IN VITRO UTILIZATION OF UNIFORMLY LABELLED $^{14}$C-GLUCOSE IN THE ADRENALS OF NORMAL AND OBESE-HYPERGLYCAEMIC MICE.

By

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ABSTRACT

The in vitro incorporation of uniformly labelled $^{14}$C-glucose in the adrenals of normal and obese-hyperglycaemic animals was studied by quantitative paper-radiochromatography. The total glucose utilization by the whole adrenal gland was found to be greater in the obese-hyperglycaemic animals. There was a lower production of $^{14}$CO$_2$ and $^{14}$C-lactic acid, however, per unit adrenal wet weight in these mice, while the formation of amino acids from the glucose tended to be greater than in normal mice. Glucose was utilized in the synthesis of the following amino acids in the adrenals: proline, alanine, aspartic acid, glutamic acid, glutamine and arginine. The formation of relatively large amounts of proline was characteristic of the amino acid pattern in the adrenals.

In recent years detailed studies have been made of the utilization of glucose in different endocrine organs. It has been shown that the pituitary gland (Beloff-Chain et al. 1959; Andersson et al. 1961), the pineal gland (Hellman & Larsson 1961 a) and the pancreatic islet tissue (Hellman & Larsson 1961 b) all exhibit a characteristic pattern with regard to those amino acids, which are formed in vitro from the carbon atoms in the glucose molecule. In the present investigation these studies have been extended to the adrenals of mice both with a normal blood sugar level (AN-mice) and with congenital hyperglycaemia (AO-mice).

Previous investigations have shown that the volume of the adrenal cortex
in adult male AO-mice is, on an average, double that in AN-mice of the same age (Hellerström et al., unpublished). From in vitro studies of the biosynthesis of steroids during ACTH stimulation, it was also found that the total quantity of corticosterone produced per gland was much greater in the AO- than in the AN-mice (Carstensen et al. 1961).

MATERIAL AND METHODS

Altogether 18 male mice, 6–8 months old, were used for the investigation. They were given free access to food. Six mice were obese-hyperglycaemic (AO-mice) of the American variety from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine — while the other 12 were normoglycaemic litter mates of ordinary weight (AN-mice). The animals were killed by cervical dislocation, after which the adrenals were quickly dissected out, weighed and transferred to ice-cold Warburg vessels (volume approximately 4 ml). In this way each of the four Warburg vessels used, contained either 6 adrenals from AO-mice or 12 adrenals from AN-mice (the total weight of adrenal in each vessel being approximately the same).

The adrenals were incubated in a phosphate buffer, the composition of which was the same as previously described by Andersson et al. (1961). Uniformly labelled 14C-glucose (Radiochemical Centre, Amersham, England) was diluted with non-radioactive carrier to give a specific activity of 20 μc/mg. The concentration of glucose in the incubation medium was 0.1%, with a total radioactivity of 10 μc per vessel (= 0.5 ml solution). The centre wells of the vessels contained rolls of filter paper soaked with 30% NaOH to absorb the CO2 evolved from the tissue. All incubations were done in an atmosphere of O2 at 37°C for two hours. The 14CO2 was determined as described previously (Andersson et al. 1961; Hellman & Larsson 1961 b).

After incubation the tissues were homogenized and extracted, and the radioactive metabolites separated by paper chromatography. The solvents for the chromatography, as well as the other details in this procedure were the same as used by Chain et al. (1960). As in previous experiments the chromatograms were scanned quantitatively by a modification of the automatic device designed by Frank et al. (1959). The insoluble residues after the alcohol extraction were hydrolyzed with 6 N HCl in sealed tubes for 4 hours, after which the hydrolysate was dried over KOH, then repeatedly suspended in water, filtered, washed and dried again. The hydrolysate was then made up to volume and transferred to paper for chromatography.

RESULTS

The amount of glucose in the medium, which was converted per 25 mg, adrenal tissue (wet weight) during a period of 2 hours, is given in μg in Table 1. As regards both CO2 and lactic acid, lower values were noted in the AO- than in the AN-animals. While the mean values for CO2 in the case of the AN-mice thus corresponded to 19.0 μg glucose, the CO2 production in the AO-mice was 27% lower. For lactic acid the mean value of the glucose equivalent in the AN-mice was 77.6 μg, while the value was no less than 34% smaller for the AO-mice. The lower rate of glucose utilization in lactic acid
Table 1.
Utilization of glucose in the adrenals of normal (AN) and obese-hyperglycaemic (AO) mice. The results are expressed as μg glucose converted per 25 mg tissue (wet weight) after 2 hours' incubation with O₂ at 37°C. Glucose concentration 0.1 per cent. Total radioactivity 10 μc per vessel (= 500 μg).

<table>
<thead>
<tr>
<th>Animal</th>
<th>Number of adrenals</th>
<th>CO₂</th>
<th>Lactic acid</th>
<th>Alanine</th>
<th>Aspartic acid</th>
<th>Glutamic acid</th>
<th>Glutamine</th>
<th>Proline</th>
<th>Arginine</th>
<th>Free glucose in the extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN</td>
<td>12</td>
<td>20.8</td>
<td>79.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.7</td>
<td>0.8</td>
<td>0.4</td>
<td>7.0</td>
</tr>
<tr>
<td>AN</td>
<td>12</td>
<td>17.2</td>
<td>76.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.5</td>
<td>0.8</td>
<td>0.3</td>
<td>5.2</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>19.0</td>
<td>77.6</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.6</td>
<td>0.8</td>
<td>0.4</td>
<td>6.1</td>
</tr>
<tr>
<td>AO</td>
<td>6</td>
<td>13.5</td>
<td>43.8</td>
<td>0.9</td>
<td>0.6</td>
<td>0.5</td>
<td>0.5</td>
<td>1.4</td>
<td>0.6</td>
<td>6.7</td>
</tr>
<tr>
<td>AO</td>
<td>6</td>
<td>14.0</td>
<td>59.0</td>
<td>0.3</td>
<td>0.2</td>
<td>0.4</td>
<td>0.5</td>
<td>0.9</td>
<td>0.3</td>
<td>6.3</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>13.8</td>
<td>51.4</td>
<td>0.6</td>
<td>0.4</td>
<td>0.5</td>
<td>0.5</td>
<td>1.2</td>
<td>0.5</td>
<td>6.5</td>
</tr>
</tbody>
</table>
and CO₂ formation per unit adrenal wet weight in the AO-mice had no counterpart in the case of the amino acids, where on the contrary the values tended to be higher than in the AN-mice. If the values are expressed instead as that amount of glucose, which was converted per whole adrenal, the values in the AO-mice become completely predominant. The average adrenal weight in the AN-mice was thus only 1.8 mg, as compared with 3.5 mg in the AO-mice (cf. Figs. 1 and 2).

In both types of mice, glucose contributed to the formation of proline, alanine, aspartic acid, glutamic acid, glutamine and arginine. Proline was formed in the greatest quantities. The amount of glucose converted to proline during a period of 2 hours by 25 mg adrenal tissue (wet weight), thus corresponded to 0.8 µg for the AN-mice and to 1.2 µg for the AO-mice.

On the other hand, glucose incorporation in the protein-bound amino acids was insignificant. No radioactivity was found, apart from traces in the glutamic acid and proline, with chromatography after hydrolysis of the insoluble residues.

**DISCUSSION**

In recent years it has become more apparent that the adrenals, like the other endocrine organs, can metabolise glucose via the hexosemonophosphate path-
way. Glock & MacLean (1954) have thus demonstrated the presence of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in this organ. Field et al. (1960) indirectly pointed out the importance of this pathway in the adrenal cortex, when they found, in vitro, that the ratio between $^{14}$CO$_2$ obtained from glucose-1-$^{14}$C, to $^{14}$CO$_2$ from glucose-6-$^{14}$C, was greater than unity. The significance of the hexosemonophosphate pathway for the steroidogenesis in the adrenals has also been emphasized by Haynes et al. (1959).

In the present investigation, attention has been paid to that part of the adrenal metabolism which is concerned with the utilization of glucose in the synthesis of amino acids. Since similar investigations have been carried out previously on other endocrine organs, such as the pituitary gland (Beloff-Chain et al. 1959; Andersson et al. 1961), the pineal gland (Hellman & Larsson 1961 a) and the pancreatic islet tissue (Hellman & Larsson 1961 b), it is relevant to discuss the observations made here in the light of these experiments. It should be mentioned, that glucose utilization in adrenal amino acid production showed considerable similarities with that found in both the anterior pituitary gland and islet tissue. Glucose contributes to γ-aminobutyric acid formation in both the posterior pituitary gland and pineal body, but no $^{14}$C-γ-aminobutyric acid was found in the adrenals. The most characteristic feature of adrenal glucose utilization was the relatively intensive synthesis of proline. While this amino acid was formed in the greatest amounts from glucose in the adrenals, only a trace of radioactivity was found in proline in the anterior pituitary gland (Beloff-Chain et al. 1959).

No differences could be demonstrated in the amino acid pattern between the adrenals of the AN- and the AO-mice. The fact that the total glucose utilization was considerably higher in the AO-mice can be explained by the greater adrenal weight in these animals. It was of interest, however, that when the values were expressed per unit adrenal wet weight, the glucose incorporation into CO$_2$ and lactic acid was considerably lower in the AO-mice, despite the fact that the total amino acid production appeared to be greater than in the AN-mice.

ACKNOWLEDGEMENT

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