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The Importance of Glucose in the Oxidative Metabolism of the Testis of the Conscious Ram and the Role of the Pentose Cycle

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1. [U-^{14}C]Glucose was infused into one or both testicular arteries of ten conscious rams and the specific activity of the glucose taken up by the testis was compared with the specific activity of the carbon dioxide produced by the testis. 2. Equilibration had occurred after infusion for 3hr. when a mean of 68% of the carbon dioxide was being derived by the testis from blood glucose and 86% of the glucose taken up by the testis was being oxidized to carbon dioxide. After 5hr. infusion, these values were 71% and 83% respectively. 3. In four other conscious rams, [1-^{14}C]glucose was infused into one testicular artery and [6-^{14}C] glucose into the other and the 'specific yields' of carbon dioxide calculated for the two forms of glucose. 4. From these values, it was calculated that a mean of 9·3% of the glucose taken up by the testis was metabolized via the pentose cycle.

Glucose was at one time thought to be the most important fuel of peripheral organs. However, in recent years, it has been found that in skeletal muscle the uptake of glucose could account for only a small fraction of the oxygen uptake (Andres, Cader & Zierler, 1956) and a similar result was obtained with heart (Bing *et al.* 1953) and kidney (see Levy, 1962). Even in brain, where the uptake of glucose was sufficient to account for the oxygen uptake and carbon dioxide production (Himwich, 1951), it has been suggested that only a fraction of the glucose is oxidized (Sacks, 1957). In the testis of the conscious ram, as in the brain, the uptake of glucose was high and was sufficient to account for all the oxygen taken up (Setchell & Waites, 1964). In anaesthetized rams, however, the uptake of glucose by the testis was lower (Annison, Scott & Waites, 1963; Setchell & Waites, 1964), and only 21% of the carbon dioxide produced by the testes was derived from glucose (Annison *et al*, 1963). Because of this difference between conscious and anaesthetized rams, an attempt has been made to assess the importance of glucose in the oxidative metabolism of the testes of conscious rams. The possible errors in this assessment have been minimized by infusing [U-^{14}C]glucose directly into the testicular artery, and this technique has also been used with [1-^{14}C]-glucose and [6-^{14}C]glucose to assess the role of the pentose cycle in the metabolism of glucose by the testis, since this pathway has been demonstrated by studies *in vitro* with testis slices (Bloom, 1955; Field, Pastan, Herring & Johnson, 1960).

MATERIALS AND METHODS

Experimental animals. Fourteen Merino rams, aged 3–4 years and weighing 42–63kg., were used. They were kept in an air-conditioned room at 21° with controlled illumination of 12hr. light and 12hr. darkness. They were given 500g. of chaffed lucerne hay and 500g. of oats at 4p.m. daily. Water was available *ad lib.*

Surgical preparation. On the day before surgery water was removed at 12 noon and food was withheld. Next day at about 9.30a.m. the ram was anaesthetized with pentobarbitone sodium B.P. and anaesthesia maintained with halothane (Fluothane; Imperial Chemical Industries Ltd., Macclesfield, Cheshire). End-hole polyvinyl chloride catheters (0·2mm. internal diam., 0·5mm. external diam.) (Dural Plastics, Dural, N.S.W., Australia) were implanted in one or both testicular arteries as described by Setchell, Waites & Thorburn (1966) and polyvinyl chloride catheters (0·97mm. internal diam., 1·27mm. external diam.) were placed in one or both internal spermatic veins and one femoral artery as described by Setchell & Waites (1964). All catheters were filled with 10% EDTA (disodium salt) (British Drug Houses Ltd., Poole, Dorset) to prevent clotting. Surgery was usually complete by 11.30 a.m. The animals were allowed to recover in a sling and were fed as soon as they were completely conscious, usually about 2p.m. Food was then available *ad lib.* until the end of the experiment.

Experimental procedure. Next morning at about 9a.m., [^{14}C]glucose (The Radiochemical Centre, Amersham, Bucks.) in 0·9% NaCl (80mμc/ml. or 800mμ/cml.) was infused into the testicular artery at the rate of 0·125ml. or 0·15ml./min. with the infusion pump (Palmer, London, or Technicon, Chauncey, N.Y., U.S.A.), or [1-^{14}C]glucose was infused into one artery and [6-^{14}C]glucose into the other.

In one experiment, glucose (0·15 μc/min. after a priming dose of 15 μc) was infused into a recurrent tarsal vein.

One hour after the start of the infusion, three blood samples were taken consecutively from the internal spermatic vein. Each sample (10ml.) was drawn at a constant rate over 5min. Simultaneously, three samples were also withdrawn from the femoral-artery catheter and the other internal-spermatic-vein catheter when two were in place. Further sets of six or nine samples were taken 3 and 5hr. after the start of the infusion.

Analytical methods. Blood glucose concentration and blood CO_2 content and specific radioactivity and blood O_2 content were determined by the methods given by Annison *et al.* (1963) and Setchell & Waites (1964). The specific radioactivity of blood glucose was measured by preparing recrystallized glucosazones, which were burnt by the Schöniger-flask technique (Kalberer & Rutschmann, 1961); the CO_2 was absorbed in 12ml. of ethanolamine–methyl-Cellosolve (1:9, v/v), 10ml. of which was then added to 9ml. of toluene containing 2,5-diphenyloxazole (0·4%) and 1,4-bis-(5-phenyloxazol-2-yl)benzene (0·01%) and counted in a Packard 3000 liquid-scintillation counter (Packard Instruments Inc., La Grange, Ill., U.S.A.).

Calculations. The following were determined.

(*a*) Radioactivity (mμc) in CO_2/ml. of blood in the internal spermatic vein.

(*b*) Radioactivity in CO_2/ml. of blood in the femoral artery.

(*c*) Specific activity (mμc/g. of C) of CO_2 in blood in the femoral artery.

(*d*) Specific activity of glucose in blood in the internal spermatic vein (assumed to equal the specific activity of glucose taken up by the testis as this was so when glucose was infused parenterally; Annison *et al.* 1963; cf. ram 15).

(*e*) Specific activity of glucose in blood in the femoral artery.

(*f*) Carbon (g.) as CO_2/ml. of internal-spermatic-vein blood.

(*g*) Carbon (g.) as CO_2/ml. of femoral-artery blood.

(*h*) Carbon (g.) as glucose/ml. of internal-spermatic-vein blood.

(*j*) Carbon (g.) as glucose/ml. of femoral-artery blood.

(*k*) Mean specific activity of glucose taken up by the testis (with [1-^{14}C]- and [6-^{14}C]-glucose); after 3hr. infusion this is (2 × specific activity after 1hr. + 1 × specific activity after 3hr.)/3 and after 5hr. infusion it is (2 × specific activity after 1hr. + 2 × specific activity after 3hr. + 1 × specific activity after 5hr.)/5. This weighting was used because of the timing of the samples during the infusion.

(*m*) Rate of infusion of glucose (mμc/min.) into the testicular artery.

(*n*) Body wt. (kg.).

(*p*) Specific activity of CO_2 produced by testis given by $(a-b)/(f-g)$.

(*q*) Percentage of CO_2 derived by the testis from blood glucose, given by $100p/d$.

(*r*) Percentage of glucose taken up that was oxidized, given by $q(f-g)/(j-h)$.

(*s*) Mean specific activity of CO_2 produced by testis during [1-^{14}C]- and [6-^{14}C]-glucose infusions. Estimates of p were made after 1, 3 and 5hr. infusion, and then the weighted mean was calculated as for k.

(*t*) Estimated blood flow(ml./min.) given by $m/(d-e)j$.

(*u*) Total uptake of [^{14}C]glucose, given by $kt(j-h)$.

(*v*) Total production of $^{14}CO_2$, given by $st(f-g)$.

(*w*) Specific yield of CO_2, given by v/u (Wood, Katz & Landau, 1963). Three determinations were made for each animal during each experiment, but strictly the amount of

Table 1. *Concentration and specific activity of the glucose and carbon dioxide in blood after infusion of* [U-^{14}C]*glucose for 5 hr.*

Symbols: A, arterial blood; V, blood from the internal spermatic vein; A—V, arteriovenous difference.

Ram no.	Rate of infusion of ^{14}C-glucose (μc/min.)	Blood glucose concn. (mg./100ml.)		Sp. activity of blood glucose (mμc/g. of C)		Blood CO_2 concn. (ml./100ml.)		Sp. activity of CO_2 in blood (mμc/g. of C)		Mean sp. activity of CO_2 produced by the testis (mμc/g. of C)
		A	A—V	A	V	A	A—V	A	V	
1	5·1*	46	8	76	1480	51·4	8·0	6	119	840
2	10·0*	41	13	180	1850	55·2	9·3	6	140	930
3	11·6*	53	10	83	2700	44·2	9·0	19	380	2140
4	11·1*	30	16	283	3000	36·4	10·0	41	535	2340
5	10·5*	48	6	200	1090	51·2	7·9	28	176	1130
6	10·5*	44	7	360	1590	51·4	5·1	38	114	890
7	57†	31	L 6 R 6	1840	L 9700 R 10000	50·5	L 4·5 R 5·2	356	L 1120 R 1030	L 9700 R 7600
8	107†	41	8	3530	21900	47·1	5·9	750	3080	21900
9	91†	36	7	5100	35000	54·1	5·8	630	2620	21000
10	142†	34	6	6400	33000	58·0	7·0	790	3270	24100
15	150‡	51	6	7600	7900	45·3	5·6	345	390	7500

* Into one testicular artery.

† Into each testicular artery.

‡ Into recurrent tarsal vein after priming dose of 15 μc.

[^{14}C]glucose and $^{14}CO_2$ in the testis itself should be included. This would comprise a small and decreasing proportion of glucose taken up and the CO_2 produced by the testis.

(x) Percentage of pentose-cycle activity by formula 4 of Wood *et al.* (1963)

(y) Entry rate of glucose (1 mole≡72g.c) for the whole animal in μmoles/min./kg., given by $10^6 mh/72enj$.

(z) Percentage contribution of glucose to the oxidative metabolism of the whole animal, given by $100c/e$. This gives a slight overestimate because the testis has access to glucose of higher specific activity than the rest of the body and would therefore add CO_2 of a higher specific activity than the other tissues of the body. As the testis is only 0·4% of the body weight the error is not likely to be serious.

RESULTS

Intra-arterial infusions of [U-^{14}C]*glucose.* The specific activity of the glucose in the blood from the internal spermatic vein reached a plateau after 1 hr. of infusion except for minor fluctuations due to small changes in blood flow. The specific activity of glucose in the arterial blood was about one-tenth of that in the internal spermatic vein when only one testis was infused and one-sixth when both testes were infused (Table 1).

The specific activity of the carbon dioxide in the blood from the internal spermatic vein was much higher than that in the arterial blood (Table 1) and was less after 1 hr. infusion than after 3 and 5 hr. infusion (Fig. 1); the two later values were similar, and so it was assumed that equilibration with the bicarbonate pool of the testis had occurred 3 hr. after the beginning of the infusion. The samples obtained at 3 and 5 hr. were therefore both used to give estimates of the oxidative metabolism of glucose by the testis.

A mean of 68% of the carbon dioxide came from blood glucose and 86% of the glucose taken up was oxidized from the values after 3 hr. infusion, and 71% and 83% from the values after 5 hr. infusion. Because of the difficulty in estimating arteriovenous differences for carbon dioxide and glucose with high precision, there are many possibilities for error, which probably explain the values greater than 100%.

Parenteral infusion of [U-^{14}C]*glucose.* The specific activity of the glucose in blood from the femoral artery and the internal spermatic vein was the same during parenteral infusions of glucose. The specific activity of the carbon dioxide was slightly higher in the venous blood than in the arterial blood (Table 1). The contribution of glucose to the oxidative metabolism of the testis, as calculated by Annison *et al.* (1963), was 64% after 3 hr. infusion and 95% after 5 hr. (Table 2).

Intra-arterial infusions of [1-^{14}C]- *and* [6-^{14}C]-*glucose.* When [1-^{14}C]glucose was infused into one testis and [6-^{14}C]glucose into the other, the specific activity of the carbon dioxide produced by the testis receiving [1-^{14}C]glucose was always higher (Table 3) than that produced by the testis receiving

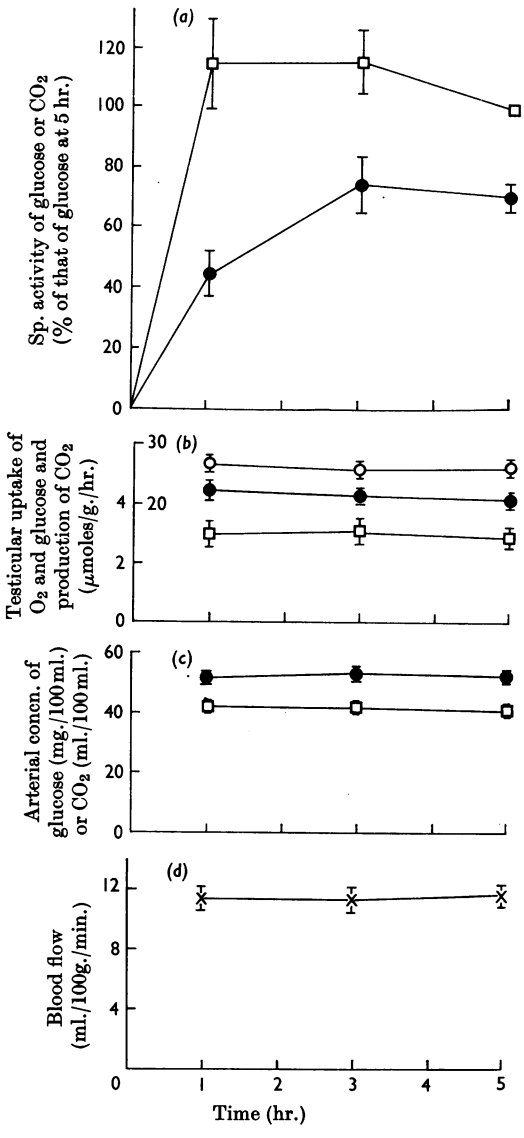

Fig. 1. (*a*) Specific activity of the glucose taken up by the testis (□) and of the CO_2 produced by the testis (●) during the infusion of [^{14}C]glucose into one or both testicular arteries. (*b*) Testicular uptake of O_2 (○) and glucose (□) and production of CO_2 (●). (*c*) Arterial concentrations of glucose (□) in mg./100 ml. and CO_2 (●) in ml./100 ml. (*d*) Blood flow in the internal spermatic vein (×) in ml. of blood/100g. of tissue drained/min. Specific activities are the means for 11 testes from ten rams; the others, the means for 19 testes from 14 rams. The vertical bars indicate the S.E.M.

Table 2. *Calculated values for glucose metabolism by the ram testis and by the whole animal*

Ram no.	CO_2 derived by testis from glucose (%) 5hr.	3hr.	Glucose oxidized (%) 5hr.	3hr.	Entry rate for glucose (whole ram) (μmoles/min./kg.)	CO_2 derived by whole ram from glucose (%)
1	57	82	77	95	12·5	8
2	50	68	48	67	10·1	3
3	79	92	96	110	25·0	23
4	76	61	64	62	6·5	14
5	103	100	136	104	10·1	14
6	56	50	55	45	8·4	10
7	L 100	36	100	85	11·5	19
	R 76	43	89	100		
8	100	90	99	115	11·9	21
9	60	63	67	92	8·0	12
10	73	66	114	105	10·2	12
11					9·8	
12					4·4	
13					8·5	
14					8·3	
15	95	64	102	105	7·3	5

[6-^{14}C]glucose; this difference was still apparent when specific yields were calculated; a mean of 9·3 ± 2·7% of the glucose taken up was oxidized by the pentose cycle.

Glucose entry rate and oxidation. The mean glucose entry rate for the 15 rams was 10·0 ± 1·2 μmoles/min./kg. (Table 2). The percentage of carbon dioxide derived from glucose in 11 of these (i.e. those receiving [U-^{14}C]glucose) was 12·8 ± 3·7%.

Blood flow, glucose and oxygen uptake and carbon dioxide production in the testes. The mean blood flow in 19 testes of 14 rams was 11·4ml./100g./min. The mean arterial glucose concentration was 40·2 mg./100ml. and the mean arterial carbon dioxide concentration was 52·2ml./100ml. Mean glucose and oxygen uptakes were 2·94 and 26·1 μmoles/g./hr. respectively and the mean production of carbon dioxide was 21·5 μmoles/g./hr. There was little variation in these parameters during the experiments and such variation as did occur was random (Fig. 1).

These later values are higher than those previously reported (Setchell & Waites, 1964; Waites & Setchell, 1964), possibly because control of the temperature of the testes was not possible in the present experiments.

DISCUSSION

These experiments demonstrate clearly that glucose is the major fuel for the testis of the conscious ram and that most of the glucose taken up by the testis is oxidized. As the technique depends on the measurement of arteriovenous differences of glucose and of carbon dioxide, there is inevitably some variation in the results, but such errors could be expected to be random. The only source of non-random error in these experiments is dilution of the venous blood from the testis by venous blood from the epididymis, which would not have received the infusion of [^{14}C]glucose. The epididymis of the ram weighs about one-sixth the weight of the testis (Setchell & Waites, 1964). It may be assumed that the half of the epididymis nearest the testis drains into the internal spermatic veins; the blood flow per unit weight through this portion is about 1·5 times that through the testis (Setchell, Waites & Till, 1964). Consequently about 12% of the blood at the point of sampling will be from areas not receiving the infusion of radioactive glucose. However, the specific radioactivity of both glucose and carbon dioxide would probably be decreased by dilution, so that the error could be expected to be considerably less than 12%. The constancy of the values for oxygen and glucose uptake and carbon dioxide production during the experiments (Fig. 1) suggest that testicular metabolism was steady during the experiments. This is very important with this type of experiment (Zierler, 1961). The similarity of the results after 3 and 5hr. infusion suggest that most of the metabolic pools of any quantitative importance would be close to equilibrium with the blood glucose. Other possible substrates in the arterial blood reaching the testis would be insignificantly

Table 3. *Arteriovenous differences and specific activities of carbon dioxide and glucose during infusion of* [1-^{14}C]*glucose into one ram testis and* [6-^{14}C]*glucose into the other*

Ram no.	Duration of infusion (hr.)	Position of ^{14}C in glucose	CO_2		Glucose		Estimated total uptake of [^{14}C]glucose (mμc)	Specific yield of CO_2	Pentose cycle (%)
			Sp. activity (μc/g. of C)	Arteriovenous difference (ml./100 ml.)	Sp. activity (μc/g. of C)	Arteriovenous difference (mg./100 ml.)			
11	1	1	1·08	6·1	5·8	9·0	210	0·170	3
		6	0·47	6·7	4·8	9·0	227	0·098	
	3	1	2·01	7·3	5·8	8·5	532	0·238	4
		6	0·89	9·1	4·6	9·1	495	0·144	
	5	1	2·78	5·0	6·2	8·1	820	0·310	2
		6	1·57	6·7	4·9	8·1	790	0·258	
12	1	1	0·48	14·6	3·2	11·0	205	0·279	9
		6	0·14	12·0	3·1	13·0	284	0·056	
	3	1	2·58	7·7	3·5	11·0	566	0·540	24
		6	0·71	7·2	3·9	11·0	697	0·110	
	5	1	1·88	7·1	3·3	6·2	810	0·670	34
		6	1·08	7·2	3·6	8·2	840	0·170	
13	1	1	0·60	6·6	5·5	5·0	124	0·190	2
		6	0·25	7·1	4·4	6·0	111	0·090	
	3	1	1·23	7·7	5·9	5·2	350	0·270	5
		6	0·73	6·5	4·2	7·1	311	0·150	
	5	1	1·78	7·5	6·4	6·1	570	0·362	8
		6	1·08	5·8	4·7	4·2	500	0·204	
14	1	1	0·42	6·2	2·0	8·3	145	0·240	5
		6	0·19	6·6	2·1	6·1	112	0·131	
	3	1	1·25	5·7	2·0	6·9	342	0·320	5
		6	0·61	6·3	2·1	6·9	256	0·221	
	5	1	1·46	6·0	2·6	5·2	530	0·514	10
		6	1·17	6·0	2·5	5·2	460	0·346	

labelled when compared with the glucose. If these unlabelled substrates were utilized to any extent, the specific activity of the carbon dioxide produced by the testis should be proportionately less than that of the glucose taken up by the testis.

Intra-arterial infusions were only feasible because of the relative constancy of testicular blood flow (Setchell & Waites, 1964; Setchell *et al.* 1966) and were preferred on two grounds; first, the labelling of other compounds in arterial blood was much less, and, secondly, the calculation of the importance of glucose in oxidative metabolism was not so sensitive to small errors in the measurement of the specific activity of the carbon dioxide in arterial and venous blood.

The main source of the difference between the present value of 70% and that of 21% found by Annison *et al.* (1963) is probably due to the use of anaesthetized animals in the earlier experiments. The difference is not due to the different infusion technique, as in one ram, with parenteral infusion as used by Annison *et al.* (1963), we obtained a value of 80%. The uptake of glucose by the testis of anaesthetized rams, measured as the product of the arteriovenous difference and blood flow, is about one-half of that in conscious rams (Setchell & Waites, 1964), and barbiturates have been shown to have marked effects on glucose metabolism by brain slices (Jowett & Quastel, 1937; Webb & Elliott, 1951) and on brain metabolism *in vivo* (Lindsay & Bachelard, 1966). The use of heparin in earlier experiments may also have introduced some errors because of its effect in activating lipoprotein lipase (Robinson & Harris, 1959) and hence stimulating utilization of lipid instead of glucose.

Incorporation of ^{14}C into other substances by other organs in the body would be minimal in the present studies but could have been significant in the earlier studies. However, this should have led to an overestimate of the importance of glucose in testicular metabolism in the earlier studies. Similarly, it is difficult to see how the difference could be due to the abnormally high testicular blood flow that resulted from the posture of the animal in the earlier experiments (Setchell & Waites, 1964).

The glucose entry rates for the whole animal are of the same order as those found in conscious ewes by Annison & White (1961) and are considerably higher than those for anaesthetized rams reported by Annison *et al.* (1963). Also, in the present series of rams the contribution of glucose to the oxidative metabolism of the whole body was closer to the value found for conscious ewes (Annison & White, 1961) than that for anaesthetized rams (Annison *et al.* 1963). Thus pentobarbitone anaesthesia appears to depress the utilization of glucose by other parts of the body as well as by the testis.

In fact there seems to be a relationship ($r = 0{\cdot}50, P < 0{\cdot}02$) between the glucose uptake by the testis and the percentage of the carbon dioxide derived by the testis from glucose (Fig. 2). This relationship seems to be an extension of the relationship between the glucose entry rate for the whole body and the percentage of carbon dioxide derived by the whole body from glucose. The same relationship seems to apply to conscious sheep (Annison & White, 1961; Bergman, 1963; J. B. Bassett, personal communication; and the present work), to anaesthetized rams and to the testes of these rams (Annison *et al.* 1963) (Fig. 2). In tissues such as the lactating mammary gland, the relationship does not apply probably because of the large proportion of glucose converted into milk constituents, but the non-lactating mammary gland fits the relationship reasonably well (Annison & Linzell, 1964). A similar relationship has been demonstrated in rats, but the glucose entry is higher for a given percentage of carbon dioxide derived from glucose (Depocas, 1964). The relationship suggests that the entry of glucose into the cells may be a major determinant of the importance of glucose in the oxidative metabolism of those cells.

The results with [1-^{14}C]- and [6-^{14}C]-glucose indicate that there is a significant contribution by the pentose cycle to the metabolism of glucose. This is to be expected after the demonstration of pentose-cycle activity in testis *in vitro* (Bloom, 1955; Field *et al.* 1960), but contrasts with the absence of such activity in the spermatozoa collected directly from the testis (Voglmayr, Scott, Setchell & Waites, 1967). In an endeavour to determine the quantitative contribution of pentose-cycle activity to glucose metabolism, 'specific yields' of carbon dioxide and pentose-cycle activity as defined by Wood *et al.* (1963) were calculated for testes infused with [1-^{14}C]glucose and [6-^{14}C]glucose. Three similar values for each animal were obtained during the experiments; the overall mean of 9·3% suggests that the pentose cycle is quantitatively a minor pathway of glucose dissimilation in the testis, unlike the lactating mammary gland (Wood, Peeters, Verbeke, Lauryssens & Jacobsen, 1965), where the pentose cycle accounts for most of the glucose oxidized. Adipose tissue has also a significant activity in the pentose cycle (Landau & Katz, 1964), but the results for brain are contradictory (Sacks, 1957; Moss, 1964).

The earlier calculation of the percentage of the carbon dioxide derived from blood glucose is not comparable with the calculation of the specific yield. The latter is less because it takes account of the [^{14}C]glucose that is stored in other compounds in the testes during equilibration whereas the former is calculated after equilibration with these compounds has occurred.

Apart from testis, studies with [^{14}C]glucose on

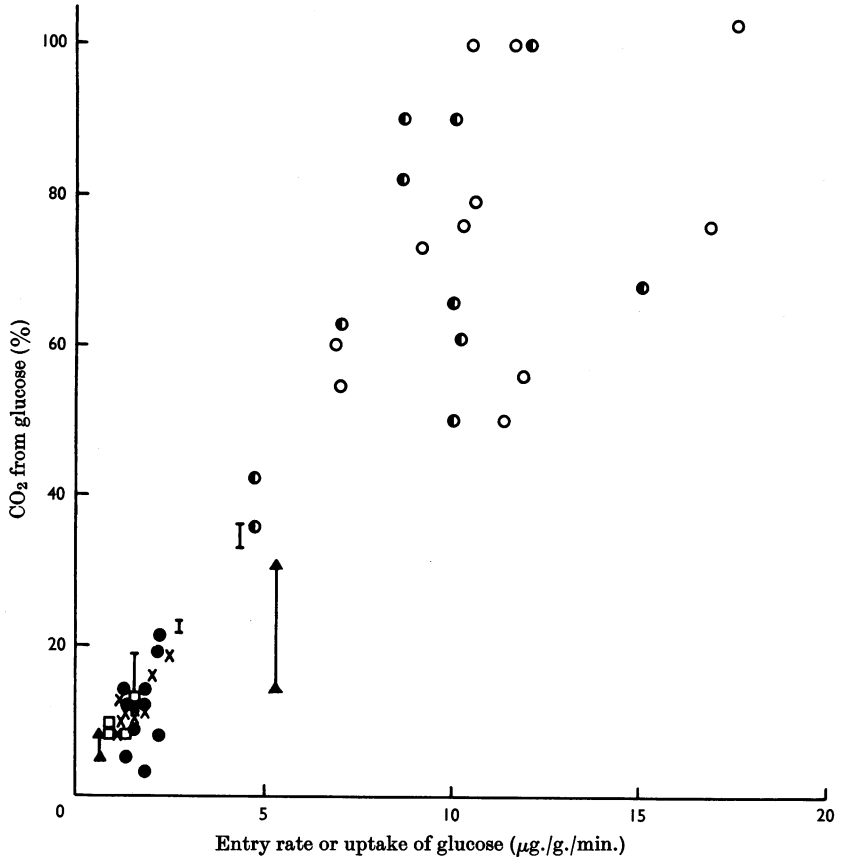

Fig. 2. Relationship between the entry rates for glucose in rams or the uptake of glucose by the testes of rams and the percentage of CO_2 derived from glucose. Present experiments with conscious rams: ◐, testes after 3hr. infusion; ○, testes after 5hr. infusion; ●, whole bodies. □, Results for conscious ewes of Bergman (1963). ×, Results for conscious ewes of J. B. Bassett (personal communication). I, Results for conscious ewes of Annison & White (1961); from left to right, starved, fed and glucose-loaded. ↕, Results of Annison *et al.* (1963) for testes (right) and whole bodies (left) of anaesthetized rams.

the quantitative importance of glucose in the oxidative metabolism have been made only on brain and mammary gland. Sacks (1957, 1961, 1965) has suggested that the brain derives only about 54% of its carbon dioxide from glucose but this value is calculated in a way not strictly comparable with our results. An exact estimate calculated in the same way from Sacks' results is not possible because of the decreasing specific activities, but the value would be close to 100%. In the lactating mammary gland only about 40% of the carbon dioxide is derived from blood glucose (Annison & Linzell, 1964). Other organs studied do not take up sufficient glucose to account for more than a small fraction of the carbon dioxide produced. There is a barrier in both brain and testis to injected Rb^+ ions (Waites & Setchell, 1966) and it may be that the dependence of these organs on glucose is simply a permeability effect.

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