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THE METABOLISM OF KIDNEY SLICES FROM NEW-BORN AND FULL-GROWN RATS

By M. CUTTING (*Beit Memorial Research Fellow*) and R. A. McCANCE
From the Department of Medicine, University of Cambridge

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In the last few years the infantile human kidney has been shown to function in many respects rather differently from the adult organ. Thus, in the infant, the glomerular filtration rate seems to vary with the hydration of the body, and, on the basis of surface area, the glomerular filtration rates and also the urea clearances are lower (Barnett, 1940; Barnett, Perley & McGinnis, 1942; McCance & Young, 1941; Young & McCance, 1942). There is a structural basis for some of these functional differences, for Clara (1936) and Grunewald & Popper (1940) have shown that the glomerular tufts at birth are covered with thick columnar or cubical cells through which filtration must be relatively slow. These cells were described and pictured at least 72 years ago in an English text-book of histology (Klein, Burdon-Sanderson, Foster & Brunton, 1873), so that they are no new discovery (McCance, 1943), but it has taken physiologists a long time to appreciate their importance. Furthermore, the Na, K and Cl clearances are lower at birth than the corresponding clearances in later life, and this suggests tubular differences, a view which is supported by studies of the osmotic pressure of the urine in infancy and also by observations on the response to posterior pituitary extract (Heller, 1944). In order to investigate some of these aspects of developmental physiology from another angle it was decided to compare the O_2 uptake and the metabolism of the kidneys in new-born and mature animals by means of tissue slices. The experiments described in this paper have been carried out on rats, but kidneys from cats, pigs and human beings are also being investigated.

Method

Technique

The adult animals were killed by severing the carotid artery. The new-born animals, which were always used within 24 hr. of birth, were killed by decapitation. The kidneys were removed immediately and transferred to a dish containing a Ringer-phosphate solution. This was a modification of that used by Krebs (1933) and was made up in the following way: To 103 vol. of 0·9% NaCl were added 4 vol. of 1·15% KCl, 1 vol. of 3·82% $MgSO_4, 7H_2O$, 1 vol. of 2·11% KH_2PO_4

and 21 vol. of phosphate buffer, *p*H 7·4, prepared by taking 40 c.c. 0·025 M-Na_2HPO_4 and 0·5 c.c. 2 N-HCl and diluting to 100 c.c. Slices of kidney, approximately 0·3 mm. in thickness, were cut, trimmed as quickly as possible and transferred to the metabolism bottles of a Barcroft apparatus. The total volume of fluid in the outer compartment was 2·7 c.c., the basis of which was Ringer phosphate. The inner compartment contained 0·3 c.c. of 20% NaOH and a small roll of Whatman no. 40 filter paper (Dixon, 1943). The bottles were then attached to the manometers, the whole apparatus evacuated and then filled with O_2 before being transferred to a water bath at 38° C. (Dixon & Tunnicliffe, 1923). The bottles moved to and fro 125 times per min., and after 5 min. for equilibration, the taps were closed and the first reading taken. This procedure was followed in each experiment and the interval between the death of the animal and the first reading was always about 40 (±5) min.

The O_2 uptakes have been measured at 15 min. intervals for an hour, and a further reading taken after 2 hr., (*a*) without any additions at *p*H 7·4 and 6·8, (*b*) after adding the following hormones at *p*H 6·8 and 7·4: 0·3–0·9 unit of posterior pituitary extract ('Pitoxylin', manufactured by Messrs Oxo) and 0·05–0·5 mg. desoxycorticosterone phosphate (presented by Messrs Organon Ltd.), (*c*) in the presence of the following substrates at *p*H 7·4: 0·02 M-Na lactate, 0·02 M-Na succinate and 0·02 M-*dl*-methionine. R.Q. determinations were made by the Dixon-Keilin method (Dixon, 1943) and all estimations of N were carried out by the micro-Kjeldahl technique. All figures given in this paper are the average results of 6–10 experiments. Owing to the inherent variability of excised tissues, the control for each experimental substrate has always been set up from the kidneys of the same animal (or, if newly born, from the kidneys of litter mates). Consequently, figures given for the control experiments in Tables 2–5 are not always the same.

Expression of results

In work with tissue slices the respiration rate is usually denoted by the symbol Q_{O_2} and is defined as the c.mm. of oxygen (at N.T.P.) taken up per hr. per mg. dry weight of tissue. This is an unsatisfactory method for two reasons. First, the rate at which O_2 is taken up by kidney slices usually falls off with the passage of time so that measurement over the first 15 min. or 30 min. gives a result very different from the one given by measurement over the first hour or over the two hours together. In these experiments, readings have been taken every 15 min. at least for the first hour, and the rate over each interval of time expressed as the c.mm. of O_2 taken up per min. In the second place the slices tend to lose weight as they are shaken (Bach, 1944) and, in consequence, the results obtained vary with the time at which shaking has been stopped and the slices removed for drying and weighing. Some workers have preferred to express their O_2 uptakes per mg. initial wet weight (Bach, 1944; Elliott, Grieg & Benoy, 1937; Field, Belding & Martin, 1931; and others). This is a great improvement in principle but it is not an easy matter to weigh the freshly cut slices accurately, and so, in order to avoid both these difficulties, the present O_2 uptakes have been expressed per mg. of N in the freshly cut slices. This figure was obtained in the following way: At the end of the experiment the N was determined both in the slices and in the fluid in which they had been respiring. The analyses were made separately, and thus the total N originally present in the slices and the percentage of N which had diffused out of them during the experiment could be ascertained.

Table 1 shows the results expressed both per mg. final dry weight and per mg. total N in the slices taken for metabolism. Each comparison was made on kidneys from the same rat. The figures in the 1st and 2nd columns represent the O_2 uptakes per mg. of the final dry weight over the first 30 min. The figures

TABLE 1. A comparison of the O_2 uptakes of rat kidney slices expressed as c.mm./mg. final dry weight—or as c.mm./mg. N in the original slices. The figures represent the O_2 uptake during the first 30 min. of experiment

	c.mm./mg. final dry weight		c.mm./mg. total N			
	Slice weighed after 30 min.	Slice weighed after 120 min.	N determined after 30 min.	N determined after 120 min.	% loss of N in 30 min.	% loss of N in 120 min.
	11·6	16·9	81·5	82·5	18·9	38·6
	12·4	15·3	82·0	82·5	23·4	37·4
	12·3	14·1	81·0	77·5	19·3	34·4
	10·1	14·2	78·5	83·5	15·0	32·5
	11·5	17·0	80·0	90·0	13·4	35·4
	9·3	10·7	67·0	65·0	14·9	28·0
	11·2	13·3	82·0	81·5	17·8	29·8
	9·7	14·1	71·0	69·5	16·9	43·0
Av.	11·0	14·5	77·9	79·0	17·5	34·9

in the 1st column were obtained by removing the slices and drying them at the end of 30 min., and those in the 2nd column by removing them after they had been shaken for 2 hr. The figures in the 2nd column are higher because the slices from which these figures were derived had been allowed a further 1½ hr. in which to lose weight, and consequently their dry weights were proportionally lower than those of the slices which had only been shaken for 30 min. This is proved by columns 3 and 4, in which the same O_2 uptakes are expressed per mg. N in the tissues taken for metabolism. It will be seen that on this basis the fictitious differences have disappeared. Columns 5 and 6 show the amount of N that the tissues lost after being shaken for 30 and 120 min. respectively.

Stating the O_2 uptakes per mg. of N in the fresh slices has its advantages also when the organs of new-born animals are being compared with those of adults, for the tissues are more hydrated at birth than at maturity (Shohl, 1939). Indeed, the kidney of the new-born rat may contain only some 15–16% of dry matter, whereas that of an older animal as much as 25%. Hence fresh weight would be an unsatisfactory basis on which to compare these organs. It would, however, if used have the effect of lowering all the O_2 uptakes for the new-born tissues given in this paper.

RESULTS

Kidney slices

O_2 *uptake in Ringer phosphate without added substrate.* Fig. 1 shows the rates of O_2 uptake at *p*H 7·4 by slices of the kidney from mature and new-born rats. It will be noted that during the first 15 min. the adult tissues took up O_2 at

the rate of 3·0 c.mm./min. and those from the newly born animals at the lower rate of 2·0 c.mm./min. With the passage of time, however, the O_2 uptake of the adult kidneys fell off rapidly while that of the new-born did not, so that at the end of 1 hr. the uptakes of both were almost the same, and during the second hour the slices from the new-born rats took up O_2 more rapidly than those from the full-grown animals. Slices from rats aged 3 weeks showed characteristics both of the adult and new-born organs. Their O_2 uptakes

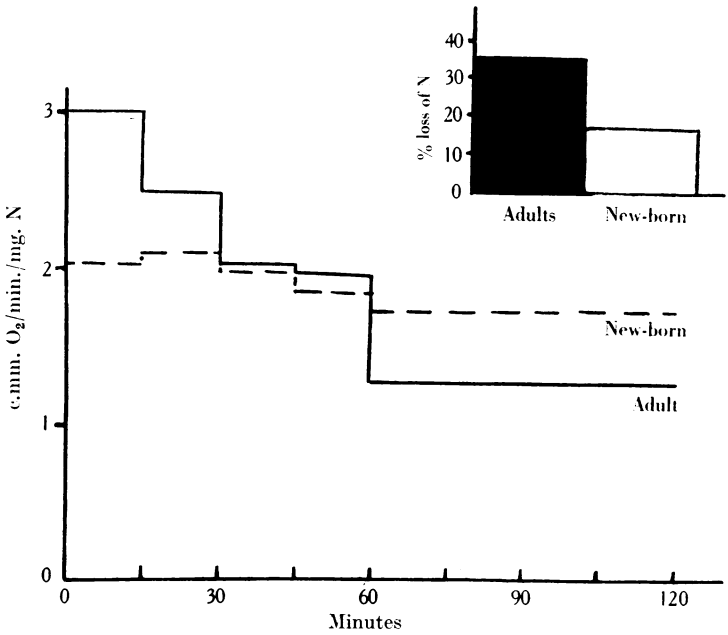

Fig. 1. The uptake of oxygen and loss of nitrogen by slices of rats' kidneys at *p*H 7·4.

averaged 3·2 c.mm./min. in the first 15 min., a little higher than those of the full-grown animals, but they fell off very slowly, a characteristic of the new-born, and were still 2·85 c.mm./min. in the last hour.

The percentage of N diffusing out of the slices taken from fully grown rats was large. In the experiments shown in Fig. 1 the average loss over a period of 2 hr. was 35%, but in other grouped experiments losses have averaged as much as 49%. Much less N came away from the kidneys of young animals. Slices from rats aged 3 weeks lost 27% of their N during the 2 hr. experiments, and those from new-born rats only 16·4%. Thus the amount of N which the slices lost depended very much upon the age of the animal, but the nature of the N did not, for at all ages 60% was in the form of protein (i.e. was precipitated by trichloroacetic acid), and there was practically no free NH_3 in the non-protein fraction.

Effect of pH. The experiments just described were carried out at pH 7·4. When the pH was lowered to 6·8, less N came away from the tissues of mature rats (Table 2). The O_2 uptake was little affected by this change for the first

TABLE 2. Effect of pH on the O_2 uptake and loss of N

		Rate of O_2 uptake, c.mm./min./mg. N					
Age	pH	0–15 min.	15–30 min.	30–45 min.	45–60 min.	60–120 min.	% loss of N
Mature rats	7·4	2·70	1·95	1·55	1·31	1·00	48·9
	6·8	2·75	2·25	1·85	1·73	1·50	35·8
New-born rats	7·4	2·3	2·11	1·94	1·99	1·85	16·4
	6·8	2·04	1·97	1·84	1·84	1·51	17·3

½ hr. but it fell off less rapidly in the last 1½ hr. so that the final O_2 uptake was a little higher. Lowering the pH from 7·4 to 6·8 slightly reduced the O_2 uptake of slices from new-born rats (Table 2). It did so equally over the first 15 min. and during the last hour. It had, however, no significant effect upon the loss of N.

Posterior pituitary extract. 0·3, 0·6 and 0·9 unit of posterior pituitary extract (Pitoxylin) were added to the Ringer phosphate solution in which the tissues were respiring. 0·9 unit appeared to be the most effective. This dose of Pitoxylin was investigated at pH 6·8 and 7·4, and may be said to have had three distinct but probably related effects upon the metabolism of the tissues from fully grown animals (Table 3). First, the O_2 uptake was considerably raised at both pH's, the initial level being slightly higher at pH 7·4.

TABLE 3. The effect of 0·9 unit Pitoxylin on the O_2 uptake and loss of N

		Rate of O_2 uptake, c.mm./min./mg. N					
Age	Additions and pH	0–15 min.	15–30 min.	30–45 min.	45–60 min.	60–120 min.	% loss of N
Mature rats	None at 7·4	2·70	1·95	1·55	1·31	1·00	48·9
	Pitoxylin at 7·4	4·15	3·54	3·45	2·86	2·26	38·9
	None at 6·8	2·75	2·25	1·85	1·73	1·50	35·8
	Pitoxylin at 6·8	3·87	3·70	3·62	3·44	3·30	23·4
New-born rats	None at 7·4	2·31	2·39	2·08	2·11	1·82	17·5
	Pitoxylin at 7·4	2·98	2·40	2·29	2·27	2·05	16·5
	None at 6·8	2·04	1·97	1·84	1·84	1·51	17·3
	Pitoxylin at 6·8	2·24	2·05	2·08	1·94	1·94	13·1

Secondly, at pH 6·8 the O_2 uptake fell off very slowly and finished considerably higher than it did at pH 7·4, or when no Pitoxylin had been added. In this the posterior pituitary extract appeared to enhance the effect of pH alone. Thirdly, Pitoxylin prevented the cells losing N. This may have been the essential feature of its action, and it certainly offers some explanation of the first two effects.

Pitoxylin slightly raised the O_2 uptake of slices from the kidneys of the new-born rats during the first 15 min. of the experiment when the pH was 7·4, but after that time the rate of respiration was very little greater than that of

the control slices to which no hormone had been added. At *p*H 6·8 Pitoxylin had similar effects, but there was a smaller increase during the first 15 min., and a slightly larger one during the second hour. When Pitoxylin was added to the slices from new-born animals, it did little to reduce their small losses of N. A small effect, however, was observed at *p*H 6·8.

Some of these effects on the adult tissues were undoubtedly due to the acetic acid which is present in commercial extracts of posterior pituitary. The *British Pharmacopoeia* specifies that sufficient acetic acid should be added to bring the extracting fluid to *p*H 3·0, and a concentration of 0·0038 M is sufficient to effect this. Such a concentration of acetic acid appreciably raised the O_2 uptake of the slices (Table 4) but did not raise it so high as Pitoxylin,

TABLE 4. Effect of Pitoxylin, acetic acid, extract of dried pituitary, etc. upon the tissues of mature rats at *p*H 6·8

Additions	Rate of O_2 uptake, c.mm./min./mg. N					
	0–15 min.	15–30 min.	30–45 min.	45–60 min.	60–120 min.	% loss of N
None	3·0	2·34	2·32	2·16	1·68	32·2
Commercial extract (Pitoxylin)	4·21	3·69	3·58	3·62	3·25	24·5
Acetic acid	3·52	2·94	2·91	2·96	2·41	31·4
Extract of dried gland	4·0	3·8	3·8	3·06	2·85	23·1
Extract of dried gland + acetic acid	4·15	3·90	4·04	4·07	3·78	21·6

nor did it prevent the slices from losing N. In the hope of disentangling some of these effects, acetone-dried posterior pituitary lobe was obtained. 0·3 g. of this material was well shaken with 9 c.c. 0·9% NaCl and centrifuged. 0·3 c.c. of the supernatant fluid was added to 2·4 c.c. of the Ringer phosphate solution in which the tissues were to respire. Respiration was measured in the presence of this extract of posterior pituitary lobe both with and without the addition of 0·0038 M-acetic acid and the results are shown in Table 4. The extract alone raised the O_2 uptake, but the combination of acetic acid and the saline extract not only raised the O_2 uptake slightly more than the posterior pituitary alone, but succeeded in maintaining it at the raised level. This double effect is characteristic of the commercial extract, Pitoxylin, at *p*H 6·8 (Table 3). All posterior pituitary extracts seemed to decrease the loss of N by the slices.

The oxytoxic and pressor activities of a posterior pituitary extract are destroyed by adding an equal volume of 2 N-NaOH to it, allowing to stand for 1 hr. and then neutralizing before use, but this treatment when applied to Pitoxylin did not destroy the factor, or factors, affecting the metabolism of the kidney slices. It is concluded, therefore, that the respiratory effects were brought about by some unrecognized substances in the extracts. It is possible that the effects were due simply to the addition of respirable nitrogenous material, but if so 0·9 unit of Pitoxylin, which contained only 0·06–0·07 mg. N, must have provided enough to saturate the enzyme systems, for the saline extracts contained from 0·25 to 0·37 mg. N, and their effects were very much the same.

Desoxycorticosterone phosphate. The best known salt of desoxycorticosterone is the acetate, but this is practically insoluble in water and was, therefore, unsuitable for the purpose in hand. The phosphate, however, is soluble and its effects were investigated at two concentrations and at *p*H 6·8 and 7·4. When 0·5 mg. of the hormone was added to the Ringer phosphate solution it was found always to lower the O_2 uptake of the adult tissues, slightly at *p*H 6·8 and very considerably at *p*H 7·4 (Fig. 2). In this connexion it is interesting

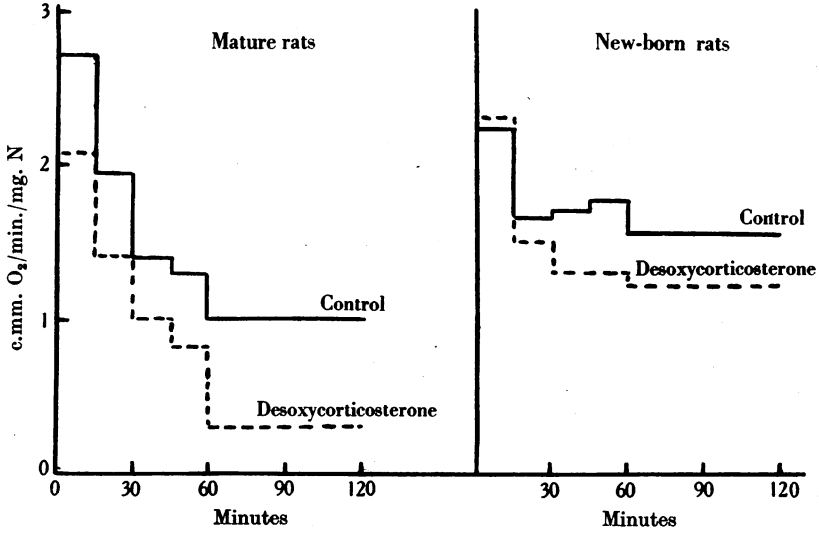

Fig. 2. The effect of 0·5 mg. of desoxycorticosterone phosphate on the uptake of oxygen by kidney slices at *p*H 7·4.

to note the toxicity of large doses upon the kidney of the intact animal (Selye, Hall & Rowley, 1945). 0·5 mg. slightly lowered the respiration rate of slices from new-born rats but its effect was less than in later life. Whatever the age of the animals 0·05 mg. did not alter the O_2 uptake at either *p*H. This hormone had little influence on the loss of N by kidney slices though at all ages it tended to decrease it at *p*H 6·8 when the dose was 0·5 mg.

Succinate, lactate and dl-*methionine.* The effect of adding 0·02 M concentrations of these compounds to the Ringer phosphate solution in which the slices from adult animals were respiring is shown in Table 5. These experiments have only been carried out at *p*H 7·4, and except in the case of lactate, at only one concentration. Doubling the dose of lactate had no greater effect. All these substrates raised the O_2 uptake considerably. Their effects, however, were rather different. Lactate raised it in the first 15 min. only from 3·0 to 4·0 c.mm./min., but the rate was still above 3 in the second hour, whereas the uptake in Ringer phosphate alone had fallen to 1·27. Succinate and methionine raised the initial rate to about 5·2 and 4·3 c.mm./min. respectively, but they

TABLE 5. The effect of lactate, succinate and *dl*-methionine at *p*H 7·4

Age	Substrate	Rate of O_2 uptake, c.mm./min./mg. N: 0–15 min.	15–30 min.	30–45 min.	45–60 min.	60–120 min.	% loss of N
Mature rats	None	3·01	2·48	2·02	1·97	1·27	35·3
	0·02 M-lactate	4·02	3·59	3·73	3·34	3·32	32·0
	0·02 M-succinate	5·16	4·55	3·74	3·60	2·76	37·2
	0·02 M-*dl*-methionine	4·3	3·05	2·66	1·99	1·24	38·7
New-born rats	None	2·03	2·12	1·99	1·85	1·72	17·6
	0·02 M-lactate	2·72	2·58	2·58	2·64	2·44	18·7
	0·02 M-succinate	3·40	3·00	2·94	2·79	2·53	22·0
	0·02 M-*dl*-methionine	2·11	2·18	2·05	2·03	1·86	18·4

did not maintain it nearly so well as lactate. The O_2 consumption with succinate fell to 2·5 c.mm./min. in the second hour, but with methionine it fell to 1·24, the same as that of the control. Table 5 also shows that these 3 compounds did little to raise the O_2 uptakes of the tissues of new-born animals. If each result is considered as a percentage of the control value, the initial effects of succinate and lactate were not so different from those found for the mature animals, but, however the data be expressed, the influence of the substrates during the 2nd hour was very much less. The uptake in the presence of lactate fell off very little, if at all, and the uptake in succinate was passably well maintained, but the uptake of the controls also remained relatively steady. The effect of methionine was trifling throughout. The substrates did not appreciably alter the losses of N either at birth or maturity.

Liver slices from adult and new-born rats

The O_2 uptake of liver slices taken from mature and new-born animals has also been studied. In Ringer phosphate solutions at *p*H 7·4 the rate at which mature liver tissue took up O_2 did not fall off as it did so characteristically with the kidney (Table 6). Furthermore, the O_2 uptake of the tissues from the new-

TABLE 6. Rate of O_2 uptake by liver tissue, c.mm./min./mg. N

Age	Substrate	0–15 min.	15–30 min.	30–45 min.	45–60 min.	60–120 min.	% loss of N
Adult rats	None	1·52	1·31	1·31	1·33	1·21	29·5
	Pitoxylin, 0·9 unit	1·75	1·41	1·31	1·33	1·20	27·3
New-born rats	None	1·78	1·73	1·75	1·73	1·46	32·0

born rats was as high or higher throughout the whole period of observation. Posterior pituitary extract has also been added to the liver slices taken from adult animals—largely to control the observations made on the kidney. The effects were negligible. The loss of N from the liver slices was not so great as it was from adult renal tissues; it was the same at birth as in adult life, and was unaffected by pituitary extracts.

Discussion

These experiments have demonstrated that the kidney slices of a new-born animal may behave very differently from those of an adult of the same species. These differences are certainly complicated and they may be interrelated. Considered as a whole the metabolism of the new-born kidney seems to be characterized by a stability and independence which are absent from that of the adult organ. Thus the O_2 uptake is well maintained without the addition of substrates, responds poorly or not at all to substances which raise the uptake of the adult organ, and resists agents which depress the adult rate. In fact, most of the present results could be explained by supposing that the new-born cells survived removal, section and shaking in Ringer phosphate solution much better than the adult tissues. How far some of these differences can be related to the fact that the new-born tissues lose less N to the surrounding medium it is not possible to say. It is, however, certain (from the examination of the sections) that the adult tissues undergo profound histological disorganization during the two hours in the respiration chamber, whereas the new-born tissues remain relatively normal.

It is generally recognized (Needham, 1931; Lightbody, 1938) that the enzymes characteristic of the adult state appear at different ages, and develop at different rates: the small response of the new-born tissues to added substrates suggests that their specific enzyme systems are not yet fully developed at birth. It seems probable, however, that the respiration of the kidney slices from young and old rats follows the same final path, for 0·001 M-CN prevented any O_2 being taken up at either age. Furthermore, the R.Q. of the tissues at birth, determined over a 30 min. period, averaged 0·913 (0·88 and 0·95) and at maturity 0·920 (0·90 and 0·94), which indicates that similar materials were being oxidized at both ages. At neither age was there any aerobic glycolysis. Nevertheless, young animals may have a source of energy other than the usual aerobic processes. Anaerobic glycolysis by the rat's kidney has been shown to be greatest before birth and is still active at birth although negligible later in life (Needham, 1931). The work of Himwich and others (Fazekas, Alexander & Himwich, 1941; Himwich, Baker & Fazekas, 1939; Himwich, Bernstein, Herrlich, Chester & Fazekas, 1942; Himwich, Fazekas & Alexander, 1941) has shown that the prolonged survival time of infant rats in an atmosphere of N is due to their low cerebral metabolism and to some anaerobic source of energy.

It has previously been considered that the O_2 consumption is higher the younger the animal (Davis, 1937; Schuler, 1943; Pearce, 1936), but these authors did not measure the metabolism of rats (or mice) under the age of 4 weeks. Tyler & van Harreveld (1942), however, measured the respiration of the developing rat's brain, and showed that the O_2 uptake was lowest during the first 7 days of life, after which it rose, and reached a maximum between

the 4th and 7th week. Thereafter it gradually fell off, but always remained considerably higher than it had been at birth and stabilized itself at the adult level at about the 20th week of life. These results are to be compared with the present findings that over the first 15 min. in the manometer the O_2 uptake by kidney slices is lowest at birth, but may be as high or higher at the age of 3 weeks than at maturity.

SUMMARY

1. A study of tissue slice technique has shown that it is better to express results per mg. total N in the fresh slices rather than per mg. final dry weight.

2. A comparison of the O_2 taken up in Ringer phosphate solutions by kidney slices from new-born and mature rats has shown that these tissues differ in the following ways:

(*a*) At maturity, the O_2 uptake begins at about 3 c.mm./min./mg. N but soon declines, and the slices lose from 40–50% of their N in 2 hr. A change in *p*H from 7·4 to 6·8 has little effect on the O_2 uptake, but decreases the amount of N lost. At birth, the O_2 uptake is initially less rapid, but this lower rate is well maintained for 2 hr., and the loss of N is only some 17%. The uptakes and losses of N are almost unaffected by a change in *p*H from 7·4 to 6·8.

(*b*) Posterior pituitary extracts diminish the loss of N and raise the O_2 uptake, and large doses of desoxycorticosterone phosphate depress the O_2 uptake of mature tissues. These additions make little difference to the renal tissues of newly born rats. Possible reasons for these and other effects of these substances have been discussed.

(*c*) The addition of various substrates such as 0·02 M-lactate, -*dl*-methionine, or -succinate raises the O_2 consumption of kidney slices from mature rats, but has less effect on those from new-born rats.

3. Slices from the livers of new-born rats took up as much or more O_2 as those from the livers of mature animals over the whole period of observation, and lost about the same percentage of their N. Posterior pituitary extracts had no effect upon the O_2 uptake or the loss of N.

Messrs Oxo, Ltd. generously presented us with the dried post pituitary gland and Messrs Organon, Ltd. the desoxycorticosterone phosphate.

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