and 2 summarize the data of two representative experiments. Data of an animal in which the simultaneous administration of cortisone and glucagon was assayed, are charted in Fig. 1. On the diet of 40 ml. daily, this animal exhibited a slight glycosuria and a maximal hourly glycemia at 135 mg. per cent. The administration of cortisone increased slightly the urine and blood sugars, the latter up to 155 mg. per cent, and markedly decreased the body weight. When given cortisone and glucagon together, this animal displayed an increased glycosuria up to 1200 mg. daily, and a higher glycemia up to 250 mg. per cent. After discontinuing the glucagon treatment a prompt fall both of the urine and blood sugars was observed.

Figure 2 is drawn from the data obtained from an animal successively treated with ACTH + glucagon and STH + glucagon. In this long standing experiment it can be observed that ACTH and STH, when administered alone, had some effect on the glycosuria and glycemia of the force-fed rat, the former decreasing and the latter increasing the body weight. In this animal, when glucagon was simultaneously administered, the blood sugar was markedly increased, while the glycosuria was scarcely influenced.
Table 2.
Mean changes of the urine and blood sugars and of the body weight in force-fed rats under combined hormonal treatment; standard errors and significances of the differences.

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Treatment</th>
<th>Glycosuria mg./day</th>
<th>Mean change</th>
<th>S. E.</th>
<th>Glycemia mg. per cent</th>
<th>Mean change</th>
<th>S. E.</th>
<th>Body weight gm./day</th>
<th>Mean change</th>
<th>S. E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>STH</td>
<td>298</td>
<td></td>
<td>163</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+4.77</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>STH + glucagon</td>
<td>340</td>
<td>+42 ± 8.1</td>
<td>224</td>
<td>+61 ± 3.1</td>
<td>+2.23</td>
<td></td>
<td></td>
<td>--2.54 ± 1.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(P' &lt; .001)</td>
<td></td>
<td>(P' &lt; .001)</td>
<td></td>
<td></td>
<td></td>
<td>(P' &gt; .05)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>ACTH</td>
<td>161</td>
<td></td>
<td>130</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>--0.85</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>ACTH + glucagon</td>
<td>227</td>
<td>+66 ± 10.5</td>
<td>178</td>
<td>+48 ± 1.8</td>
<td>--0.50</td>
<td></td>
<td></td>
<td>+0.35 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(P' &lt; .001)</td>
<td></td>
<td>(P' &lt; .001)</td>
<td></td>
<td></td>
<td></td>
<td>(P' &gt; .05)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Cortisone</td>
<td>383</td>
<td></td>
<td>154</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>--1.94</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Cortisone + glucagon</td>
<td>888</td>
<td>+505 ± 9.8</td>
<td>251</td>
<td>+97 ± 2.1</td>
<td>--2.14</td>
<td></td>
<td></td>
<td>--0.20 ± 0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(P' &lt; .001)</td>
<td></td>
<td>(P' &lt; .001)</td>
<td></td>
<td></td>
<td></td>
<td>(P' &gt; .05)</td>
<td></td>
</tr>
</tbody>
</table>
**Fig. 1.**

- Blood sugar 1st p.o. mg.
- Urine glucose/24h g.
- Body weight g.

**Fig. 2.**

- Blood sugar 1st p.o. mg.
- Urine glucose/24h g.
- Body weight g.
DISCUSSION

From the above results it is apparent that glucagon is highly effective as diabetogenic agent in the intact force-fed rat. When compared with other diabetogenic preparations, such as cortisone, ACTH and STH, glucagon showed an enhancing effect on urine and blood sugars practically of the same magnitude. Glucagon was more effective on the glycemia than the other hormones tested and, as glycosuria-enhancing factor, was second only to cortisone.

In vitro and in vivo experiments have shown that glucagon acts as an active glycogenolytic factor on the liver (Sutherland & Cori, 1948; Pincus, 1950); it was also demonstrated that this principle lowers the glucose uptake in the isolated diaphragm of the rat under the influence of insulin (Candela et al., 1951) and that it can antagonize the hypoglycemic effect of insulin in the normal animal (Tyberghein, 1952). It is very likely that in force feeding experiments these mechanisms are highly operative, since under the influence of glucagon the glucose entering the liver can not be stored and the circulating glucose is not utilized by the peripheral tissues. Since the glucagon we used was impure and since some inactivation of this factor is claimed to occur when preparing it from insulin by treatment with alkali (Sutherland & De Duve, 1948), it can be assumed that with pure preparations the effects would be even more enhanced.

The synergic effect of glucagon on the diabetogenic activity of the other hormonal preparations is closely reminiscent of the similar phenomenon observed by Engel et al. (1951, 1952) in intact rats force-fed a high carbohydrate diet simultaneously treated with ACTH and STH, and by Reid (1951) in intact cats subjected to the same combined treatment. In our experiments the maximal potentiation was obtained when glucagon and cortisone were administered together.

The most likely explanation for the diabetogenic effect of glucagon may be an increased breakdown of glycogen to glucose by the liver. If it is given simultaneously with cortisone or ACTH the breakdown is perhaps enhanced by the greater glycogen storage caused by these hormones. This explanation can not be extended to STH.

The present data suggest that under favourable conditions glucagon, like cortisone, ACTH and STH, can act as hyperglycemia and glycosuria inducing factor and can potentiate the effects of other diabetogenic preparations.

SUMMARY

Adult intact male rats, force-fed on a high carbohydrate liquid diet and given cortisone, ACTH, STH or glucagon, showed an increase of the urine and blood
sugars. This effect was the most evident in cortisone and glucagon treated groups.

When glucagon was administered simultaneously with cortisone, ACTH or STH to the force-fed rat, it showed a high enhancing effect on glycosuria and glycemia. The combined treatment with glucagon and cortisone was the most effective in provoking a temporary diabetic condition.

It is concluded that glucagon may be an important diabetogenic factor under favourable conditions.

ACKNOWLEDGMENTS

The authors are greatly indebted to Dr. G. Galansino and Dr. R. Fior for their cooperation. The hormones used in the investigation were kindly supplied by Dr. W. R. Kirtley (Eli Lilly a. Co., Indianapolis). Dr. H. de Jager (N. V. Organon, Oss) and Dr. A. Choay (Lab. Choay, Paris).

REFERENCES