An Investigation of Renal Function during Histogenesis in the Late Foetal and Neonatal Rat by means of the Uptake and Autoradiographic Localization of Radioactive Potassium

by J. G. BEARN[1] *and* F. T. C. HARRIS[2]

From the Departments of Anatomy and Biology as Applied to Medicine, The Middlesex Hospital Medical School

WITH ONE PLATE

INTRODUCTION

EXPERIMENTAL surgery on the foetal rat in the last 2 days of gestation shows that a hypotonic urine is produced by the kidney (Wells, 1946). A study has been made with radioactive potassium (K^{42}) of the rate of exchange, and also the localization, of this isotope in the kidney of the 20- and 21-day foetal and of the neonatal rat.

The rate of exchange of potassium from the blood-stream to the tissues can be studied by injecting small amounts of the radioactive isotope as bicarbonate into the circulation. The rate at which the adult rat kidney exchanges K^{42} is higher than that of any other organ (Walker & Wilde, 1952; Ginsburg & Wilde, 1954). In the first 2 minutes following intravenous injection, the radioactivity of the kidney is eight times that of the liver (Ginsburg & Wilde, 1954), and the uptake of the isotope is mainly restricted to the adult cortex (Morel & Guinnebault, 1956). Within the cortex the potassium is cleared from the plasma through the glomeruli into the lumen of the proximal convoluted tubules. Here it is reabsorbed from the glomerular filtrate and then, depending on its concentration in plasma, the potassium may be excreted through the distal tubules (Walker *et al.*, 1941). The localization of radioactive potassium in the cells of the adult proximal and distal tubules has been demonstrated by autoradiography of kidney sections taken 2 minutes after intravenous injection (Eisen & Harris, 1957).

Owing to the importance of conserving potassium it might be expected that the new-born rat has already developed this capacity for concentrating potassium in the tubular cells. This has been investigated using both the exchange rate of K^{42} by the kidney relative to the liver, and autoradiography.

Authors' addresses: [1] Department of Anatomy, and [2] Department of Biology as Applied to Medicine, The Middlesex Hospital Medical School, London, W. 1, U.K.

J. Embryol. exp. Morph. Vol. 9, Part 2, pp. 335–341, June 1961]

MATERIAL AND METHODS

For this investigation both foetal and neonatal kidneys were studied. The foetal kidneys were taken from gravid females which were within 1 or 2 days of estimated term, and the neonatal kidneys were taken from young rats 3 hours after delivery.

The determination of the uptake of K^{42} by liver and kidney

The foetal rats were obtained from twelve Wistar females for whom the time of mating had been confirmed by the presence of sperm in vaginal smears. The carrier for radioactive potassium was potassium carbonate, which was dissolved in physiological saline and diluted to contain 100 μc. of the isotope in each ml. at a standard reference time. One ml. of the solution was injected into the tail vein of each pregnant rat under light ether anaesthesia. Seven gravid females were injected on the 20th day and five on the 21st day of pregnancy. Two minutes after the end of the injection two foetuses were removed by caesarian section from each rat. The injection took 15 seconds to complete. Each foetus was killed immediately by an incision into the heart, and both kidneys were removed together with two liver specimens and placed on frozen carbon dioxide immediately to prevent loss of weight due to dehydration. The time taken to kill the foetus and extract the tissues varied between $1\frac{1}{2}$ and 2 minutes.

Six new-born rats were obtained from three litters and 0·1 ml. of a solution of the isotope containing 25 μc./ml. was injected into a superficial neck vein of each new-born rat. Kidney and liver tissue were obtained from these animals by the procedure described.

The tissues were then weighed to the nearest 0·1 mg. on a torsion balance. They were digested in 1 ml. of concentrated nitric acid and incubated for 1 hour at 50° C. The samples were made up to 5 ml. with distilled water and their radioactivity determined in a Beta Particle G.M. counter. The results were expressed as the ratio of the number of counts of K^{42} per minute per milligramme of foetal kidney to the number of counts of K^{42} per minute per milligramme of foetal liver.

The determination of the potassium content of liver and kidney

The total amount of potassium per milligramme of foetal kidney and liver was estimated in six foetuses and six new-born animals, using a Warren Flame Spectrophotometer (Warren, 1959). It is not advisable to use strongly acidic extracts in spectrophotometers, and, to avoid the possible errors that might be associated with dilution, neutralization, and subsequent concentration of nitric acid digests, an alternative technique was used in the case of these estimations. The liver and kidneys were ashed in a muffle furnace and extracted with potassium-free dilute acetic acid, and the total potassium then determined with the flame spectrophotometer. All dilutions were made with glass distilled water stored in polythene containers. The potassium content of the distilled water used in each

series of dilutions was estimated on a blank sample of distilled water and was allowed for. In order to check the accuracy of the spectrophotometric analysis, the estimations were made on two dilutions of each solution, one series of dilutions being twice that of the other, and three readings were made for each dilution.

The histological localization of the site of exchange of K^{42}

Autoradiograms of rat adult kidney sections were prepared by the technique described by Eisen & Harris (1957) to determine the intrarenal distribution of K^{42}. Autoradiograms of rat foetal kidney were prepared by a modification of this technique. The foetal kidneys were removed 2 minutes after the intravenous injection of 100 μc. of K^{42} into the tail vein of the mother.

RESULTS

The results of the determination of the total potassium content per milligramme of liver and of kidney showed no significant difference between these two organs. In view of this finding, it was unnecessary to determine the specific activity of the liver and kidney and their radioactivity was compared directly.

The radioactive determinations for the 20- and 21-day foetuses showed a significant difference between the rates of exchange of potassium by the kidney and liver, the rate of exchange of the kidney being almost twice that of the liver. The new-born rats also showed a significant difference in these relative rates of exchange, that of the kidney now being less than that of the liver (Table 1).

The distribution of K^{42} in the autoradiogram of the adult kidney showed a concentration of the isotope in the proximal and distal tubular cells (Plate, figs. A, B), this finding being similar to that of Eisen & Harris (1957). In the autoradiogram of the 21-day foetal kidney, the isotope was found to be uniformly distributed throughout the kidney (figs. C, D). No concentration could be demonstrated in the cells.

TABLE 1

The relative uptake of K^{42} *by the liver and kidney of the rat foetus 2 minutes after intravenous injection into the mother at 20 and 21 days of pregnancy, and into the new-born rat*

	Number of foetal or neonatal rats	*Number of kidneys*	*Number of liver samples*	**R* ±*S.E.*
20-day foetus .	14	27	27	1·95 ±0·10
21-day foetus .	10	20	20	1·86 ±0·12
Three hours after birth . .	6	10	10	0·39±0·10

**R* = The ratio of the counts of K^{42}/min./mg. of kidney to the counts of K^{42}/min./mg. of liver.

DISCUSSION

This investigation shows that the 20- and 21-day foetal metanephros does not have the high relative rate of K^{42} exchange found in the adult kidney, which exchanges K^{42} eight times faster than the liver (Walker & Wilde, 1952). We find that the rate of exchange in the late foetal kidney is twice that of the foetal liver and in the neonatal animal is less than that of the neonatal liver. In addition, the autoradiograms of the foetal kidney do not show any localization of the isotope in the foetal tubular cells.

These results indicate that the capacity for the high relative exchange rate and the ability to concentrate radioactive potassium in the proximal and distal tubular cells develops postnatally in the rat. At their face value the results suggest that this mechanism for potassium regulation does not exist in the foetal and neonatal rat kidney.

The comparison of exchange rates has been between kidney and liver. As the kidney exchanges K^{42} with plasma, ideally the radioactivity of the kidney should have been compared with plasma, but the technical difficulties of estimating the radioactivity of foetal plasma were too great. It is possible that the change in relative exchange rates is due to the foetal liver having a higher exchange rate than the adult liver. As no autoradiographic localization of K^{42} occurred in the foetal kidney, this suggests that the alteration in relative exchange rates is due to decreased activity in the kidney.

The capacity for a high early exchange rate in the adult is associated with the localization of the isotope in the proximal and distal tubular cells (Eisen & Harris, 1957). Studies on the state of histogenesis of the late foetal and neonatal kidney indicate that apparently mature tubular cells are present and capable of function at and before birth. Baxter & Yoffey (1948) found that in the neonatal rat the glomeruli are at different stages of maturity depending on their position in the cortex. The most mature nephrons are in the juxta-medullary region, whereas the more peripheral nephrons are still undergoing histogenesis. The tubular cells in the juxta-medullary region are capable of storing trypan blue, this storage being associated with a PAS-positive brush border. Davies (1954) studied the development of the rat kidney by histochemical techniques and found PAS-positive granules in the tubular cells of the foetal metanephros, and took this as indicative of reabsorption of protein from the glomerular filtrate by these cells.

Although these studies demonstrate the presence of apparently mature and functioning tubular cells in the foetal and neonatal kidney, in the 1st week after birth the renal cortex consists largely of glomerular tissue (Arataki, 1926). He found 1,000 glomeruli in each cubic millimetre of infant cortex compared with 50/cu. mm. in the adult, the infant glomerular diameter being half that of the adult. From this it appears that the tubular development of the foetal and neonatal kidney is very limited when compared with the adult, although it is possible that the mature tubules of the juxta-medullary region are capable of

concentrating potassium. If this were so, it might explain the higher K^{42} exchange rate of the foetal kidney compared to the foetal liver. However, localization of the isotope in the juxta-medullary region was not detected in the autoradiograms.

Care must be taken when making functional predictions based on evidence of morphological maturity. For example, in the rabbit, although the 19- and 21-day foetal kidneys appear morphologically identical, only the latter are capable of glomerular filtration and tubular reabsorption (Gersh, 1937). In spite of the maturity of the juxta-medullary tubular cells at birth (Baxter & Yoffey, 1948) the negative birefringence of the brush border is not fully developed until the 8th day of postnatal life in the rat (Olivecrona & Hillarp, 1949). The functioning of the kidney depends not only on its structural maturity but also on other factors such as the presence of hormones in the foetal blood and the circulation rate. In this connexion Hoy & Adolph (1956) found no response of the neonatal kidney to anti-diuretic hormone, and Barcroft (1946) has shown in the sheep that the foetal blood-pressure is lower than that of the postnatal animal.

We do not consider that the evidence presented in this paper is adequate to explain the decrease in the relative exchange rates of potassium in the neonatal animal compared to the foetus, an interval of only 24 hours. It is possible that this change may be due to alteration in the circulation through the liver occurring at birth rather than to change in renal function. Changes in renal function at birth have been shown by physiological studies. A sharp drop in the ratio of the clearance of insulin to phenol red occurs at birth in the rabbit and is due to an increase in the clearance of inulin, which almost doubles (Levine & Levine, 1958). The clearance of inulin steadily increases towards the adult value, having increased some tenfold by 20 days' postnatal life. The clearance of phenol red increased twenty-fivefold over this period, suggesting that tubular function is minimal in the rabbit just before and after birth.

The time chosen for estimating the radioactivity of the organs was 2 minutes after the intravenous injection of the isotope. This time interval was chosen because studies in the adult have indicated that the radioactivity of the kidney is at its peak within the first 2 minutes after injection (Walker & Wilde, 1952). In a preliminary study it was found that the radioactivity of the foetal kidney behaves in a similar manner.

The validity of the early exchange rate of radioactive potassium by the kidney as an index of its function has been considered by Black, Davies, & Emery (1955). They suggested that the high early exchange was an indication of the high blood-flow of the kidney, which receives 20 per cent. of the resting cardiac output. However, the observations of Eisen & Harris (1957), who found the isotope localized in the cytoplasm of the proximal and distal tubular cells, indicate that the high early exchange rate is not only a matter of blood-flow.

It is important to study the time of postnatal alteration in the rate of potassium exchange and localization and to correlate these with histogenesis. In particular

the period round about 8 days of postnatal growth in which the brush border shows an alteration in its submicroscopical structure (Olivecrona & Hillarp, 1949) may be of critical significance. In this connexion it is of interest that Hoy & Adolph (1956) found that whilst the young rat has a barely demonstrable water and salt diuresis 6 hours after birth, this diuresis increases to the adult magnitude after 7 days.

SUMMARY

1. A technique has been developed for studying the uptake of K^{42} by the foetal rat kidney.

2. Using this technique the exchange rate for K^{42} of the foetal and neonatal metanephros relative to the foetal and neonatal liver has been investigated. It was found that the metanephros of the 20- and 21-day foetus shows a rate of exchange which is twice that of the liver, whereas the neonatal metanephros shows a rate of exchange which is less than half that of the liver.

3. The results of this investigation demonstrate that the ratio of K^{42} uptake by the metanephros of the 20- and 21-day rat foetus, relative to that of the foetal liver, is one-quarter of that reported for the adult rat.

4. An autoradiographic study of the distribution of K^{42} in the foetal kidney has shown that histological localization of the isotope in the proximal and distal tubules, found in the adult, does not occur.

RÉSUMÉ

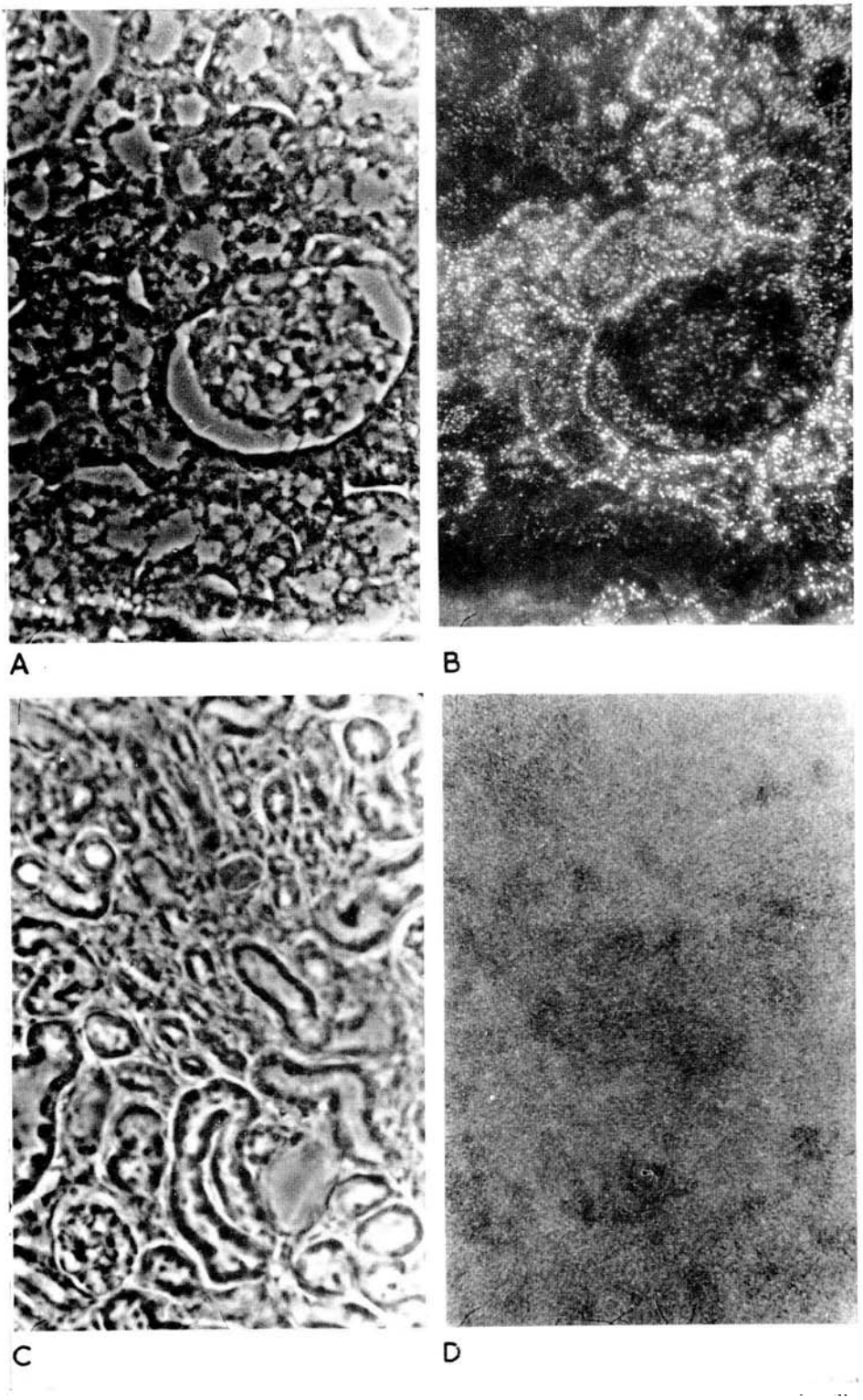
Étude de la fonction rénale pendant l'histogenèse du fœtus avancé et du nouveau-né chez le rat, par la méthode de l'absorption et de l'autoradiographie du potassium radioactif

1. Description d'une technique pour l'étude de l'absorption de K^{42} par le rein du fœtus de rat.

2. Au moyen de cette technique, on étudie le taux des échanges de K^{42} du métanephros du fœtus et du nouveau-né, en comparaison avec le foie du rat de même âge. Le métanephros du fœtus de 20 et 21 jours montre un taux d'échanges 2 fois plus élevé que le foie, tandis que le métanephros du nouveau-né montre un taux d'échanges plus faible que celui du foie.

3. Cette étude démontre que le taux d'absorption de K^{42} par le métanephros du fœtus de 20 et 21 jours, en comparaison avec le foie fœtal, est égal à un quart de celui du rat adulte.

4. Une étude histo-autoradiographique de la distribution du K^{42} dans le foie fœtal montre que la localisation de l'isotope ne se produit pas, comme chez l'adulte, dans les tubes proximaux et distaux.


A
B
C
D

ACKNOWLEDGEMENTS

We wish to thank Professor E. W. Walls and Professor D. R. Newth for their advice and encouragement. We also wish to thank Professor J. E. Roberts for making radioactive potassium available to us, and Mr. R. Ekins and Mr. R. Warren, who carried out the radioactive and photometric measurements respectively.

One of us (J. G. B.) is indebted to the Medical Research Council for a grant towards apparatus.

REFERENCES

ARATAKI, M. (1926). On the postnatal growth of the kidney, with special reference to the number and size of the glomeruli (albino rat). *Amer. J. Anat.* **36,** 399–436.

BARCROFT, J. (1946). *Researches on Pre-Natal Life.* Oxford: Blackwell.

BAXTER, J. S., & YOFFEY, J. M. (1948). The postnatal development of renal tubules in the rat. *J. Anat. Lond.* **82,** 189–96.

BLACK, D. A. K., DAVIES, H. E. F., & EMERY, E. W. (1955). The disposal of radioactive potassium injected intravenously. *Lancet,* **1,** 1097–9.

DAVIES, J. (1954). Cytological evidence of protein absorption in foetal and adult kidneys. *Amer. J. Anat.* **94,** 45–72.

EISEN, V. D., & HARRIS, F. T. C. (1957). Demonstration of early renal uptake of potassium 42 by an autoradiographic method for water soluble isotopes of short half-life. *Nature, Lond.* **180,** 440–1.

GERSH, I. (1937). The correlation of structure and function in the developing mesonephros and metanephros. *Contr. Embryol. Carneg. Instn.* **26,** 33–58.

GINSBURG, J. M., & WILDE, W. S. (1954). Distribution kinetics of intravenous radiopotassium. *Amer. J. Physiol.* **179,** 63–75.

HOY, P. A., & ADOLPH, E. F. (1956). Diuresis in response to hypoxia and epinephrine in infant rats. *Amer. J. Physiol.* **187,** 32–40.

LEVINE, J., & LEVINE, A. D. (1958). Excretion of phenol red and inulin by foetal and newborn rabbit. *Amer. J. Physiol.* **193,** 123–8.

MOREL, F., & GUINNEBAULT, M. (1956). Origine tubulaire du potassium excrété par le rein: Étude expérimentale à l'aide du radio-potassium K^{42} chez le lapin. *Helv. physiol. acta,* **14,** 255–63.

OLIVECRONA, H., & HILLARP, N. (1949). Studies on the submicroscopical structure of the epithelial cells of the intestine, pancreas and kidney in rats during histogenesis. *Acta anat.* **8,** 281–5.

WALKER, A. M., BOTT, P. A., OLIVER, J., & MACDOWELL, M. C. (1941). The collection and analysis of fluid from single nephrons of the mammalian kidney. *Amer. J. Physiol.* **134,** 580–95.

WALKER, G. W., & WILDE, W. S. (1952). Kinetics of radiopotassium in the circulation. *Amer. J. Physiol.* **170,** 401–13.

WARREN, R. L. (1959). A versatile micro-spectrophotometer. *Abstr. Proc. Colloq. Spectroscop. Lucerne.*

WELLS, L. J. (1946). Observations on secretion of urine by kidneys of fetal rats. *Anat. Rec.* (Abstract). **94,** 504.

EXPLANATION OF PLATE

FIG. A. Microphotograph of an unstained section of adult rat kidney cortex seen by phase-contrast illumination. ×800.

FIG. B. Microphotograph of an autoradiogram corresponding to Fig. A seen by dark-ground illumination. ×800. The silver grains in the emulsion show as points of light over the proximal and distal tubules associated with the glomerulus.

FIG. C. Microphotograph of an unstained section of a 21-day foetal rat kidney cortex seen by phase-contrast illumination. ×800.

FIG. D. Microphotograph of an autoradiogram corresponding to Fig. C seen by dark-ground illumination. ×800. The uniform distribution of silver grains is in marked contrast to that shown in Fig. B.

(Manuscript received 2: xi: 60)