RATES OF OXYGEN CONSUMPTION AND OF ANAEROBIC GLYCOLYSIS IN RENAL CORTEX AND MEDULLA OF ADULT AND NEW-BORN RATS AND GUINEA-PIGS

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SUMMARY

1. Rates of oxygen uptake and of anaerobic glycolysis were estimated in slices from the renal cortex and medulla (*a*) of adult rats and guinea-pigs, (*b*) of new-born (1-, 5- and 21-day-old) rats and of guinea-pigs of 1, 12, 21, 24 and 120 hr age.

2. In the adult rat, Q_{O_2} values for the cortex were $12 \cdot 55 \pm 0 \cdot 20$ (22) and for the medulla: $8 \cdot 56 \pm 0 \cdot 17$ (22) μl./hr.mg dry weight, while in the new-born rat (24 hr old) they were $10 \cdot 99 \pm 0 \cdot 46$ (12) and $9 \cdot 33 \pm 0 \cdot 18$ (9) μl./hr.mg dry weight respectively.

3. Values for $Q_{CO_2}^{N_2}$ (anaerobic glycolysis) in the 14 hr old new-born rat were in the renal cortex $9 \cdot 65 \pm 0 \cdot 35$ (5) and in the medulla $7 \cdot 39 \pm 0 \cdot 43$ (5) μl./hr.mg dry weight; while in the adult they were $2 \cdot 25 \pm 0 \cdot 08$ (16) and $5 \cdot 76 \pm 0 \cdot 14$ (16) μl./hr.mg dry weight, respectively.

4. In the adult guinea-pig values for $Q_{CO_2}^{N_2}$ were of the same order as in the adult rat, though the rate of O_2 uptake was for the cortex $8 \cdot 12 \pm 0 \cdot 22$ (12) and for the medulla $5 \cdot 02 \pm 0 \cdot 23$ (11) μl./hr.mg dry weight.

5. Though the Q_{O_2} values in the renal cortex and medulla were smaller in the 1 hr old new-born guinea-pig, they were already increasing in the 12 hr old neonate.

6. The results are discussed in the light of enzyme changes occurring during the process of maturation of the nephron as indicated by histochemical observations.

INTRODUCTION

Whereas adult mammals are known to have a low resistance to anoxia, new-born animals, with the possible exception of guinea-pigs, exhibit a remarkable tolerance to lack of oxygen. This is true also of their isolated tissues (Fazekas, Alexander & Himwich, 1941). In the kidneys of adult rabbits, rats and guinea-pigs, the metabolism of slices cut from the cortex

is predominantly aerobic, with high rate of oxygen consumption and low rates of anaerobic glycolysis, and it has been shown by Whittam & Davies (1954) that in slices from the renal cortex of adult guinea-pigs the energy derived by anaerobic glycolysis is only 1 % of that supplied by aerobic metabolism and is about one sixth of the minimum required for the active transport of sodium and potassium at the rates found aerobically (Whittam, 1960). In the new-born rabbit the rate of anaerobic glycolysis of the renal cortex has been found to be of the order of fivefold greater than that of adult animals (Whittam, 1961). As the age of the neonate increases, the rate of anaerobic glycolysis in slices of the renal cortex falls. Thus, in the young rabbit, it takes between 3 and 4 weeks for the high rate of glycolysis of the renal cortex to fall to values found in the adult animal (Whittam, 1961).

McCance & Stanier (1960) showed that the rabbit's foetuses, 9 days before term, and foetal pigs of 90 days gestation, excrete a urine which differs in its composition from the animal's own plasma and from the urine of the adult animal: its urea concentration is about three times that of the plasma, while its concentration of chloride (and presumably of sodium) is only half that of the blood serum. Nearly 30 years ago, McCance & Young (1941) demonstrated that the sodium and chloride clearance during the first 2 weeks of life of a baby were only about one fifth of those of adults, and that in the new-born in general the renal tubules reabsorb more of the sodium and chloride from the glomerular filtrate than adults do (McCance & Widdowson, 1953). An increased rate of tubular reabsorption assumes a greater supply of energy; this, however, is difficult to reconcile with a low oxygen uptake, which has been found in the kidney of new-born rats and rabbits (Robinson, 1949; Whittam, 1960).

Though rates of oxygen uptake and of anaerobic glycolysis have been published for the new-born guinea-pig, rabbit and rat, they were usually estimated on slices made across the kidney, thus without differentiating between the contribution from the cortex and the medulla (Robinson, 1949) or on slices cut from the cortex only (Whittam, 1960). In view of the fact that the loops of Henle may not yet be developed, as in the rat (Bogomolova, 1965) or may be functioning normally soon after birth, as in the guinea pig (Dicker & Heller, 1951), it was thought of interest to reinvestigate the problem, with a view to explaining the apparent paradox which exists in the kidney of the new-born of a high rate of sodium reabsorption accompanied by a low rate of oxygen uptake.

METHODS

Animals used: adult rats of about 250 g body weight; new-born rats of 1, 5 and 21 days of age; adult guinea-pigs and new-born guinea-pigs of 1, 12, 21 and 24 hr. They were all killed by decapitation and bled. The kidneys cleaned from the perirenal fat were quickly removed and immersed in cold Robinson Ringer solution for about 5 min. They were then cut longitudinally into two halves and using a Stadie & Riggs (1944) microtome slices of approximately 0·25 mm were cut from the renal cortex and medulla, the slices from the medulla being cut at right angles to the cortico-papillary axis. Slices from the medulla were taken usually from the upper two thirds of the axis, though in a few experiments in adult animals slices from the lower third were also made. For the adult about 30 mg of cortical and 40 mg of medullary tissue were put in the Warburg flasks while for new-born animals the weight of slices did not exceed 20 mg (Dicker & Shirley, 1970).

For measurement of oxygen uptake Robinson (1949) medium, buffered at pH 7·4 was used. Its composition was in terms of mM: Na^+: 140, K^+: 5, Ca^{2+}: 2·5, Mg^{2+}: 1, Cl^-: 144, PO_4^3: 3 and SO_4^2: 1. Glucose (1 mg/ml.) was added. The medium was gassed with 100 % oxygen. For the estimation of rate of glycolysis Whittam's (1960) solution was used. The latter contained in terms of mM: NaCl: 150, KCl: 5, $NaHCO_3$: 25, glucose 10.

For measurements of both rates of oxygen uptake and of anaerobic glycolysis, a Warburg apparatus was used, and the standard method followed. All estimations were made at 37° C, but for those experiments in which the effects of temperature on the Q_{O_2} were investigated, in which the temperature of the bath of the Warburg apparatus was 25 or 15° C. For water content estimations, slices from the renal cortex and medulla of known weight were dried in an oven at 104° C for 48 hr, by which time the weights were found to be constant.

RESULTS

The results represented in Table 1, 2 and 3, show that in the adult rat and guinea-pig the oxygen uptake of slices from cortex was between 40 and 60 % higher than in slices from the medulla. In twelve experiments in which slices of the medulla were taken from the lower third of the cortico-papillary axis, this ratio increased to about 100 % in the rat, thus indicating a clear gradient between the low rate of oxygen consumption in the inner medulla ($1·4 \pm 0·12$ (9) μl./hr.mg wet tissue) and the higher rate in the cortex.

In the new-born rat, the Q_{O_2} (μl./hr.mg) was significantly lower than in the adult animal, and was of a similar magnitude in both the cortex and the medulla. It was only when neonate rats were 21 days old that values for Q_{O_2} were comparable with those observed in the adult (Table 2). Lowering the temperature of the Warburg bath to 15° C resulted in a decrease of oxygen uptake of the cortex of some 52 % and of the medulla 65 %, for the adult rat. In the 5-day-old neonate, however, the decrease was of the order of 80 % for both cortex and medulla (Table 1). Though in the 1 hr old guinea-pig values for Q_{O_2} in the cortex were lower by some 30 % than in the

TABLE 1. Effect of temperature on the rate of oxygen consumption by slices from the renal cortex and medulla of adult and new-born rats and guinea-pigs

Temperature (°C)	Rats		Guinea-pigs	
	Cortex	Medulla	Cortex	Medulla
	(μl./hr.mg dry wt.)		(μl./hr.mg dry wt.)	
Adult				
37	12·55 ± 0·20 (22)	8·56 ± 0·17 (22)	8·12 ± 0·22 (12)	5·02 ± 0·23 (11)
25	5·42 ± 0·22 (12)	4·12 ± 0·13 (12)	3·47 ± 0·08 (12)	1·80 ± 0·07 (12)
15	2·38 ± 0·08 (12)	1·34 ± 0·04 (11)	—	—
New-born*				
37	10·10 ± 0·23 (17)	10·24 ± 0·30 (14)	9·70 ± 0·44 (5)	5·80 ± 0·29 (5)
15	1·24 ± 0·10 (10)	1·33 ± 0·17 (10)	—	—

Q_{O_2} is expressed as μl./hr.mg dry wt. Values are means ± S.E.
* The new-born rats were 5 days old, while the guinea-pigs were 24 hr old only. In parentheses, number of experiments.

TABLE 2. Rate of oxygen uptake by slices from the renal cortex and medulla of new-born and adult rats and guinea-pigs expressed per mg wet and mg dry tissue weight

	Cortex (μl./hr.mg wet wt.)	Medulla (μl./hr.mg wet wt.)	Cortex (μl./hr.mg dry wt.)	Medulla (μl./hr.mg dry wt.)	Water content Cortex (%)	Water content Medulla (%)
Rat						
Adult	2·85 ± 0·05 (22)	1·85 ± 0·04 (22)	12·55 ± 0·20 (22)	8·56 ± 0·17 (22)	77·3 ± 0·5 (5)	78·5 ± 0·3 (5)
24 hr	1·88 ± 0·08 (12)	1·53 ± 0·03 (9)	10·99 ± 0·46 (12)	9·33 ± 0·18 (9)	82·9 ± 0·2 (5)	83·6 ± 0·2 (5)
5 days	1·95 ± 0·04 (17)	1·69 ± 0·05 (14)	10·10 ± 0·23 (17)	10·24 ± 0·30 (14)	80·7 ± 0·1 (8)	83·5 ± 0·1 (8)
21 days	2·54 ± 0·09 (9)	1·38 ± 0·14 (9)	11·54 ± 0·41 (9)	7·08 ± 0·71 (9)	77·2 ± 0·3 (6)	82·1 ± 0·6 (6)
Guinea-pig						
Adult	1·99 ± 0·06 (12)	1·06 ± 0·05 (11)	8·12 ± 0·22 (12)	5·02 ± 0·23 (11)	75·5 ± 0·1 (8)	78·9 ± 0·3 (8)
1 hr	1·23 ± 0·08 (5)	0·70 ± 0·05 (6)	6·18 ± 0·38 (5)	4·32 ± 0·30 (5)	80·1 ± 0·2 (8)	83·8 ± 0·2 (8)
12 hr	1·72 ± 0·08 (6)	0·82 ± 0·02 (6)	8·82 ± 0·40 (6)	5·00 ± 0·12 (6)	80·5 ± 0·1 (6)	83·6 ± 0·3 (6)
21 hr	1·76 ± 0·09 (6)	0·82 ± 0·04 (6)	8·71 ± 0·47 (6)	4·82 ± 0·22 (6)	79·8 ± 0·2 (6)	83·0 ± 0·4 (6)
24 hr	1·96 ± 0·09 (5)	0·98 ± 0·05 (5)	9·70 ± 0·44 (5)	5·80 ± 0·29 (5)	79·8 ± 0·4 (6)	83·1 ± 0·4 (6)

All measurements were made at the temperature of 37° C, and results are given as means ± S.E. In parentheses, number of experiments.

adult animal, in the 12 hr old neonate the Q_{O_2} in the cortex was already of the same order of magnitude as in the adult.

Whittam (1961) had shown that in slices from the renal cortex of the new-born rabbit, there was an exponential fall in $Q_{CO_2}^{N_2}$ with age. Similar results were found for the new-born rat and guinea-pig. In the day-old rat, the rate of anaerobic glycolysis in the cortex was some fivefold that in the adult, and in the 1 hr old guinea-pig the $Q_{CO_2}^{N_2}$ was of the order of four times that in the adult animal (Table 3).

TABLE 3. Rate of glycolysis ($Q_{CO_2}^{N_2}$) in the renal cortex and medulla in new-born and adult animals

$Q_{CO_2}^{N_2}$ in $\mu l./hr.mg$ dry wt.

	Cortex	Medulla
Rat		
Adult	$1 \cdot 91 \pm 0 \cdot 9$ (10)	$6 \cdot 52 \pm 0 \cdot 29$ (10)
New-born (1 day)	$9 \cdot 65 \pm 0 \cdot 35$ (5)	$7 \cdot 39 \pm 0 \cdot 43$ (5)
New-born (5 days)	$5 \cdot 08 \pm 0 \cdot 21$ (9)	$7 \cdot 45 \pm 0 \cdot 42$ (11)
Guinea-pig		
Adult	$2 \cdot 25 \pm 0 \cdot 08$ (16)	$7 \cdot 76 \pm 0 \cdot 14$ (16)
New-born 1 hr	$8 \cdot 89 \pm 0 \cdot 15$ (6)	$13 \cdot 33 \pm 0 \cdot 43$ (6)
New-born 24 hr	$5 \cdot 14 \pm 0 \cdot 25$ (6)	$11 \cdot 48 \pm 0 \cdot 30$ (6)
New-born 120 hr	$3 \cdot 68 \pm 0 \cdot 13$ (6)	$6 \cdot 97 \pm 0 \cdot 15$ (6)
Rabbit*		
Adult*	$3 \cdot 70 \pm 0 \cdot 50$ (7)	—
New-born*	$25 \cdot 00 \pm 2 \cdot 00$ (5)	—

Results are given as means $\pm$ S.E. In parentheses, number of estimations.
* These data have been taken from Whittam (1961) and are given as a comparison with those on rats and guinea-pigs.

DISCUSSION

Rates of aerobic metabolism and of anaerobic glycolysis were estimated not only in slices from the renal cortex but also from the medulla of the adult and new-born rat and guinea-pig, and the present data fill a gap, since no simultaneous values for Q_{O_2} and $Q_{CO_2}^{N_2}$ appear to be available for the medulla of rats or for the new-born rat and guinea-pig. Values for rate of oxygen consumption in the cortex of adult rats and guinea-pig were of the same order, though not identical with those reported by Whittam (1960) though values for anaerobic glycolysis reported here are lower than those found by him in the adult animal. However, values for $Q_{CO_2}^{N_2}$ in the new-born rat reported here are not markedly different from those published by György, Keller & Brehme (1928) and Whittam (1960). Though no explanation can be offered for these variations it will be remembered that similar variations have been reported by other authors using the same manometric method (Wu, 1965).

It is clear from the results presented that the rate of oxygen uptake of slices cut through the renal cortex of new-born animals, rats and guinea-pigs, is markedly lower than that of adult animals though for the guinea-pig this seems to be true only during the first few hours after birth. In both species, the low rate of oxygen consumption found in both the cortex and the medulla is associated with a high water content of the tissue and this is interesting in view of the well established inverse relation that seems to exist between tissue water content and their rate of respiration (Robinson, 1950). In the rat, it would appear that it takes about 3 weeks before values for Q_{O_2} and water content become comparable with those of the adult animal.

The problem that faces the new-born rat, and to some lesser extent the guinea-pig during its first hours of life, is that of the energy necessary for the active transport of electrolytes, since from all evidence available the rate of tubular reabsorption of sodium and chloride appears to be greater in the neonate than in the adult. It will be remembered that oxidative enzymes, particularly succinic dehydrogenase and acid phosphatase are not only active in the renal tubules of the new-born rat and rabbit but that their presence has been interpreted as an expression of immaturity, since with the development of zonal differentiation this enzyme activity disappears (Wachstein & Bradshaw, 1965). On the other hand, no adenosine triphosphate has been detected in the tubules. Bogomolova (1965) who also made a thorough study of the histochemical changes with age of the kidney of the rat, described in the new-born rat the presence of glycogen in the apical part of the cells lining the tubules and the collecting ducts, and commented on its disappearance when functional and anatomical differentiation of the nephron is achieved. As for the kidney of the guinea-pig which is usually described as mature at birth, it is of interest to note that it lacks glomerular adenosine triphosphatase activity, though as a whole its pattern of tubular enzymic activity resembles that found in adult mammals.

The presence in the tubules of kidneys of new-born rats of glycogen and oxidative enzymes and their progressive disappearance during the 2–3 weeks following birth are closely correlated with the initial high rate of anaerobic glycolysis and its subsequent decrease to levels found in the adult animal. It will be remembered that 57 kcal of free energy are released in the fermentation of a 6-carbon unit of glycogen, but that nearly two thirds of the free energy is retained in a form suitable for use in coupled reaction. Since the product of both respiration and anaerobic glycolysis is adenosine triphosphoric acid (ATP) it may be assumed that a supply of ATP from either metabolic pathway may be used for active transport systems.

Whittam & Davies (1954) have shown that it is not possible to calculate energy requirements for steady-state exchanges of ions from kinetic and thermodynamic data without information on the mechanisms responsible for the exchange. It will, however, be evident from the present results, that in the new-born rat the lack of energy resulting from a lower rate of oxygen uptake is amply made up by the increase in the rate of anaerobic glycolysis in the renal cortex and to a lesser extent in the medulla. This might be equally true for the new-born guinea-pig, but only during the first hours after birth. The observation that immediately after birth, the kidneys of the guinea-pig, as assessed by their rate of aerobic and anaerobic metabolism and their water content, are not fully mature is of interest, and agrees with the observations of Dicker & Heller (1951). Of equal interest, of course, are the observations showing that changes in enzyme activity in the kidneys of the new-born guinea-pig occur in a matter of a few hours after birth, whereas similar changes appear to take up to 21 days in the new-born rat and rabbit (Whittam, 1961).

From the present investigation, it would appear then that the apparent paradox observed in new-born animals of excreting a urine poor in sodium chloride is explained by the greater amount of energy derived from anaerobic glycolysis which would supplement the deficiency of energy if the latter was derived from aerobic metabolism only. This then would explain the observation by McCance & Young (1941) and by McCance & Widdowson (1953) that in the new-born, in general, the renal tubules reabsorb more sodium and chloride from the glomerular filtrate than adults do.

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