Observations on the Respiratory Metabolism of Rat-Adrenal Cortex

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Study of the respiratory and carbohydrate metabolism of adrenal-cortex tissue may be expected to yield information of interest in connexion with the growth and secretory function of the gland. Indications of the quantitative changes in oxidative activities which may take place under the influence of regulatory hormones have already been given. Carpenter, Macleod & Reiss (1946) showed that the oxygen uptake by rat-adrenal cortices *in vitro* in Ringer-glucose solution was higher with animals treated with adrenocorticotrophic hormone than with normal animals. Sourkes & Heneage (1951) found that homogenates of whole adrenals from cortisone-treated rats showed decreased rates of endogenous respiration, and decreased ability to oxidize various intermediates of the citric acid cycle.

This investigation was begun in order to obtain information on the respiration of rat-adrenal-cortex tissue in the presence of various substrates. Some observations on the glycolytic activities of the tissue were also made.

METHODS

Preparation of tissue. Female rats of about 200–250 g. body wt. were used exclusively. They were killed by decapitation, the carcasses were exsanguinated and the adrenals were rapidly removed, cleaned from fat, opened laterally with fine scissors and carefully bisected. The medullae could then be dissected out, and though a part of the zona reticularis is unavoidably lost, these demedullated bisected adrenals may be regarded as intact adrenal-cortex tissue. According to Carpenter *et al.* (1946) cortices prepared in this way satisfy the conditions required for diffusion in manometric experiments. The tissue was washed once by placing it in ice-cold Ringer solution, then blotted and transferred to the Warburg vessels.

Medium. The modified Ringer solution of Krebs & Henseleit (1932) was used, containing as buffer either 0.05 M-phosphate (pH 7.4) or 0.027 M-bicarbonate.

Measurements of oxygen uptake. These were carried out by the Warburg manometric technique at 37°. The tissue was allowed to respire in Krebs-Ringer-phosphate solution with the appropriate substrate in an atmosphere of O₂, CO₂ being absorbed by KOH soln. on filter paper in the central compartment of the vessel. In some preliminary experiments the bisected cortices were mixed indiscriminately, but in all the later experiments it was found advantageous to distri-

bute the halves from a number of adrenals between a pair of Warburg vessels in such a way that the half of an adrenal in one vessel was matched by the corresponding other half in the second vessel. Portions of tissue distributed between a pair of vessels in this way are referred to as 'paired bisects'. Six bisects per vessel were normally used, and measurements of uptake of O₂ were carried out for 3 hr. The tissue in each vessel was then removed, blotted with filter paper and dried at 100°.

Glycolysis. This was measured at 37° in Krebs-Ringer-bicarbonate solution in an atmosphere of CO₂ + N₂ (5:95) or CO₂ + O₂ (5:95). In the anaerobic experiments yellow phosphorus was placed in the central compartment of the vessel. Measurements were made at 37° for 2 hr. The pieces of tissue were then removed for determination of dry wt., and the remaining liquid was centrifuged. Lactic acid was estimated on 1 or 2 ml. samples by the method of Barker & Summerson (1941).

Respiratory quotient. This was determined at 37° both by Warburg's (1914) direct method (correction being made for retention of CO₂) and by the method of Dickens & Šimer (1930*a*), with incubation periods of 2 and 3 hr. for each method.

Materials. D-Glucosamine hydrochloride and hexose diphosphate were obtained commercially. The dicalcium salt of the latter was converted into the sodium salt before use.

RESULTS

Endogenous respiration

In the first experiments, each vessel contained six bisects (total dry wt. 20–30 mg.) which were mixed indiscriminately. Later, the use of correctly paired bisects was tried and was found to give more consistent duplicates. This method was therefore used in all the subsequent work. Representative results of a series of experiments showing the variations in endogenous respiration between sets of paired bisects, with different numbers of bisects, are given in Table 1.

The values for Q_{O_2} measured with different amounts of tissue are reasonably close, and this perhaps offers some support for the view of Carpenter *et al.* (1946) that the demedullated bisects of rat adrenals are suitable as a form of tissue with which to study the respiratory metabolism of surviving adrenal cortex.

Effects of various substances on the respiration

Each substrate was added to a set of paired bisects, with the corresponding set of paired bisects acting as the control. The results are shown in Table 2.

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Table 1. *Endogenous respiration of paired rat-adrenal cortices in Krebs-Ringer-phosphate medium (pH 7.4) in oxygen at 37°*

No. of bisects/vessel	Total dry wt. of tissue (mg.)		-Q _{O₂}					
			1st hr.		2nd hr.		3rd hr.	
	1st halves	2nd halves	1st halves	2nd halves	1st halves	2nd halves	1st halves	2nd halves
8	30.9	24.6	3.1	3.4	2.1	2.3	1.03	1.3
8	22.9	27.6	3.2	3.3	1.6	1.8	0.8	0.9
6	17.6	18.3	3.5	3.4	1.5	1.7	0.6	0.7
4	14.4	11.2	3.8	3.5	2.0	2.3	0.9	0.9
2	11.3	10.2	2.2	2.2	0.9	0.6	0.3	0.5

Table 2. *Influences of various substrates on the respiration of paired rat-adrenal cortices in Krebs-Ringer-phosphate medium (pH 7.4) in oxygen at 37°*

Substrate and concn. (M)	-Q _{O₂}					
	1st hr.		2nd hr.		3rd hr.	
	Without substrate	With substrate	Without substrate	With substrate	Without substrate	With substrate
Glucose, 0.01	2.9	4.1	1.3	4.0	0.6	3.7
Fructose, 0.01	2.5	3.0	1.3	1.7	0.5	1.0
Galactose, 0.01	2.4	3.1	1.6	2.4	0.7	1.5
Lactose, 0.01	2.8	3.2	1.6	2.0	0.6	1.0
Glycogen, 0.1 %	3.2	2.8	1.5	2.4	1.1	1.5
D-Glucosamine, 0.02	2.7	5.0	1.3	4.1	0.7	3.0
Sodium lactate, 0.02	2.7	5.1	2.4	4.9	1.1	4.1
Sodium succinate, 0.02	4.5	12.1	3.4	7.4	2.6	7.8
Sodium pyruvate, 0.02	3.8	4.8	2.2	3.5	1.0	2.6
Sodium α-oxoglutarate, 0.02	3.4	4.9	1.8	2.8	0.7	1.8
Sodium α-glycerophosphate, 0.01	2.5	3.4	1.7	1.7	0.9	0.9
Sodium glutamate, 0.01	3.5	3.9	1.8	2.5	1.0	1.6

Of the carbohydrates tried, glucose was by far the most effective in enhancing respiration. The respiration without substrate showed the usual steady decline over the 3 hr. period, but in the presence of glucose it remained practically constant during that time, the effect being particularly marked during the third hour. Fructose, galactose and lactose produced much lower effects. Glycogen produced a positive effect in the second hour only, and this is of doubtful significance. The respiration rate in the presence of glucose is comparable with the rates quoted by Carpenter *et al.* (1946), i.e. $-Q_{O_2}$ 2.82–4.18 for normal rats. There was a considerably increased uptake of O_2 in the presence of D-glucosamine. Of the non-carbohydrates, succinate produced the greatest effect; pyruvate, α-oxoglutarate and lactate also increased the uptake of O_2 markedly. α-Glycerophosphate produced a positive effect in the first hour, and glutamate caused a definite, though small, increase, in agreement with the observation of Sourkes & Heneage (1951).

The following substances, at the molar concentrations indicated, increased the rates of tissue respiration by less than 10 % over the first and second hours, and by less than 20 % in the third hour: sucrose (0.01), hexose diphosphate (0.02), the

sodium salts of malic, citric, acetic, butyric, oleic and gluconic acids (0.02), alanine (0.02), choline (0.03), glycerol (0.01), ethanol (0.02) and cholesterol (saturated). The effects of these substances are accordingly regarded as insignificant. The results for citrate, malate, acetate and butyrate are at variance with those of Sourkes & Heneage (1952), who found that all of these substances were oxidized by ox-adrenal slices, and that citrate and malate were oxidized by homogenates of rat and ox adrenals.

Aerobic and anaerobic glycolysis

In view of the high rate of oxidative catabolism of glucose, it was of interest also to investigate aerobic and anaerobic lactic acid formation by the paired-bisect technique. Results are shown in Table 3. (Figures for Q_{CO_2} for the aerobic experiments are not included because the differences in manometer-level readings were so small that the calculations for CO_2 output cannot be regarded as accurate.) Close agreement was obtained between sets of paired bisects for endogenous-acid formation both in terms of CO_2 output and of lactic acid. In the presence of glucose or hexose diphosphate, the anaerobic output of CO_2 was markedly increased, and the figures thus obtained for $Q_{CO_2}^N$ are similar to those reported by Dickens & Šimer (1930b) for liver and

Table 3. *Aerobic and anaerobic acid production by paired rat-adrenal cortices in Krebs-Ringer-bicarbonate medium*Tissues were respiring in either $\text{CO}_2 + \text{O}_2$ or $\text{CO}_2 + \text{N}_2$ at 37° .

Conditions	Substrate	$Q_{\text{CO}_2}^{\text{N}_2}$				Lactic acid ($\mu\text{g./mg. dry wt./2 hr.}$)	
		1st hr.		2nd hr.		Without substrate	With substrate
		Without substrate	With substrate	Without substrate	With substrate		
Anaerobic	Glucose, 0.01 M	2.1	3.7	0.9	2.3	4.1	14.2
Anaerobic	Hexose diphosphate, 0.01 M	1.2	3.6	1.2	4.6	2.6	13.6
Anaerobic	Glycogen, 0.2 %	2.1	1.3	1.1	1.2	1.7	2.4
Aerobic	Glucose, 0.01 M	—	—	—	—	1.9	6.2

Table 4. *Respiratory quotients of paired rat-adrenal cortices at 37°*

Method	Incubation period (hr.)	Respiratory quotients	
		Without glucose	With glucose (0.01 M)
Warburg direct	2	0.77	0.74
Warburg direct	3	0.73	0.76
Dickens & Šimer (1930a)	2	0.68	0.84
Dickens & Šimer (1930a)	3	0.70	0.71

Table 5. *Comparison between endogenous respiration rates of intact adrenal cortices and homogenized adrenal cortices*Tissues were respiring in Krebs-Ringer-phosphate medium, pH 7.4, in O_2 at 37° .

	— $\mu\text{l. of O}_2/\text{mg. wet wt.}$	
	Intact cortices	Homogenized cortices
1st hr.	0.44	0.06
2nd hr.	0.40	0.03
3rd hr.	0.29	0.02

kidney. Hexose diphosphate seemed to be slightly though not significantly more active than glucose in stimulating CO_2 output, but the figures for estimated lactic acid were not appreciably different. With glycogen as substrate, neither the CO_2 nor lactic acid content was significantly increased. Lactic acid formation under aerobic conditions in the presence of glucose was comparable with that reported by Carpenter *et al.* (1946), and it appears to be somewhat high in relation to the results obtained under anaerobic conditions.

Respiratory quotient

The relatively high rates of endogenous respiration and the low rates of anaerobic glycolysis in the presence of glucose made it of interest to obtain further information regarding the importance of glucose as an oxidizable substrate, and measurements of the respiratory quotients, in the presence and absence of glucose, were undertaken. The results are shown in Table 4. The quotients, both with and without glucose, were well below unity in every case, and in most cases had values which may be regarded as consistent with a fat metabolism.

Comparison between endogenous respiration of bisects and homogenates

The rates of endogenous respiration for the first hour were distinctly higher than those previously reported by Sourkes & Heneage (1952). These workers found Q_{O_2} values of -1.1 (on a calculated dry wt. basis) for rat-adrenal homogenates and of -2.0 for ox-adrenal-cortex slices. It seemed probable that the respiratory activity of the rat tissue, without addition of substrates, was largely lost on homogenizing. A direct comparison was therefore made between the endogenous respiration of bisects and homogenates. One set of paired bisects was used intact, after weighing on a torsion balance. The other set was also weighed wet, then homogenized in the minimum volume of Krebs-Ringer-phosphate medium and finally transferred, with washing, to a Warburg vessel, and made up to the appropriate volume. Each vessel was gassed with O_2 , and the respiration measured as usual. Typical results are shown in Table 5. It is evident that homogenization much decreases the ability of the tissue to oxidize its endogenous substrates, the rate of endogenous respiration being reduced to less than 10 % of the value for intact tissue.

Depletion of endogenous substrates

The presence of appreciable amounts of endogenous oxidizable material may mask the effects produced by added substrates; it is possible that the differences between the present results and those of Sourkes & Heneage (1952) might be due to this. It was decided to determine whether fresh, intact tissue could be depleted of its endogenous substrates by pretreatment with O_2 at 37° . This technique was

found to be of value in the study of the oxidative metabolism of the mammary gland (Turner, 1952). One set of paired bisects was treated with O_2 for 1 hr. at 37° as described by Turner, while the other set was kept in ice-cold Krebs-Ringer solution. The two sets of bisects were then transferred to Warburg vessels containing the buffered medium, the vessels were gassed with O_2 , and the respiration rates measured. The pretreatment with O_2 resulted in a substantial fall in endogenous respiration to about 50 % in the first hour and 37 % in the second hour. In a second experiment, $-Q_{O_2}$ values for pretreated tissue were 1.55 (first hour) and 0.7 (second hour), and these increased to 2.4 (first hour) and 2.15 (second hour) in the presence of 0.01 M-glucose, indicating that the depleted tissue is still able to oxidize glucose. It is probable that the depletion technique could be used with advantage in studying the oxidative metabolism of this tissue.

DISCUSSION

Of the carbohydrates studied, glucose is evidently the most readily utilized. Though there is some evidence that fructose, galactose and lactose may be oxidized, their rates of oxidation are in any case considerably lower than that for glucose. Apart from oxidative catabolism, glucose appears to be utilizable by an anaerobic glycolytic mechanism, presumably through a series of reactions on the lines of the Embden-Meyerhof scheme, since lactic acid is formed from hexose diphosphate to approximately the same extent as from glucose. There is little or no glycolytic activity with glycogen as substrate; the relative inefficiency of glycogen, compared with glucose, in promoting glycolysis in adrenal-cortex tissue has already been observed by Haarmann (1932). The glycolytic rates in the presence of glucose, however, are not high, and it seems likely that the anaerobic catabolism of glucose is of minor importance. It is of interest in this connexion that Glock & McLean (1954) found that glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities are very high in adrenal-cortex tissue. Kelly, Nielson, Johnson & Vestling (1955) have deduced from this, and from other evidence, that the direct oxidative pathway of glucose catabolism is of particular importance in the adrenal cortex.

These observations have a bearing on the energy requirements of the gland in connexion with steroid synthesis. As far as is known, the adrenal cortex does not store glycogen and would depend, in large measure, on the rapid oxidation of circulating glucose in the blood. The high rates of oxidation of some of the intermediates of carbohydrate metabolism found in the present investigation, and found by Sourkes & Heneage (1951, 1952), are in accordance with this. A possible connexion

between carbohydrate metabolism and corticosteroid synthesis has previously been indicated by Vogt's (1951) finding that adenosine triphosphate and creatine phosphate caused a marked increase in the secretion of corticosteroids by perfused adrenals of dogs.

At the same time, the low values found for the respiratory quotients, both in the presence and absence of glucose, would seem to point to the oxidation of much fat, although the present investigation did not produce evidence that added fatty acids are oxidized by the tissue under the particular conditions used.

The observation that glucosamine is oxidized by the adrenal cortex is of interest, since this substance is known to be oxidized at appreciable rates by only three other tissues: brain cortex, kidney cortex and testis (Lutwak-Mann, 1941). Comparing the present measurements with those reported by Lutwak-Mann, the rate of oxidation of glucosamine for adrenal-cortex tissue appears to be comparable with that for brain cortex, but lower than that for kidney cortex and testis.

SUMMARY

1. The influence of various substances on the respiration of intact rat-adrenal cortex has been studied.

2. Glycolysis in this tissue is stimulated by glucose and hexose diphosphate under anaerobic conditions, and by glucose under aerobic conditions.

3. The respiratory quotient, in the presence and absence of glucose, is consistent with the oxidation of much fat.

4. The endogenous respiration is considerably reduced when the tissue is homogenized, or when it is pretreated with oxygen at 37° .

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