of agents capable of reducing the ferric component in the reagent. Ascorbic acid, hydroxymethylglyoxal (reductone) and cysteine can bring about fading of the canavanine colour, and if present in sufficient quantity can inhibit completely the colour test. These, and possibly other reducing factors, must be considered in testing biological material for canavanine compounds; their effects can be overcome by pretreatment with aqueous iodine.

(3) Competitors. No guanidinoxy reaction could be observed with any animal secretion or tissue examined (milk, blood serum, cerebrospinal fluid, urine, liver, muscle, kidney), and low recovery values were obtained for canavanine added to these materials. Pretreatment with aqueous iodine had little if any effect on the low recovery, which suggested that it was not caused by destruction of the canavanine by arginase or inactivation of the reagent by reducing agents. Individual tests in presence of the chief urinary solutes showed that creatinine strongly inhibits the PCAF reactions with guanidinoxy compounds. This inhibition can be overcome by addition of an excess of the reagent, which indicates that the creatinine inhibition effect may arise from competition with the canavanine.

**SUMMARY**

1. Methods are described for the detection and estimation of guanidinoxy compounds, including canavanine, in biological material.
2. Canavanine has been isolated from the seeds of the bladder senna, *Colutea arborescens*.

3. A guanidinoxy compound, similar in its colour reactions to canavanine, has been detected in the seeds of *Medicago arborea*, *M. echinus*, and *Ornithopus perpusillus*.

**REFERENCES**


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**The Influence of Previous Diet on the Fasting Blood-Sugar Level and on Glucose Utilization in the Rat and Hamster**

**By R. J. Garner and R. Roberts**

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(Received 11 August 1954)

Observations have been made from time to time on the effect of previous diet upon carbohydrate metabolism in animals. Samuels, Gilmore & Reinecke (1948) found that, after short periods of fasting, rats on a high-fat diet had lower blood-sugar levels than those on a high-carbohydrate diet. A reduced tolerance in man to orally or intravenously administered glucose (Sweeney, 1927; Himsworth, 1933) and depressed glucose utilization by isolated rat diaphragm (Lundbaek & Stevenson, 1948; Gilmore & Samuels, 1949; Hansen, Rutter & Samuels, 1951) has also been observed as a result of feeding a diet high in fat and low in carbohydrate. In these respects, fat-fed non-ruminant animals show a striking resemblance to the adult ruminant (Reid, 1950a, b; 1952).

The purpose of the present work was to confirm these findings in rats and hamsters with a view to reproducing the conditions obtaining in the adult ruminant in non-ruminant animals by suitable modification of the diet. The effect of feeding various diets upon the fasting blood-sugar level, upon glucose tolerance, and upon glucose utilization and glycogen deposition in isolated diaphragm has therefore been examined. Studies on the hexokinase activity of various tissues were included in the hope that they would throw light upon the mechanisms involved.

While the work reported here was in progress, Long (1953) published his results on the hexokinase activity of tissues from rats fed from weaning on a variety of diets. Using homogenates of intestinal mucosa, highest activity was found in animals fed a high-carbohydrate, fat-free diet and lowest in those fed a high-fat, carbohydrate-free ration. As recorded below, similar results have been obtained using homogenates of rat and hamster kidney.
EXPERIMENTAL

Animals and diets

Hooded rats and hamsters were used. They were introduced to the synthetic diets (Table 1) immediately after weaning (at 22 days old). The mean weights of the rats after 13 weeks on the high-carbohydrate, high-fat and high-protein rations were 164, 133 and 136 g., respectively.

Experiments with isolated diaphragm

Animals which had been maintained on the appropriate diet for 3 months were fasted for 48 hr. (this rather lengthy period of starvation being found necessary to obtain approximately the same initial glycogen content in each diaphragm) and killed by decapitation and exsanguination. The diaphragm was immediately removed and placed in ice-cold Krebs phosphate-Ringer solution. Warburg flasks to receive the hemidiaphragm contained, in the main compartment, 2-5 ml. phosphate-Ringer with glucose (80 mg./100 ml.), and 0-2 ml. 10% (w/v) KOH in the centre well. The diaphragm was divided along the mid-line, and the two portions placed in separate Warburg vessels. The flasks and attached manometers were gassed for 15 min. with O2 and then placed in the water bath at 38° for 10 min. to equilibrate. At the end of this period one-half of each diaphragm was removed for determination of the initial glycogen content. The other half of the diaphragm was incubated for a further 2 hr., the flasks being shaken at approximately 100 oscillations/min. The hemidiaphragm was then removed for glycogen determination. The initial and final glucose concentrations of the medium were determined in 0-5 ml. of fluid.

Determination of hexokinase activity

The kidneys and brain from the animals used for the isolated diaphragm experiments were removed as rapidly as possible and immersed in ice-cold water. The tissues were then homogenized with phosphate-fluoride buffer in a glass-Perspex homogenizer of the Potter & Elvehjem (1936) type; the technique employed was essentially that described by Long (1952). The incubation fluid contained the following components (final concentrations in brackets): glucose (0-0012 m), potassium adenosine triphosphate (ATP) (0-005 m), KF (0-05 m), potassium phosphate buffer, pH 7-8 (0-04 m), MgCl2 (0-005 m) and KCl (0-042 m). The amount of glucose disappearing in 5 min. (brain) or 10 min. (kidney) at 30° in air was determined by the Harding-Nelson method (King & Garner, 1947). Hexokinase activity was estimated by the difference in glucose utilization in corresponding preparations with and without ATP.

Determination of glucose and glycogen

Blood glucose was determined by the titrimetric method described by King (1951) on a protein-free filtrate prepared according to Somogyi (1945). Blood for glucose determination was obtained by heart puncture under Nembutal (sodium pentobarbitone) anaesthesia, precipitation of proteins being carried out within 1–2 min. of obtaining the blood sample. Glycogen determinations were made by the method of Good, Kramer & Somogyi (1933).

RESULTS

Effect of previous diet on fasting blood-sugar levels

In both rats and hamsters, the blood-sugar level of fat-fed animals is lower after 17 hr. fasting than that of corresponding controls receiving the diet containing 56% carbohydrate (Table 2A). The differences recorded in rats are statistically significant at both 7 and 10 weeks (P < 0.001). As is also shown in this table, the blood sugar of the fat-fed animals can be restored to the level of that of the controls by reversing the diets.

Blood samples were also taken from three groups of thirteen rats before killing for removal of diaphragm and kidneys. These animals had been maintained on high-carbohydrate, high-fat and high-protein diets (nos. 3–5, Table 1) for 3 months and the mean figures for blood glucose were 85, 89 and 99 mg./100 ml., respectively. The results previously obtained are apparently completely reversed. However, these rats had been starved for 48 hr. as compared with 17 hr. for the first set. Since Samuels et al. (1948) had found that rats receiving a high-fat diet exhibited lower blood-sugar levels than those on a high-carbohydrate diet only for the first 6 hr. of fasting, the position being reversed after 12–16 hr., it seemed likely that the lengthier period of fasting was responsible for the discrepancy. This was confirmed by taking blood samples after 17, 24 and 41 hr. fasting (Table 2B).

Table 1. Composition of diets

One part of monotype punchings was added to 19 parts of diet. The salt mixture was prepared according to Osborne & Mendel (1913).

<table>
<thead>
<tr>
<th>Diet (g./100 g.)</th>
<th>No. 1</th>
<th>No. 2</th>
<th>No. 3</th>
<th>No. 4</th>
<th>No. 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>carbohydrate</td>
<td>5-7</td>
<td>32</td>
<td>9</td>
<td>56</td>
<td>9</td>
</tr>
<tr>
<td>fat</td>
<td>4-3</td>
<td>24</td>
<td>23</td>
<td>23</td>
<td>70</td>
</tr>
<tr>
<td>Hydrogenated palm oil</td>
<td>—</td>
<td>—</td>
<td>9</td>
<td>56</td>
<td>9</td>
</tr>
<tr>
<td>Starch</td>
<td>56</td>
<td>10</td>
<td>57</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Casein</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>70</td>
</tr>
<tr>
<td>Marmite</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Cod-liver oil</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>NaCl</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Bioch. 1955, 59
Table 2. Effect of feeding high-carbohydrate and high-fat diets from weaning on fasting blood-sugar levels of rats and hamsters

Figures given are mean ± s.d.

A. Effect of diet on changes in fasting blood-sugar level with age

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. of animals</th>
<th>Blood glucose (mg./100 ml.) at (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 1 (high carbohydrate)</td>
<td>11</td>
<td>70±8.4</td>
</tr>
<tr>
<td>No. 2 (high fat)</td>
<td>11</td>
<td>70±10.9</td>
</tr>
<tr>
<td>Diets interchanged at 7 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 1 (high carbohydrate)</td>
<td>6</td>
<td>66±5.8</td>
</tr>
<tr>
<td>No. 2 (high fat)</td>
<td>6</td>
<td>58±5.2</td>
</tr>
<tr>
<td>Hamsters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 3 (high carbohydrate)</td>
<td>5</td>
<td>87±2.4</td>
</tr>
<tr>
<td>No. 4 (high fat)</td>
<td>6</td>
<td>85±1.8</td>
</tr>
</tbody>
</table>

B. Effect of diet on changes in blood-sugar level of 13 week-old rats with varying periods of fasting

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. of animals</th>
<th>Blood glucose (mg./100 ml.) after fasting (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>No. 3 (high carbohydrate)</td>
<td>5</td>
<td>84±9.1</td>
</tr>
<tr>
<td>No. 4 (high fat)</td>
<td>6</td>
<td>74±11.4</td>
</tr>
</tbody>
</table>

25% (w/v) glucose solution was injected at the rate of 0.3 g./kg. body weight. Further blood samples were taken from the heart over a period of 2.5 hr. The results are recorded in Fig. 1. This procedure was invariably fatal, one animal dying during the course of the test and the remaining three a few hours after the test had been completed, presumably due to repeated damage to the heart.

Effect of previous diet on glucose utilization and glycogen synthesis in isolated rat diaphragm

In a preliminary experiment using three rats from each of the groups on diets nos. 1 and 2 (high-carbohydrate and high-fat, respectively) it was confirmed that fat feeding depresses both glucose utilization and glycogen synthesis (Table 3). In order to determine whether the effect was due to the high fat or low carbohydrate content, a further experiment was carried out including an additional group of rats on a diet containing 70% protein (diet no. 5, Table 1). The results are summarized in Table 3.

Effect of previous diet on hexokinase activity of brain and kidney homogenates

Two groups of rats and one group of hamsters, all of which had been maintained on the appropriate diets for 3 months were used. The animals were fasted for 48 hr. and duplicate (later triplicate) estimations on kidney and, in some cases, brain activity of one animal on each diet were carried out simultaneously. Table 4 lists the mean results for all groups.

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**Fig. 1.** Effect of previous diet on tolerance to intravenous glucose (0.3 g./kg.) in rats; ●, high-carbohydrate diet; ○, high-fat diet.

**Effect of previous diet on glucose tolerance in rats**

Four 4-month-old rats (two on high-carbohydrate diet no. 1, two on high-fat diet no. 2) which had been fasted for 17 hr. were used. Under Nembutal anaesthesia a blood sample was taken by heart puncture and then, without removing the needle,
The effect of varying the blood-sugar level of rats on high-carbohydrate and high-fat diets is similar to, but not identical with, that described by Samuels et al. (1948). From an inspection of Table 2B it is apparent that similar blood-sugar values would be reached in the two groups of rats after approximately 20 hr. fasting, as opposed to 6–12 hr. as found by Samuels et al. As mentioned above, it was found necessary to starve the rats used in the present work for 48 hr. in order to reduce the initial glycogen content of the diaphragm to similar levels in all animals. It was also found that up to 72 hr. fasting was required to deplete the liver glycogen of similar rats on a stock diet. This is considerably longer than the 24 hr. usually considered necessary, and may reflect some peculiarity of the particular strain used. As it is during the period of glycogen depletion that the blood sugar falls, the discrepancy between the results reported here and those of Samuels et al. may be due simply to strain differences.

The fall in blood sugar with age seen in hamsters is of considerable interest as it is believed that this phenomenon is otherwise confined to ruminants and related species. The plasma glucose concentration of the fat-fed animals at 10 weeks appears to be of the same order as that recorded for the adult ruminant (Somogyi, 1933).

All workers agree that glucose utilization is depressed by high-fat, low-carbohydrate diets and this reduced utilization is clearly demonstrated by the intravenous glucose-tolerance tests (Fig. 1) and less clearly by the isolated diaphragm experiments. It may be argued that the reduced tolerance to glucose in the fat-fed rats reflects a terminal rise in blood sugar, but no similar rise occurred in the controls, which also died a few hours after the test had been completed.

Glycogen synthesis by rat diaphragm has been studied by several workers but the results obtained have been equivocal. According to Lundbaek & Stevenson (1948) the previous diet has no effect on the amount of glycogen synthesized, which suggests that a high-fat diet exerts a sparing action on glucose oxidation. However, Hansen et al. (1951) report that less glycogen is formed on a high-fat diet, although again their results indicate a sparing action. In the present work glycogen synthesis has been found to be profoundly depressed by both high-fat and high-protein diets, indicating that it is carbohydrate deprivation which is the operative factor. Some of these discrepancies are capable of an explanation. Hansen et al. believe that the initial glycogen content of the diaphragm determines to some extent whether glycogen synthesis or breakdown shall occur. In the light of observations made during the course of the work presented here it is considered that the glucose level of the blood may also be a determining factor.

Depressed glucose utilization suggests depressed hexokinase activity. The negative findings in the first series of rats used (Table 4) are difficult to explain in view of the positive results obtained with kidney preparations from the second series of rats and from hamsters. The reduction in activity has since been confirmed in a different strain of rats.
during the course of further work to be reported at a later date. It is certain that there are differences in the effect of diet on different tissues as Long (1953) has found that, although the hexokinase activity of intestinal mucosa is depressed by a low-carbohydrate diet, that of intestinal muscle is unaffected.

In so far as attempts to reproduce the anomalies of carbohydrate metabolism observed in the adult ruminant are concerned, these have been successful and it may be concluded from the similarities between the adult ruminant and the fat-fed non-ruminant that many of the apparent abnormalities in the former are due to the peculiar digestive processes of the ruminant rather than to specific hormonal or enzymic differences.

SUMMARY

1. After 17 hr. fasting, blood-sugar levels from rats and hamsters fed on a high-fat diet from weaning are consistently lower than those from similar animals on a high-carbohydrate diet.
2. The reduced tolerance to intravenously injected glucose induced by a high-fat diet has been confirmed using rats.
3. Restriction of the carbohydrate intake by feeding excess fat or protein depresses both glucose uptake and glycogen synthesis by isolated rat diaphragm.
4. The hexokinase activity of kidney homogenates from rats and hamsters is depressed by high-fat diets.

We are indebted to Messrs J. Bibby and Sons Ltd. for supplies of arachis oil and hydrogenated palm oil, and to Messrs C. Tinling and Co. Ltd. for monotype punchings. Our thanks are also due to Professor R. A. Morton, F.R.S., for his constant interest, advice and encouragement. This work was supported by a Research grant from the Agricultural Research Council.

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Evidence for the Occurrence of γ-Methylene-α-oxoglutaric Acid in Groundnut Plants (Arachis hypogaea)

BY L. FOWDEN AND J. A. WEBB
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(Received 12 August 1954)

The isolation of two new amino acids, γ-methylenglutamic acid and γ-methyleneglutamine, from groundnut plants (Arachis hypogaea) was described by Done & Fowden (1951, 1952). More recently these two compounds have been isolated from tulips (Tulipa gesneriana) by Zacharius, Pollard & Steward (1954), and their presence has been indicated in hops (Humulus lupulus) by Harris & Tatchell (1953). Although these compounds have a limited distribution within the plant kingdom, there are indications that they are of considerable importance in the metabolism of those species in which they occur. γ-Methylenglutamic acid can, under the influence of the transaminase systems present in groundnut plants, transfer its α-amino group to α-oxoglutaric, oxalacetic or pyruvic acids (Fowden & Done, 1953a), whilst γ-methyleneglutamine appears to be important in the transport of nitrogen within this species (Fowden, 1954).

α-Oxo acids have been the subject of several detailed investigations, and α-oxoglutaric, oxalacetic, pyruvic and glyoxylic acids have, by characterization as their 2,4-dinitrophenylhydrazine (DNPH) derivatives, been found to be widely