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The Role of Glucose and Acetate in the Oxidative Metabolism of the Testis and Epididymis of the Ram

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Information on the metabolism of ejaculated spermatozoa is extensive (Mann, 1954), but little is known about the metabolic activity of the testis in which the spermatozoa are formed or of the epididymis in which they mature and are stored. Studies with tissue slices have failed to reveal differences in the metabolic rate of these structures (T. Mann, cited by Cross & Silver, 1962). An attempt has been made to investigate the metabolism of the testis and epididymis *in vivo* by using catheterization techniques that have been successfully applied to the study of other organs, e.g. heart (Bing *et al.* 1953), liver (Kolodny, Kline & Altszuler, 1962), brain (Sacks, 1956) and kidney (Levy, 1962). Most studies have been based on estimates of the net uptake of a particular substrate by the organ. These are calculated from the arteriovenous differences of that substrate, and the total oxidative activity of the tissue under investigation may be calculated from blood-gas exchanges measured at the same time. In the present studies glucose and acetate metabolism by the testis and epididymis of the anaesthetized ram were examined under controlled temperature conditions by measurements of arteriovenous differences and also by the use of ^{14}C-labelled substrates. These were continuously infused to achieve constant specific radioactivities of substrate in the circulation, when arteriovenous differences in the concentration and specific radioactivity of the CO_2 of the blood passing through the testis and epididymis allowed the direct contribution of substrate to the oxidative metabolism of the tissues to be calculated.

A preliminary report of this work was presented to the Biochemical Society (Annison, Scott & Waites, 1962).

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MATERIALS AND METHODS

Experimental animals. Seventeen Merino rams, aged 3–5 years and weighing 43–75 kg., were used. They were housed indoors for at least 1 week before the experiment and given 400 g. of lucerne chaff and 400 g. of oats per day. Food was withheld for 24 hr. before the experiment.

Surgical preparation. Anaesthesia was induced with an intravenous dose (20–25 mg./kg.) of Nembutal (pentobarbitone sodium) and light anaesthesia was maintained by additional doses injected through a catheter in the left external jugular vein. All catheters were of flexible silicone-treated polythene tubing (external diam. 1·5 mm.; internal diam. 1·0 mm.) and filled with heparin dissolved in 0·9% NaCl (3 mg./ml.). The rams lay on an electric warming pad with one hind limb raised. A catheter 22–25 cm. long was pushed upstream in a femoral artery so that the tip lay in the aorta 4–7 cm. proximal to the origin of the internal spermatic arteries (Fig. 1A). Catheters 7–12 cm. long were introduced into a vein of one or both spermatic cords through incisions in the anterior scrotal skin and pushed downstream so that the tips then lay in the internal spermatic veins inside the abdomen (Fig. 1, V). Venous blood from both the testis and epididymis drains into the multiple venous network of the pampiniform plexus (see Fig. 1 in Waites & Moule, 1961) before passing into the internal spermatic veins from which the venous samples were withdrawn. In another study (B. P. Setchell & G. M. H. Waites, unpublished work) blood flow was measured by the method of Huckabee & Walcott (1960) which required frequent blood samples to be withdrawn through catheters identical with those used here, and located exactly as in the present studies. Estimates of blood flow agreed well with simultaneous direct measurements of flow, and the similarity of the blood flow observed in both internal spermatic veins of the same ram provided good evidence that mixing was complete at this level. All skin incisions were closed with Michel clips.

Attempted isolation of the epididymis. The vascular anatomy suggested that the epididymis could be isolated for a separate metabolic study by tying off the testicular artery to exclude the testis. A ligature was passed under this artery exposed on the testis surface through short paramedian incisions in the posterior scrotal skin (Fig. 1, L_t). This ligature was tied on 18 occasions after which the blood removed through the catheter in the internal spermatic veins was assumed to be representative of the venous outflow from the epididymis. In nine of these preparations the artery and vein running with the vas deferens were also ligated (Fig. 1, L_d) to remove this alternative channel for venous drainage of the epididymis. The weights of individual epididymides and testes were $29 \pm 1·1$ g. (19 samples; range 20–38 g.) and $168 \pm 6·9$ g. (19 samples; range 126–215 g.) respectively, so that the epididymis represented only 15% of the total tissue. The oxygen uptake with the testicular artery tied off varied from 1·1 to 3·8 vol./100 vol. and represented $34 \pm 5·6$% (5 determinations; range 15–47%) of the uptake of the combined tissue. The mean R.Q. of this preparation was 1·13 (range 0·37–2·63) and the extreme variability suggested that other than metabolic factors were influencing gas exchange after the tying of the testicular artery. For this reason, subsequent studies were confined to the combined testicular and epididymal tissues.

Blood flow. The blood flow through the combined tissue was measured at the end of four experiments by either opening the internal spermatic veins and collecting the outflow or by folding a pneumatic cuff around the veins, inflating it to 30–40 mm.Hg and removing the venous outflow through the sampling catheter. The mean flow was 15 ml./min./100 g.

Haematocrits. These were determined by centrifuging at 2000 *g* for 30 min.

Experimental procedure. Temperatures were recorded from the subcutaneous tissues of the scrotum by thermistors contained in hypodermic needles to within 0·25°, or by 38 S.W.G. copper–constantan thermocouples in the tips of hypodermic needles (20 S.W.G.) recording on a potentiometric recorder (Leeds and Northrup, Speedomax G, 1 mv full-scale deflexion) to within 0·1°. Rectal temperatures were measured with a clinical thermometer. In most experiments, the temperature of the scrotum was controlled within the normal range (32·6–34·4°; Waites & Moule, 1961) by a radiant heater and a cooling fan.

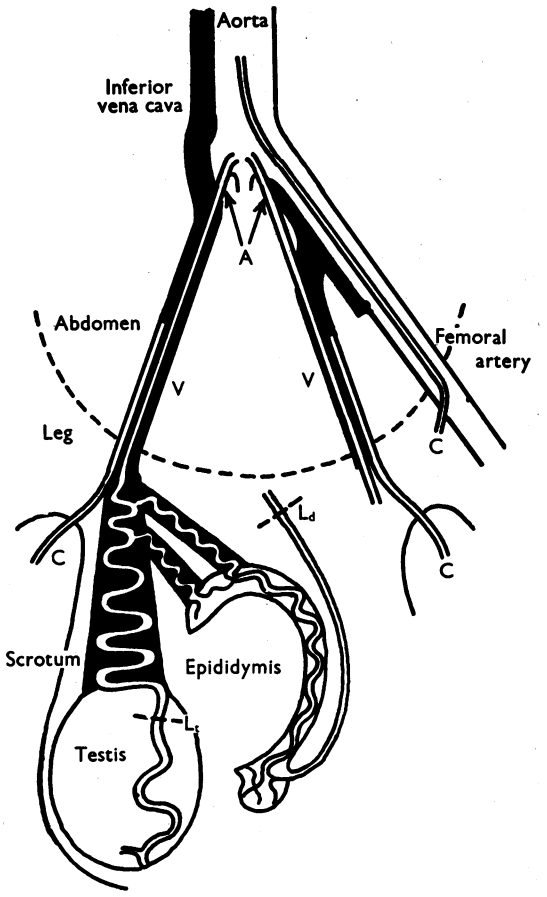


Fig. 1. Diagram to show the position of the sampling catheters (C). A and V, Internal spermatic arteries and veins respectively; L_t and L_d, position of ligatures around testicular artery and deferential (vasal) vessels respectively.

Substrate infusions were made through a catheter in the right external jugular vein with an injection apparatus (Palmer Instrument Ltd., London).

[^{14}C]Glucose was infused at a rate of 0·17 μc/min. for 150–230 min. after a priming dose of 15 μc (Annison & White, 1962). [^{14}C]Acetate was infused at a rate of 0·34 μc (0·13 m-mole)/min. for 120–130 min., and $NaH^{14}CO_3$ at a rate of 1 μc/min. Blood samples (5–20 ml.) for chemical analysis were withdrawn through the catheters simultaneously and at a slow rate (about 4 ml./min.) to minimize interference with blood flow. Samples (5 ml.) for gas analysis were drawn anaerobically into syringes lubricated with liquid paraffin and containing 3 mg. of heparin; the syringes were sealed with mercury-filled caps. The samples for chemical and for gas analysis were taken within 8 min. Gas analyses were completed within 6 hr. of collection. At the end of the experiment, the rams were killed, and the testes and epididymides were removed, blotted dry and weighed.

Plasma glucose concentration and specific radioactivity. The glucose content of blood and plasma was determined with glucose oxidase (Huggett & Nixon, 1957). Glucose was isolated from plasma as the glucosazone and assayed for radioactivity as described by Annison & White (1961).

Blood acetate. Acetate was isolated from blood and assayed for specific radioactivity by using procedures described by Annison & White (1962).

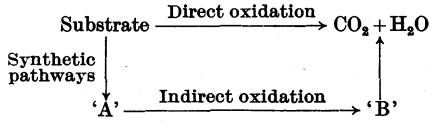
Blood lactate. This was determined by the method of Barker & Britton (1957).

Blood gases. Analyses for O_2 and CO_2 were made on the same blood sample by using the procedures of Peters & Van Slyke (1932).

Specific radioactivity of blood carbon dioxide. This was determined by the procedure described by Annison & Lindsay (1961). The standard error of the method was determined by carrying out 12 analyses on a blood sample containing added $NaH^{14}CO_3$ (6·2 μmc/ml.). The mean value for the specific radioactivity of blood CO_2 was 15·1 ± 0·27 (S.E.) μc/g. of C.

Radioactive materials. Sodium [1-^{14}C]acetate, sodium [2-^{14}C]acetate, uniformly labelled D-[^{14}C]glucose and $NaH^{14}CO_3$ were obtained from The Radiochemical Centre, Amersham, Bucks.

Theoretical considerations. Arteriovenous differences in the concentration of substrates provide a measure of net uptake or production of substrate if the blood flow is known, but assessment of the contribution of substrate to tissue oxidation requires in addition the use of labelled materials. When constancy of specific radioactivity of substrate in the circulation has been achieved by the continuous infusion of ^{14}C-labelled substrate, the contribution of substrate to the oxidative metabolism of a tissue may be assessed by direct comparison of the specific radioactivities of the substrate in the circulation and of the CO_2 produced by the tissue (calculated from the concentrations and specific radioactivities of CO_2 in venous and arterial blood) if this has reached a constant value. A limitation of this procedure arises when substrate oxidation proceeds by indirect pathways. Oxidation of substrate might occur through a number of intermediates, perhaps involving equilibration with several pools (see Scheme 1). Although equilibrium with respect to direct oxidation might be reached fairly quickly, indirect oxidation of substrate through a number of intermediates will result in a slow increase in the specific radioactivity of CO_2 produced by the tissue, and mask measurement of the contribution of substrate to direct oxidation. In addition, the possible production of a ^{14}C-labelled blood metabolite at a site other than the tissue under investigation, and its subsequent oxidation by that tissue (resulting in rising values for the specific radioactivity of CO_2 produced by the tissue), must be borne in mind.



Scheme 1

Identical specific radioactivities of substrate in arterial blood and in venous blood leaving a tissue indicate the absence of substrate production by that tissue. Lower but constant specific radioactivities of substrate in the venous blood relative to arterial blood indicate substrate production by the tissue. The presence of tissue pools that equilibrate slowly with blood, and that give rise to low but steadily rising specific radioactivities in venous blood, are more easily detected during the early stages of infusion of ^{14}C-labelled substrate.

RESULTS

Physiology of the preparation. Surgical intervention in the present studies was minor since all the vessels required for catheterization were superficial and outside the abdomen. The volume of blood removed during sampling (115–270 ml.) represented only 5–9 % of the estimated blood volume (Schambye, 1952). The packed-red-cell volume did not differ between arterial and venous samples removed simultaneously and there was no progressive change during the experiments; the fall with anaesthesia was within the range reported by Turner & Hodgetts (1959). The temperature of the testes of conscious rams is about 5° less than deep body temperature (39·6°), and the subcutaneous temperatures of the scrotum were shown to be a good index of testicular temperature (Waites & Moule, 1961). The subcutaneous temperatures of the scrotum were kept within the range 30·0–34·8° in ten experiments and 33·8–36·8° in four experiments. Body temperature was maintained in the normal range, 38·7–40·5° (mean 39·4°). Arterial blood glucose and lactate concentrations were 1·90–2·56 μmoles/ml. and 0·70–0·90 μmole/ml. respectively, which are within the normal range for sheep (Annison, Lindsay & White, 1963). The arterial oxygen content was 11·1 ± 0·14 vol./100 vol. (11 determinations; range 8·3–13·5 vol./100 vol.), which is approx. 3 vol./100 vol. lower than the mean arterial oxygen content reported for sheep immediately after thiopentone anaesthesia (Halmagyi & Colebatch, 1961).

Blood-gas exchanges across the testis and epididymis. The oxygen uptake by the combined testi-

cular and epididymal tissue estimated from serial blood samples was $5{\cdot}9 \pm 0{\cdot}46$ vol./100 vol. (9 determinations; range 4·3–8·1 vol./100 vol.), and the coefficient of oxygen utilization (oxygen uptake/arterial oxygen content) was $56 \pm 4{\cdot}7$ % (9 determinations). The corresponding R.Q. values were $0{\cdot}93 \pm 0{\cdot}024$ (9 determinations; range 0·79–1·05). The mean testicular and epididymal tissues of a 50 kg. ram weigh about 400 g. The total blood flow through this tissue is approx. 60 ml./min., and, as the oxygen uptake is about 6 ml./100 ml. of blood, then 3·6 ml. of oxygen is being removed/min. If it is accepted that a ram of this weight anaesthetized with barbiturate would consume about 200 ml. of oxygen/min. (Halmagyi & Colebatch, 1961), then the testicular and epididymal tissues would account for about 2 % of the total oxygen consumption.

Bicarbonate pools. The presence of bicarbonate pools in testicular and epididymal tissue was demonstrated by measurement of the specific radioactivities of CO_2 in arterial and venous blood during the infusion of ^{14}C-labelled bicarbonate. When $NaH^{14}CO_3$ was infused into the whole animal at a rate of 1 μc/min. the specific radioactivities of CO_2 in venous blood were 29, 55, 60 and 76 % of the corresponding arterial values at 5, 10, 15 and 21 min. respectively after the start of the infusion. Long-term infusions (270–430 min.) of $NaH^{14}CO_3$ confirmed previous results (Annison & Lindsay, 1961) indicating that substantial equilibrium between bicarbonate pools in the whole animal was reached within about 300 min. (Fig. 2). The mean difference in the specific radioactivities of arterial and venous blood draining the testis and epididymis (after 300 min.) was roughly accounted for by dilution with unlabelled CO_2 produced by the tissue (Fig. 2).

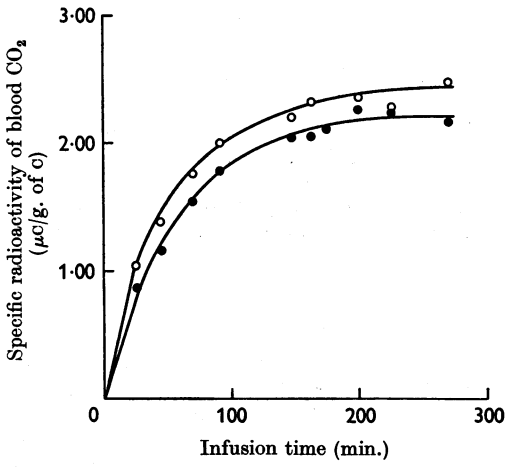


Fig. 2. Specific radioactivities of arterial and venous blood CO_2 after the infusion of $NaH^{14}CO_3$ (1 μc/min.) into the jugular vein of a ram. Experimental details are given in the text. ○, Specific radioactivity of arterial CO_2; ●, specific radioactivity of venous CO_2.

Metabolism of glucose. Relatively large uptakes of glucose by combined testicular and epididymal tissues were revealed by examination of arterio-venous differences. In six experiments in which whole blood was analysed, the mean uptake was $0{\cdot}3 \pm 0{\cdot}04$ μmole/ml.; in a further six experiments in which plasma was examined, the mean uptake was $0{\cdot}61 \pm 0{\cdot}09$ μmole/ml. These values correspond to 5·0 μmoles/min./100 g. of tissue if it is assumed that blood flow through the testis and epididymis was 15 ml./min./100 g.

Application of the ^{14}C-labelling technique indicated substantial glucose oxidation by testicular and epididymal tissue, the specific radioactivity of the CO_2 in venous blood being significantly higher than that of arterial blood removed simultaneously. The mean values for the contribution of glucose to tissue oxidation in four experiments were 14, 16, 22 and 31 % (mean 21 %) when measured over the period 190–230 min. after the start of infusion of [^{14}C]glucose. The mean uptake of blood glucose (0·3 μmole/ml.) indicated that glucose, if fully oxidized, would yield 1·8 μmoles of CO_2/ml. or 70 % of the total CO_2 production (mean value, 2·5 μmoles/ml.). By using the mean value for the contribution of glucose to tissue oxidation (21 %) it was calculated that 0·51 μmole of CO_2 arose from glucose, or that $(0{\cdot}51/1{\cdot}8) \times 100$ % (28 %) of the glucose removed by the testis and epididymis was oxidized. The results of one experiment are shown in Table 1.

The specific radioactivity of glucose in arterial blood, and in blood draining the testis and epididymis, was not detectably different during the infusion of [^{14}C]glucose, indicating the absence of glucose production by the tissues (Table 1).

In all experiments the specific radioactivity of the CO_2 produced by the tissues slowly increased with time, resulting in an apparently increased glucose oxidation (Table 1).

Total glucose entry rates in the present studies were $4{\cdot}8 \pm 0{\cdot}3$ μmoles/min./kg. body wt. (6 determinations). These values were considerably lower than those reported by Annison & White (1961) for unanaesthetized sheep [$8{\cdot}3 \pm 0{\cdot}5$ μmoles/min./kg. (13 determinations)]. Comparison of the specific radioactivities of arterial-blood CO_2 and glucose in five experiments indicated that only 5–8 % of the total body CO_2 was derived from glucose. Values of 11–19 % were obtained with unanaesthetized sheep by Annison & White (1961).

The lactic acid content of venous and arterial blood in two experiments gave no evidence of lactate production from glucose (Table 1), but the results did not exclude the slight possibility of identical rates of lactate uptake and production.

Table 1. *Metabolism of glucose by testicular and epididymal tissues of the ram*

Uniformly labelled [^{14}C]glucose (0·17 μc/min.) was infused for 230 min. after the injection of a priming dose (12·5 μc). Blood samples were withdrawn simultaneously from the aorta (A) and right spermatic veins (RV) (see Fig. 1). Experimental details are given in the text.

Sample	Time after start of infusion (min.)	Blood O_2 (ml./100 ml.)	Blood CO_2 (ml./100 ml.)	Specific radioactivity of blood CO_2 (μc/g. of C)	Blood glucose (μmoles/ml.)	Blood lactate (μmole/ml.)	Specific radioactivity of plasma glucose (μc/g. of C)	Specific radioactivity of CO_2 produced by tissue (μc/g. of C)	Contribution of glucose to tissue oxidation (%)	Percentage of glucose uptake that is oxidized
A 1	190	11·6	61·0	0·72	2·31	0·84	9·7	2·6	27	—
RV 1		6·3	65·0	0·84	2·06	0·93	9·8	—	—	23
A 2	208	11·8	60·1	0·75	—	—	—	3·1	31	—
RV 2		6·6	64·4	0·91	—	—	—	—	—	—
A 3	230	12·0	63·2	0·76	2·42	0·79	9·9	3·5	35	—
RV 3		6·4	65·7	0·95	2·02	0·81	10·0	—	—	16

Acetate metabolism. Comparison of the acetate content of blood samples withdrawn simultaneously from the aorta and internal spermatic veins indicated acetate uptakes of 0·1–0·3 μmole/ml. Complete oxidation of acetate would have accounted for only about 20 % of the CO_2 produced by the tissues.

During the infusion of [^{14}C]acetate, no differences could be detected in the specific radioactivities of CO_2 in arterial blood, and in blood draining the testis and epididymis, i.e. the specific radioactivity of CO_2 produced by the tissues was not measurably different from that of arterial CO_2, and the contribution of acetate to oxidation in the tissues was not detectably different from that of acetate oxidation in the whole animal. The results of a typical experiment are shown in Table 2. The mean value for acetate uptake from blood by testicular and epididymal tissue was $0·21 \pm 0·03$ μmole/ml. (5 determinations) or about 3 μmoles/min./100 g. if the blood flow is assumed to be 15 ml./min./100 g. The contribution of acetate to tissue oxidation calculated from the relative specific radioactivities of CO_2 produced by the gland and that of the acetate in the circulation was about 23 % (3 determinations) when measured 180–220 min. after the start of the infusion of [^{14}C]acetate. By using the mean value for CO_2 production by the tissues (2·5 μmoles/ml. of blood) it was calculated that CO_2 production due to acetate was $2·5 \times 23/100$ or 0·57 μmole/ml., which is equivalent to 0·28 μmole of acetate, a value somewhat greater than the measured uptake (0·21 μmole/ml.). These results suggest that acetate taken up by the tissue was completely oxidized.

The specific radioactivities of blood acetate in venous and arterial blood were not measurably different, indicating the absence of acetate production by the tissues (Table 2).

Total acetate entry rates in the whole animal were calculated from the specific radioactivities of acetate in the circulation during the continuous infusion of [^{14}C]acetate (Annison & Lindsay, 1961) and corrected for the carrier acetate infused with the labelled material. A mean value of $6·8 \pm 2·34$ μmoles/min./kg. (4 determinations) was obtained. The contribution of acetate to total oxidation was within the range 13–23 % in four experiments.

DISCUSSION

The simultaneous use of techniques based on isotope dilution and arteriovenous differences has provided quantitative information on the metabolism of glucose and acetate by the testis and epididymis. Glucose was shown to be a more important metabolite than acetate, rates of utilization for these substrates being about 5·0 and 3·0 μmoles/min./100 g. of tissue respectively or 8 and 3·5 % respectively of total substrate entry (4·78 and 6·83 μmoles/

Table 2. *Acetate metabolism by testicular and epididymal tissue of the ram*

Uniformly labelled [^{14}C]acetate was infused at a rate of 0·34 μc (0·13 m-mole)/min. for about 230 min. Blood samples were withdrawn simultaneously from the aorta (A) and from the left (LV) and right (RV) spermatic veins (see Fig. 1). Experimental details are given in the text.

Sample	Time after start of infusion (min.)	Blood O_2 (ml./100 ml.)	Blood CO_2 (ml./100 ml.)	Blood acetate (μmole/ml.)	Specific radioactivity of blood acetate (μc/g. of C)	Specific radioactivity of blood CO_2 (μc/g. of C)
A1		8·7	62·6	0·83	9·8	2·24
RV1	198	3·5	66·1	0·76	10·2	2·23
LV1		3·6	66·7	0·60	10·5	2·09
A2		—	—	0·83	10·2	2·42
RV2	204	—	—	0·74	9·7	2·31
LV2		—	—	0·72	9·4	2·46
A3		7·9	61·6	0·82	10·8	2·26
RV3	226	2·7	67·2	0·66	10·9	2·28
LV3		4·1	65·8	0·68	—	2·25

min./kg.) in the anaesthetized sheep. When expressed in terms of body weight, glucose uptake by the testicular and epididymal tissue was 0·39 μ-mole/min./kg. body wt. and acetate uptake by these structures was 0·23 μmole/min./kg. Comparison of the specific radioactivities of the substrates in the circulation and of CO_2 produced by the testis and epididymis during the continuous infusion of [^{14}C]-acetate and [^{14}C]glucose revealed that, whereas only about one-third of the glucose removed by the tissues was directly oxidized, all of the acetate taken up contributed to CO_2 production. The preferential utilization of glucose relative to acetate is in contrast with findings with ram spermatozoa *in vitro*, which oxidize acetate in preference to glucose (Scott, White & Annison, 1962).

The metabolic interrelations of glucose and acetate in isolated ruminant and non-ruminant tissues have been discussed by Scott *et al.* (1962), but information on intact organs is scanty. Hardwick & Linzell (1962) reported that the isolated perfused goat udder oxidized both glucose and acetate, but that glucose made a greater contribution to total oxidation. The heart is known to utilize both glucose (Bing *et al.* 1953) and acetate (Cavert & Johnson, 1956), but the metabolic interrelations of these substrates were not reported.

The steady increase with time in the specific radioactivities of CO_2 produced by the testis and epididymis during the period of constant specific radioactivity of glucose in the circulation (Table 1) almost certainly indicated increasing $^{14}CO_2$ production by these tissues from the indirect oxidation of ^{14}C-labelled metabolites. Values for the apparent contribution of glucose to oxidative metabolism must be regarded as maximal for direct (and minimal for direct plus indirect) oxidation.

Sacks (1956, 1957) studied the cerebral metabolism of [^{14}C]glucose in human subjects by measuring the changes in specific radioactivity of arterial-blood and venous-blood CO_2 after the intravenous injection of a single dose of [^{14}C]glucose, and concluded that about one-half of the cerebral CO_2 was derived from the oxidation of glucose. Continuous-infusion procedures, which formed the basis of the present studies, offer many advantages over the single-injection method. Specific radioactivities of the substrate in the circulation remain constant for long periods, allowing substantial equilibration of body CO_2 pools. The specific radioactivity of the CO_2 produced by the tissue is easily determined and this value, when related to the specific radioactivity of the substrate in the circulation, provides a direct measure of substrate oxidation by the gland. Also, the metabolic activity of the tissue may be compared with the substrate entry rate and the overall oxidation in the whole animal.

In metabolic studies in specific tissues this approach may be used in conjunction with experiments designed to measure net uptake or production of substrate by arteriovenous differences. The difficulties associated with procedures based on arteriovenous differences have been enumerated and discussed by Zierler (1961), who concluded that avoidance of changes in metabolic rate or blood flow were prerequisites of successful experimentation. In the present studies temperature changes, which markedly affect metabolic rates, were avoided, and blood samples were drawn slowly through flexible catheters to minimize possible effects on blood flow. Total glucose and acetate entry rates in the present studies, and total glucose (but not acetate) oxidation, were significantly lower than values observed with unanaesthetized sheep by Annison & Lindsay (1961) and Annison & White (1961, 1962). It is impossible to distinguish between the effects of the anaesthetic on the metabolic rate, which would be expected to lower glucose entry, and its possible inhibitory effects on glucose

metabolism in certain tissues. Cross & Silver (1962) reported that the administration of Nembutal to rams did not change tissue oxygen concentrations in the testis and epididymis, and this anaesthetic was reported to be without effect on hepatic blood flow in dogs (Gilmore, 1958). Diminished acetate entry was probably due to impaired absorption from the rumen, since inhibition of rumen movements caused by anaesthesia would prevent adequate mixing of rumen contents. Acetate oxidation in rats was reported to be unaffected by Nembutal (Williams & Van Bruggen, 1956).

SUMMARY

1. A general procedure for studying the oxidative metabolism of substrates by specific tissues is described with particular reference to the metabolism of glucose and acetate by the testis and epididymis of the anaesthetized ram.

2. Continuous infusion of $NaH^{14}CO_3$ demonstrated the presence of bicarbonate pools in testicular and epididymal tissue. Equilibrium conditions between bicarbonate pools in the whole sheep were reached within about 300 min. after the start of infusion.

3. Glucose is a more important metabolite than acetate in testicular and epididymal metabolism, values for glucose and acetate uptake from blood based on arteriovenous differences being 5·0 and 3·0 μmoles/min./100 g. of tissue respectively.

4. The contributions of glucose and acetate to the overall oxidative metabolism of the testis and epididymis calculated by comparing the specific radioactivities of CO_2 produced by the tissue with those of the substrates in the circulation were 30 and 20% respectively. About one-third of the glucose and all of the acetate taken up from blood by the testis and epididymis was directly oxidized.

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The Effect of Ethanol and Electrical Stimulation on the Amino Acid Metabolism of Rat-Brain-Cortex Slices *in vitro*

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The object of the present study was to reproduce *in vitro* the changes in the concentrations of amino acids which had been observed in the brains of living rats during intoxication by ethanol (Häkkinen & Kulonen, 1961): a temporary increase in the concentrations of γ-aminobutyric acid, glutamic acid and aspartic acid and a decrease in the concentration of glutamine.

The metabolic relationships of glutamic acid and γ-aminobutyric acid have attracted great interest