Metabolic Activities of the Isolated Perfused Rat Kidney

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1. A technique for perfusing the isolated rat kidney is described. It is primarily designed for the study of renal metabolism but is also suitable for studying some aspects of the secretory function; this was normal with respect to minimal glucosuria. The glomerular filtration rate as measured by creatinine clearance was lower than *in vivo* and slowly decreased with time. 2. Gluconeogenesis from a variety of precursors was rapid and similar to that in kidney-cortex slices, in contrast with liver where the perfused organ is more effective than slices. Whereas the maximal rates of gluconeogenesis from glycerol and pyruvate were similar in liver and kidney, the rates from succinate, malate and fumarate were 14–20 times, and those from glutamate and aspartate about three times, as high in the kidney. 3. The oxygen consumption of the perfused organ was about twice that of cortex slices, presumably because of the secretory work done in the perfused organ but not in slices. 4. The rate of acetoacetate oxidation was about the same in the perfused organ and in slices but, because of the higher rate of oxygen consumption, the percentage contribution of acetoacetate to the fuel of respiration was lower in the perfused organ. The results suggest that acetoacetate can supply energy for the basal requirements and for gluconeogenesis but not for the secretory work. 5. Glutamine was formed at a high rate from glutamate and at a lower rate from aspartate. The high rates indicate that, in the rat, the kidney is a major source of body glutamine.

The maximal rates of gluconeogenesis in the perfused rat liver are for most substrates three to five times as high as the rates observed in rat liver slices (Ross, Hems & Krebs, 1967). In view of these differences it seemed desirable to compare also the rates of gluconeogenesis in the perfused rat kidney and in kidney slices. A method of rat kidney perfusion was therefore developed. It is based on the techniques used by Weiss, Passow & Rothstein (1959) for the perfusion of rat kidney and by Miller, Bly, Watson & Bale (1951), Schimassek (1963) and Hems, Ross, Berry & Krebs (1966) for the perfusion of rat liver. A major difference between the techniques for the two organs concerns the hydrostatic pressure of the ingoing medium. Whereas liver perfusion through the portal vein requires a pressure about 20cm. water, perfusion of the kidney through the renal artery requires a pressure about 120cm. water.

In contrast with hepatic gluconeogenesis, renal gluconeogenesis from a variety of precursors was found to be similar in the perfused organ and in slices.

MATERIALS AND METHODS

Perfusion apparatus. The apparatus was a modification of that used in this Laboratory for liver perfusion (Hems *et al.* 1966). The modifications concern the following points.

(1) The distance between the bottom of the oxygenator and the 'kidney tray' was 120cm., to obtain the required hydrostatic pressure. (2) The kidney, unlike the liver, was not perfused *in situ* but was transferred to a 'kidney tray'. This consisted of a nylon mesh stretched over a ring (7·5cm. diam.). A stainless-steel strip was mounted about 2·5cm. above the tray to support the arterial cannula. (3) The temperature of the cabinet and the circulating medium was 38–40°. (4) The arterial tube from the oxygenator was mainly of glass to avoid oxygen loss because of the permeability of polythene to gases. (5) The collecting vessel received the venous outflow via a 2ml. graduated pipette, which also served as a flow-meter. (6) The urine produced was returned to the collecting vessel to avoid loss of products of metabolism, except in experiments on the secretory function. (7) The total volume of the perfusion medium was 100ml.

Animals. Male Wistar rats weighing 350–450g. were starved for 42–45hr., because higher rates of gluconeogenesis are obtained in the kidney of starved rats (Krebs, Bennett, de Gasquet, Gascoyne & Yoshida, 1963*a*).

Operative technique. After anaesthetizing the rat with intraperitoneal nembutal (0·1ml. of 6%, w/v, solution per 100g. body wt.), an abdominal incision was made in the midline, extended laterally, and the intestine was placed to the animal's left. The right kidney was used for perfusion, because the mesenteric artery arises from the aorta at the same level as the right renal artery and a cannula

can be passed from one to the other without blood loss and without stopping the blood flow to the kidney.

To expose the major abdominal vessels and the right kidney, fat and perivascular tissue were cleared away by blunt dissection. The adrenal branch of the right renal artery was tied, and loose ligatures were placed around the following blood vessels: (1) inferior vena cava, just below the liver; (2) aorta, above the mesenteric artery; (3) mesenteric artery, near the aorta; (4) mesenteric artery, further from the aorta than (3); (5) right renal artery, at its origin from the aorta; (6) inferior vena cava, between the left and right renal vein; (7) inferior vena cava, below the left renal vein; (8) inferior vena cava, more distally still; (9) left renal vein.

The right kidney was mobilized as far as possible, leaving only its vascular and ureteric attachments. For the collection of urine the ureter was cannulated by Portex tubing (Portland Plastics Ltd., Hythe, Kent) size PP 10 (internal diam. 0·28mm., external diam. 0·61mm.) and tied in place with thread (Ethicon, R 842 3/0).

Then heparin (0·2ml., 200 units) was injected into the lower inferior vena cava; afterwards the opening in the wall of the vein was closed by means of a ligature passed over the point of the injecting needle.

Next, ligature (9) was tied. The venous cannula, which consisted of a 3cm. length of Portex tubing, size PP 270 (internal diam. 2mm., external diam. 3mm.), cut off at an angle to form a sharp tip, was inserted in the inferior vena cava and tied in places (6) and (7). With the cannula in position, the opening lay opposite the right renal vein. The other end of the cannula was temporarily closed by a loose plug of tissue paper.

The arterial cannula was made of glass tubing (internal diam. 2·8mm., external diam. 3·5mm.) drawn to a tip of 1·3mm. external diam. and 1·0mm. internal diam. It was bent to a right angle 1·5cm. from the tip and the short limb of the cannula had little or no taper. The tip of the cannula was bevelled slightly to facilitate its insertion. The cannula was filled to the tip with perfusion medium. The mesenteric artery was tied distally (4) and Spencer Wells forceps were used to maintain tension on the vessel. The artery was grasped at its origin with fine curved forceps and an incision made in the wall. The cannula was inserted and passed to meet the forceps, which were then removed. The tip of the cannula was advanced into the aorta and then into the renal artery opposite, allowing perfusion medium to flow to the kidney. The cannula was tied in place by ligatures (3) and (5).

In an alternative procedure the aorta was tied at (2) and the cannula was inserted directly into the aorta and right renal artery. A short period of ischaemia of the kidney is unavoidable with this method of cannulation; it is therefore not the method of choice.

The aorta was tied at (2) and cut below the ligature. The inferior vena cava was tied at (1) and cut above the ligatures. The perfusion medium then flowed down the venous cannula. The plug was removed and the first 8–10ml. of medium was discarded. The kidney was transferred from the animal to the nylon-mesh tray. The whole operation took about 15min. The flow rate was 16–30ml./min.

Perfusion medium. It was found necessary to modify the medium used for liver perfusion to obtain optimum rates of gluconeogenesis from added substrates and low rates from endogenous sources. The modifications concern a higher concentration of albumin (which approaches the physiological value of the colloid osmotic pressure) and dialysis of the bovine albumin (which decreases glucose formation in the absence of added precursors). The presence of red blood cells proved unnecessary for full oxygenation because of the high flow rate. The standard medium was prepared as follows: 5g. of bovine serum albumin (fraction V; Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex) was dissolved with stirring in about 70ml. of the saline of Krebs & Henseleit (1932). This solution was dialysed against 2l. of the saline (five changes) for 48hr. in a sealed vessel (to prevent loss of CO_2) at 4° with shaking. After dialysis, the perfusion medium was brought to 111ml. with the saline. The final concentration of albumin was about 4·5% (w/v).

Substrates were usually added to the perfusion medium as 2·5ml. of 0·2M neutral solution to give a final concentration about 5mM. This concentration was lower than that (10mM) used in the liver-perfusion experiments because the ratio of medium to tissue was much higher for the kidney (about 100) than for the liver (about 25). This meant that changes in the substrate concentration could not be accurately measured at 10mM when the rate of removal was relatively low. The time-course of the metabolic changes in the liver indicated that rates were still maximal at rather less than 5mM. Tests carried out on the perfused kidney showed that the rate of gluconeogenesis from glutamate was not measurably higher when the substrate concentration was raised from 5 to 10mM.

Sampling of perfusion medium. For analyses, samples of the medium (0·5ml.) were taken from the collecting vessel and pipetted directly into 4ml. of 2% (w/v) $HClO_4$. One sample was taken immediately before the addition of substrates (i.e. 3–7min. after the start of the perfusion) and another 4–5min. after substrate addition. Subsequent samples were usually taken every 10 or 15min. over a period of 2–4hr.

Creatinine clearance. Between 10 and 200mg. of creatinine was added to 100ml. of medium and urine was collected over a measured interval of time (5–20min.). The first collection was started 10–20min. after the beginning of perfusion, because urine flow was sometimes irregular in the first 10–15min. The urine flow rate was calculated by dividing the collected volume by time. For the determination of the creatinine concentration in the medium, the samples were taken from the collecting vessel at the midpoint of each urine collection period.

Analytical methods. The methods for the determination of metabolites, including the electrometric method for O_2, were as previously described (Hems *et al.* 1966; Ross *et al.* 1967). The protein content of the urine was estimated by the biuret method, and creatinine was determined by the method of Folin as modified by Vestergaard & Leverett (1958). The adenine nucleotide content of the kidney was measured in the rapidly frozen tissue as described by Hems *et al.* (1966). For the determination of adenine nucleotides before perfusion, animals were anaesthetized with nembutal and the kidney was rapidly removed through a dorsal incision and freeze-clamped.

Calculation of metabolic rates. The metabolic rates were calculated from a plot of the linear portion of the time-course, which extended over at least 60min. and usually over 2hr. unless the substrate was exhausted earlier as

with pyruvate and succinate. To compare the rates observed in the perfused organ with those previously published for slices the results are expressed in μmoles of metabolite/hr./g. dry wt. (mean ±S.E.M.), except when stated otherwise. Another reason for choosing dry weight as a reference is the weight increase which occurs in the kidney during the perfusion, discussed in the following paragraph.

Weight increase of kidney on perfusion. While the wet wt./dry wt. ratio of the rat liver changes very little on perfusion, from 3·50 before perfusion (mean of 24 observations) to 3·59 after perfusion (mean of 199 observations), that of the perfused kidney increased considerably. The wet wt./dry wt. ratio was 3·94±0·14 (8) before the perfusion and 5·63 (mean of 83 observations) after perfusion. The weight increase occurred early during the perfusion. Histological examination of the kidney before and after perfusion showed that the epithelium of the convoluted tubules became flattened and the lumen expanded. The interstitial spaces also were slightly increased in the perfused kidney. These changes occurred despite the high albumin content of the perfusing medium; Bauman, Clarkson & Miles (1963) found similar changes after perfusion with rat blood. The main cause of the increased wet weight of the perfused organ was obviously the greater fluid content of the tubules. Factors which might contribute to the accumulation of fluid are lack of nervous control of the afferent glomerular arterioles, a block of the lymphatics which are tied during the preparation and an increased venous pressure.

Effect of serum albumin on the removal of metabolites. Acetoacetate was found to undergo a relatively rapid spontaneous decomposition (probably decarboxylation) when dissolved in the saline medium containing albumin. At 39° less than 2% disappeared within 1 hr. in the absence of albumin and 11%/hr. with albumin. A correction allowing for this was used in all calculations. Pyruvate was also found to be slightly less stable in the presence of albumin. About 12% disappeared in 1 hr. at 39°, but compared with the metabolic utilization the non-enzymic disappearance (probably a formation of parapyruvate) was negligible. The kidney removed approx. 500 μmoles in 90 min. through metabolic reactions when about 40 μmoles were lost from the medium through the non-enzymic reactions. Therefore no correction was made in experiments with pyruvate.

RESULTS

Need for dialysis of albumin. When the kidney was perfused with the standard medium, without addition of substrate, no measurable amounts of glucose or lactate were formed during 2 hr. When non-dialysed medium was used glucose appeared at a rate of 93±15 μmoles/hr./g. dry wt. (three observations). The cause of this blank has not been fully investigated. It may be due to gluconeogenic amino acids contaminating the albumin and to acetate present in the albumin (35 μmoles/g., D. Keane, personal communication), which can stimulate gluconeogenesis by a sparing effect (Krebs, Speake & Hems, 1965). To avoid this 'blank' the medium was dialysed as described in the Materials and Methods section.

Time-course of glucose synthesis from pyruvate. Pyruvate, which in slices of kidney cortex forms glucose two to three times as rapidly as L-lactate (Krebs *et al.* 1963*a*), was also more effective than lactate in the perfused kidney. The time-course of glucose formation from pyruvate is shown in Fig. 1. Pyruvate (500 μmoles) was added 2·5 min. after the start of the perfusion; a high rate of pyruvate removal (5·36 μmoles/min. for this kidney) was maintained for 75 min. The rate fell at this stage when most of the added pyruvate had been used. Glucose formation was linear for the first 90 min. at a rate of 1·55 μmoles/min. It was accompanied by the formation of lactate at the rate of 1·02 μmoles/min. When a further quantity of 500 μmoles of pyruvate were added at 107 min. pyruvate was again metabolized at a high rate. Pyruvate removal, glucose formation and lactate formation were 5·50, 1·10 and 1·56 μmoles/min. respectively. Thus, after perfusion for about 2 hr. pyruvate was again removed at the same rate as initially; a little less was converted into glucose and a little more into lactate.

Time-course of oxygen consumption on addition of pyruvate. The arterial oxygen content, measured electrometrically, was constant during the course

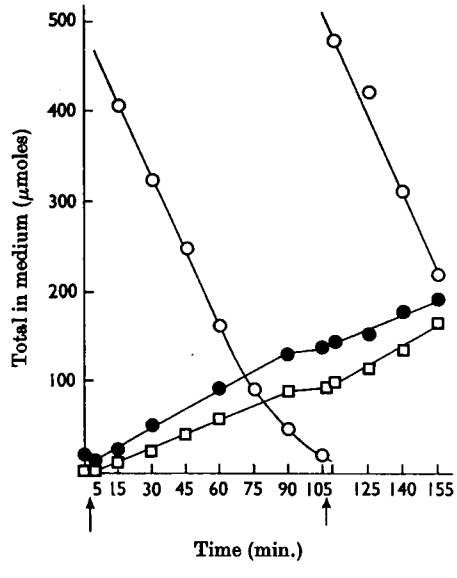

Fig. 1. Time-course of metabolism of pyruvate in the perfused rat kidney. Pyruvate (500 μmoles) was added at 2·5 min. (first arrow) after the start of the perfusion. Samples of medium were taken at 15 min. intervals for the determination of glucose, pyruvate and lactate. At 107 min. (second arrow) a further 500 μmoles of pyruvate were added. The wet weight of kidney was 1·26 g. (dry wt. 0·24 g.). ○, Pyruvate; □, lactate; ●, glucose.

of a perfusion and was therefore determined only at the beginning and end of an experiment. As the oxygen consumption varies with the plasma flow rate in the kidney (Van Slyke, Rhoads, Hiller & Alving, 1934; Kramer & Deetjen, 1964) the flow rate of medium was kept nearly constant in the present experiments. As seen in Table 1, at a mean flow rate of 24ml./min. the basal oxygen consumption without added substrate was 6·9μ-moles/min. (23μmoles/min./g. dry wt.). The addition of pyruvate (500μmoles) resulted in an increase in oxygen consumption, which reached a maximum of 40% within 10min. and began to fall after 80min., when most of the pyruvate had gone. After 125min. of perfusion the oxygen consumption returned to the basal level.

Correlation between oxygen consumption and other metabolic changes in the presence of pyruvate or lactate. The results of a series of experiments in which the rate of the various metabolic changes on addition of pyruvate or lactate were measured are shown in Table 2. Substrates (500μmoles of L-lactate or pyruvate) were added 30min. after the start of the perfusion to obtain constant values for the rate of oxygen consumption before addition of substrate. Removal of added pyruvate occurred at about the same rate as in the experiment recorded in Fig. 1, but the rate of glucose formation was lower, possibly because of the later time of addition of the substrate. The rate of the oxygen consumption before the addition of pyruvate was 1390μ-moles/hr./g. dry wt., a value nearly twice that found with slices of rat kidney cortex without added substrate (Krebs, Hems & Gascoyne, 1963*b*; Krebs *et al.* 1965). On addition of pyruvate, the rate of oxygen consumption rose by 552μmoles/g. dry wt./hr. above the basal value. This increase is similar to that found in slices. The amount of pyruvate removed was in excess (507μmoles/g. dry wt./hr.) of the amounts needed for the formation

Table 1. *Time-course of oxygen consumption and of glucose formation from pyruvate in the perfused rat kidney*

Pyruvate (500μmoles) was added 35min. after the start of the perfusion, and samples of medium taken at 15min. intervals were analysed for pyruvate, lactate and glucose content. The initial volume of the perfusion medium was 94ml. The data refer to one kidney, the weight of which was 1·52g. wet, 0·26g. dry. For other details see the text.

	Before addition of pyruvate			After addition of pyruvate					
Time after start of perfusion (min.)	27	32	35	42	60	81	110	125	140
Flow rate (ml./min.)	24	24	24	24	24	24	20	17	17
Uptake of O_2 (μmoles/min.)	6·90	6·90	6·90	9·65	9·65	9·25	7·80	6·80	—
Pyruvate in medium (μmoles)	—	0	—	372	263	140	23	13	9
Glucose in medium (μmoles)	—	17·0	—	28·7	55·0	76·0	88·3	90·0	91·5
Lactate in medium (μmoles)	—	9·5	—	17·7	38·0	61·5	84·4	85·1	81·7
Ratio $\frac{\text{lactate}}{\text{pyruvate}}$ in medium	—	—	—	0·048	0·145	0·439	3·65	6·56	9·1

Table 2. *Metabolism of* L-*lactate and pyruvate in the perfused rat kidney*

The rats were starved for 48hr. Substrate (500μmoles) was added 30min. after the start of perfusion. The data are means ± S.E.M. for five observations, except for the O_2 uptake in the lactate experiment when four observations were made. For full details see the text.

	Metabolic changes (μmoles/hr./g. dry wt.)					
				O_2		
Substrate added (5mM)	Glucose	Lactate	Pyruvate	Before addition of substrate	After addition of substrate	Increase due to substrate
L-Lactate	+ 64± 9	−419±73		−1609± 86	−2234±324	625±198
Pyruvate	+270±26	+224±28	−1271±104	−1386±158	−1938±143	552± 78

of glucose and lactate, probably because pyruvate contributed to the fuel of respiration.

When L-lactate was added, glucose synthesis was rather less consistent than with pyruvate, as indicated by the greater S.E.M. The rate was about 25% of the rate from pyruvate (Table 2). Pyruvate was formed during the first 30min. of perfusion (89 μmoles/g. dry wt./hr.; see Table 3) and was subsequently slowly removed. With lactate the increase of the oxygen uptake was similar to that with pyruvate but the maximum was not reached until 20 and 45min. after the addition of substrate, whereas the formation of glucose was maximal from the time of the addition of lactate. Variations in the rate of the oxygen uptake are reflected by the high S.E.M. of the value in Table 2.

Rate of glucose synthesis from various precursors. Glutamate, aspartate, succinate, malate and fumarate, which are not readily metabolized in the perfused liver (Ross *et al.* 1967), are highly effective gluconeogenic precursors in the perfused kidney (Table 3). The rates of gluconeogenesis from all precursors tested in the perfused kidney were somewhat lower than in kidney-cortex slices if compared on the basis of the uncorrected dry weight (Table 3 and Krebs *et al.* 1963*a*, Table 3). However, in comparing slices with the perfused organ, allowance has to be made for the fact that the perfused kidney includes the white medulla (about 10–15% of the dry weight), that the white medulla may consume glucose at a rate of 350 μmoles/g. dry wt./hr. (see György, Keller & Brehme, 1928; Dickens & Weil-Malherbe, 1936) and that slices, unlike the perfused organ, produce considerable amounts of glucose (25 μmoles/g. dry wt./hr.) from endogenous sources. Moreover, the dry weight of the slices in the experiments quoted was determined at the end of the incubation when up to 20% of the initial dry matter had been lost to the medium. When these factors are taken into consideration the differences in the rates of gluconeogenesis between slices and the perfused kidney disappear.

Several substrates formed lactate as a by-product. An exception was glycerol, which, in contrast with all the other precursors tested, did not form pyruvate—the immediate precursor of lactate—as an intermediate in gluconeogenesis. Other additional products included malate (formed from succinate and fumurate), aspartate (formed from glutamate) and glutamine (formed from glutamate and aspartate). It should be emphasized that the rates recorded for the formation of additional products refer to the first 30min. perfusion, when the output was linear. The rates subsequently decreased, with the exception of that of glutamine formation which remained linear throughout. The rate of formation of glutamine from glutamate was remarkably high: more glutamine was formed than

Table 3. *Glucose synthesis from various precursors in the perfused rat kidney*

The values are means ±S.E.M. and (numbers of observations). For the calculation of the amounts of substrates accounted for by products it was assumed that the formation of glucose requires 2 molecules of precursor, the production of glutamate and glutamine from aspartate 2 molecules and the formation of other products 1 molecule. For general procedure see the text.

Substrate added	Amount of substrate removed (μmoles/g. dry wt./hr.)	Glucose formed (μmoles/g. dry wt./hr.)	Lactate formed (μmoles/g. dry wt./hr.)	Other products formed (μmoles/g. dry wt./hr.)	Amount of substrate accounted for by products (μmoles/g. dry wt./hr.)	Not accounted for (μmoles/g. dry wt./hr.)
None		<3 (5)	<3 (5)			
L-Lactate	405±32 (10)	82± 9 (10)		Pyruvate 89±20 (3)	253	152
Pyruvate	1246±82 (10)	304±30 (10)	212±24 (10)		820	426
Glycerol	282±13 (4)	95± 7 (4)	16± 6 (4)		206	76
Succinate	1054±59 (5)	310±50 (5)	88±22 (5)	Malate 77±6 (2)	785	269
L-Glutamate	518±49 (8)	107± 9 (6)	<5 (5)	Aspartate 32± 4 (3) Glutamine 196±14 (3) Glutamate 160±19 (5)	442	76
L-Aspartate	687±59 (6)	72± 7 (7)	<3 (5)	Glutamine 67± 3 (2)	598	89
L-Malate	892±12 (4)	206±16 (5)	71±26 (4)	Fumarate Irregular (0–50)	483	409
Fumarate	837±31 (5)	265±26 (5)	98±16 (5)	Malate 150±16 (2)	778	59

Table 4. *Effect of glucagon on gluconeogenesis in the perfused rat kidney*

Substrates and glucagon were added together when the flow rate was steady. The concentrations of lactate and pyruvate were 5mM and of glucagon 5μg./ml. For further details see the Materials and Methods section.

Substances added	Substrate removed (μmoles/hr./g. dry wt.)	Glucose formed (μmoles/hr./g. dry wt.)
L-Lactate	405± 32 (10)	82± 9 (10)
L-Lactate; glucagon	500± 62 (4)	82±12 (4)
Pyruvate	1250± 82 (10)	304±30 (10)
Pyruvate; glucagon	1450±235 (3)	263±56 (3)

glucose. In most cases the products measured accounted for the bulk of the substrate removed.

Glucose uptake by the perfused kidney. Glucose added at a concentration of 5mM was removed from the perfusion medium at the rate of 54±15μ-moles/g. dry wt./hr. (three observations). This was accompanied by the formation of about the same molar quantity of lactate (55±15μmoles/g. dry wt./hr.).

Effect of glucagon on gluconeogenesis in the perfused rat kidney. Glucagon, which gave increased rates of glucose synthesis from lactate and pyruvate in the perfused rat liver (Ross *et al.* 1967), was without effect on the perfused kidney when added under the same conditions (Table 4). The slightly increased rate of substrate removal was not significant. The gluconeogenic effect of glucagon thus appears to be restricted to the liver.

Metabolism of acetoacetate in the perfused rat kidney. In kidney-cortex slices acetoacetate is readily oxidized, contributing up to 70% of the fuel of respiration, and it can accelerate the rate of gluconeogenesis from lactate (Krebs, Hems, Weidemann & Speake, 1966). The latter effect also occurs in the perfused kidney (Table 5), where the rate of glucose formation from lactate was more than doubled by acetoacetate, and that from pyruvate increased by over 50%. Acetoacetate was also readily oxidized in the perfused kidney but its contribution to the fuel of respiration was smaller than in kidney slices. The amount of acetoacetate oxidized accounted for 21·5% of the uptake of oxygen when acetoacetate was the sole substrate, and for 25·7% in the presence of acetoacetate and lactate. The corresponding figures for slices reported by Krebs *et al.* (1966), referring to rats in a different nutritional state (well fed as opposed to starved), were 67 and 71%. It is remarkable that the absolute rates of acetoacetate oxidation were not very different in slices and the perfused organ as the following comparison shows. The amount of acetoacetate oxidized (μmoles/g./hr.) were: 105 in the perfused kidney, 134 in slices (no lactate added); with 5mM-lactate 182 in the perfused kidney, 213 in slices. If allowance is made for the inclusion of the medulla in the perfused organ and for the fact that the slices were weighed at the end of the incubation when some dry matter had been lost, the rates for the perfused organ and slices appear very similar.

As in slices, acetoacetate was reduced to β-hydroxybutyrate, and the rate of reduction was increased by lactate and pyruvate.

Urine production by the perfused kidney. The results of various functional tests of the perfused kidney are summarized in Table 6. The mean rate of urine flow was 0·056ml./min./g. wet wt., a value similar to that found by Bauman *et al.* (1963). The rate was somewhat higher than that reported for the normal rat kidney (0·027–0·059ml./min./g. wet wt.; Lippman, 1947) but near to the maximum rate found *in vivo* when diuresis is induced by mannitol (Harvey & Malvin, 1965). Traces of glucose appeared in the urine but the values were lower than those in the urine of the intact rat; Cori (1925) found about 21μg./ml. in rat urine after 48hr. starvation. Proteinuria was somewhat high but this is of limited significance since the rat kidney *in vivo* excretes injected bovine albumin (Lippman, 1949).

The glomerular filtration rate, as measured by the creatinine clearance, decreased with time (Table 7). In the example quoted the clearance was 0·159ml./min./g. wet wt. at 28min. and 0·106ml./min./g. wet wt. at 88min. The same trend was observed in many experiments, whereas the absolute values and the rate of decrease of the creatinine clearance differed from experiment to experiment. On the other hand urine flow was fairly constant in each experiment, and the flow rate of medium remained constant for the first 1–2hr. and then gradually fell. As the creatinine clearance progressively decreased the value at 30min. was taken from each time-curve for comparison of several experiments (Table 6). The mean value was 0·145ml./min. for perfusions in which the creatinine concentration was 0·2mg./ml. or more.

Values reported for the creatinine clearance of rat kidney *in vivo* vary widely (Friedman, 1947; Lippman, 1947; Corcoran, Masson, Reuting & Page, 1948; Fingl, 1952; Glasser, 1961; Harvey &

Table 5. *Effect of acetoacetate on the metabolism of lactate and pyruvate in the perfused rat kidney*

The general technique was as described in Tables 1 and 2. Acetoacetate, lactate or pyruvate, when added as the sole substrate, was added after about 20 min. When acetoacetate together with another substrate was to be added the acetoacetate was added first, and the rate of O_2 uptake was measured when a stable rate of perfusion was reached (usually during 10 min.). The value for the O_2 uptake obtained for this period is included in the value for the column headed 'Acetoacetate'. When the measurement of the O_2 uptake was completed, lactate or pyruvate was then added. The O_2 consumption before the addition of any substrate was 1620 ± 88 (20) μmoles/hr./g. dry wt. Acetoacetate 'oxidized' refers to the fraction of acetoacetate not accounted for by β-hydroxybutyrate. In the calculations of the O_2 uptake accounted for by the oxidation of the various substrates a deduction has been made for the formation of β-hydroxybutyrate and lactate from the ketonic acids (0·5 μmole of O_2/μmole of β-hydroxybutyrate or lactate). When pyruvate was the substrate the assumption was made that the oxidation of pyruvate provided the reducing equivalents required for the formation of lactate and glucose.

Substrate added (all 5 mM) Metabolic changes (μmoles/hr./g. dry wt.)	Acetoacetate	L-Lactate	Acetoacetate L-lactate	Pyruvate	Acetoacetate pyruvate
Acetoacetate	−146± 9 (3)	—	−264± 35 (4)	—	−384±65 (4)
β-Hydroxybutyrate	+41± 4 (3)	—	+82± 8 (6)	—	+86± 8 (4)
L-Lactate	—	−419± 73 (5)	−465± 44 (6)	+224± 28 (5)	+344± 22 (5)
Glucose	Nil	+64± 9 (5)	+148± 22 (6)	+270± 26 (5)	+340± 34 (5)
Pyruvate	—	—	+100± 19 (6)	−1271±104 (5)	−1242± 43 (5)
O_2	−1939±116 (11)	−2234±324 (4)	−2780±216 (6)	−1938+143 (5)	−2975±182 (4)
Acetoacetate oxidized	105		182		298
Uptake of O_2 (%) accounted for by:					
Complete oxidation of acetoacetate	21·5		25·7		40·5
Formation of pyruvate			1·8		
Complete oxidation of lactate			7·4		
Complete oxidation of pyruvate				45·6	1·1

Table 6. *Creatinine clearance (glomerular filtration rate) and urine composition in the perfused rat kidney*

The tests were carried out at various creatinine concentrations. The clearance was calculated according to the standard formula (Pitts, 1963). The value at 30min. was taken from each time-course curve. For further details see the Materials and Methods section. The creatinine clearance at low creatinine concentrations (0·1mg./ml.) cannot be equated to glomerular filtration rate because the secretion of creatinine makes a significant contribution. The data for urine flow and the glucose and protein content of urine refer to a sample collected during the first 30min.

Initial creatinine concn. in medium (mg./ml.)	No. of observations	Kidney (g. wet wt.)	Urine flow (ml./min./g.)	Creatinine clearance (ml./min./g.)	Glucose in urine (μg./ml.)	Protein in urine (mg./ml.)
0·1	4	1·29±0·02	0·065±0 041	0·308±0·055	5·8±3·0	3·1±0·7
0·2	4	1·57±0·03	0·056±0·014	0·145±0·047	4·4±0·90	5·3±1·55
2·0	2	1·39±0·15	0·035±0·015	0·140±0·019		3·2±0·10

Table 7. *Time-course of creatinine clearance and urine flow in perfused rat kidney*

Creatinine (0·2mg./ml.) was added to the medium initially. Samples of medium were taken at the mid-point of each 12min. interval and clearance was calculated according to the standard formula. Kidney weight was 1·51g. (wet), 0·27g. (dry).

Time (min.)	28	40	52	64	76	88
Creatinine clearance (μl./min./g. of wet kidney)	159	148	144	126	120	106
Urine flow (μl./min./g. of wet kidney)	53	53	54	52	50	46

Malvin, 1965), but are generally higher than those found in the present experiments, perhaps because the number of functioning nephrons was decreased in the perfused organ. The higher figures shown in Table 6, obtained at creatinine concentrations of 0·1mg./ml., are probably due to active secretion of creatinine (Fingl, 1952).

The flow rate of medium in the perfused kidney (16–30ml./min./g. wet wt.) was intentionally about four to five times that found adequate by Bauman *et al.* (1963) for perfusions with whole rat blood, in order to meet the oxygen requirements of the kidney.

Adenine nucleotides in perfused rat kidney. Another measure of the functional integrity of the perfused organ is the stability of the adenine nucleotides and the ATP/ADP ratio. As shown in Table 8, the concentration of ATP had fallen and that of AMP increased at the end of the operation, presumably because of temporary anoxia during the preparation of the kidney for perfusion. Within 30min. the normal values obtained from non-perfused kidneys were restored. The total adenine nucleotide content, the ATP/ADP ratio and the concentrations of AMP were maintained rather more effectively than in the perfused liver (see Schimassek, 1963; Hems *et al.* 1966), irrespective of the addition of lactate or pyruvate. The decrease of the total adenine nucleotides after 2hr. in the presence of lactate was 15%. Although the addition of lactate caused a fall in the concentration of ATP and in the ATP/ADP ratio in the liver, there were no significant changes in this direction in the perfused kidney.

No significant difference was found between the adenine nucleotide content before perfusion of kidneys from well-fed and starved rats.

DISCUSSION

General comment on the perfused rat kidney preparation. The isolated perfused rat kidney does not seem to have been used in the past for studies of renal metabolism, though there are a few reports on the secretory function of the perfused organ (Weiss *et al.* 1959; Bauman *et al.* 1963; Tobian, Coffee, Ferreira & Meuli, 1964). The experiments reported in this paper indicate that the perfused rat kidney is a satisfactory preparation for investigations of both metabolic and secretory activities. One of the reasons for formerly using larger animals was the need for sufficient material for the chemical analyses. The modern micro methods have greatly scaled down this requirement and, since the small size of rat kidney offers no special difficulties of operative technique, the perfused rat kidney may in future conveniently replace for many purposes the use of larger animals, especially cats and dogs. Although the perfused kidney is, in respect to the secretory functions, somewhat inferior to the organ

Table 8. *Adenine nucleotides in perfused rat kidney*

Adenine nucleotides were determined in rapidly deep-cooled tissue as described for the liver by Hems *et al.* (1966). Rats were starved for 48 hr., except when stated otherwise. 'At end of operation' was the stage of removal of the kidney from the animal (see text). Data refer to dry wt. The wet wt./dry wt. ratio of the normal kidney of starved rat, not perfused, was 3·94 ± 0·14 (8). Initial substrate concentration was 5 mM.

State of tissue	Duration of perfusion (min.)	No. of observations	ATP (μmoles/g. dry wt.)	ADP (μmoles/g. dry wt.)	AMP (μmoles/g. dry wt.)	Ratio $\frac{ATP}{ADP}$	Total adenine nucleotides
At end of operation	0	4	4·81 ± 0·33	2·83 ± 0·22	2·71 ± 0·44	1·71 ± 0·14	10·84 ± 0·26
Perfused with lactate	30	4	6·33 ± 0·48	2·44 ± 0·48	0·85 ± 0·09	2·93 ± 0·52	9·62 ± 0·46
Perfused with lactate	60	4	6·87 ± 0·59	2·85 ± 0·18	0·84 ± 0·12	2·41 ± 0·06	10·56 ± 0·65
Perfused with lactate	120	3	5·57 ± 0·13	1·96 ± 0·27	0·76 ± 0·24	2·94 ± 0·29	8·30 ± 0·38
Perfused with pyruvate	30	3	6·48 ± 0·70	2·32 ± 0·29	0·66 ± 0·25	2·80 ± 0·10	9·67 ± 0·79
Perfused without substrate	30	4	6·32 ± 0·31	2·32 ± 0·13	1·04 ± 0·03	2·72 ± 0·02	9·68 ± 0·46
Not perfused (control)	—	4	7·20 ± 0·38	2·66 ± 0·07	0·78 ± 0 10	2·72 ± 0·08	10·69 ± 0·37
Not perfused (well-fed rat)	—	4	6·18 ± 0·90	2·30 ± 0·37	0·68 ± 0·13	2·74 ± 0·15	9·13 ± 1·36

in situ, there is no evidence that the metabolic functions deviate in a major way from those of the normal organ.

Comparison of gluconeogenesis in the perfused and sliced organ. The finding that the rates of gluconeogenesis from a variety of precursors were approximately the same in the perfused organ as in slices of kidney cortex is in contrast with the results obtained with rat liver, where, under optimum conditions, the rates in the perfused organs were three to five times as high as in the slices (Ross *et al.* 1967). No explanation offers itself for the fact that the gluconeogenic capacity of the liver grossly deteriorates on slicing whereas that of the kidney is well maintained.

Oxygen consumption. The oxygen consumption of the perfused organ was about twice as high as that of slices, possibly because of the secretory work done in the perfused organ but not in slices. Much of the oxygen consumption of the intact organ is known to be connected with the absorption of sodium (Kramer & Deetjen, 1964). The extra oxygen consumption which followed the addition of pyruvate was much higher than expected on the basis of ATP requirements for gluconeogenesis. Since the synthesis of one molecule of glucose requires six molecules of ATP the consumption of one molecule of oxygen per molecule of glucose formed would supply the required ATP if the P/O ratio is 3. The extra oxygen consumption observed in the presence of pyruvate was 550 μmoles/hr./g. dry wt. and in the presence of lactate 625 μmoles/hr./g. dry wt. The expected extra consumption of oxygen was 270 and 64 μmoles/hr./g. dry wt. respectively (Table 2). Similar discrepancies were observed before in slices of kidney cortex (Krebs *et al.* 1963*b*).

Acetoacetate as a fuel of respiration. The fact that the absolute rate at which acetoacetate was oxidized was about the same in slices and in the perfused organ, although the oxygen uptake of the intact kidney was much higher, suggests that acetoacetate can supply fuel for the basic respiration and for the extra ATP requirements of gluconeogenesis, but not for the energy requirements of the secretory work. This would imply that the cells or the mitochondria of the cell regions where the main secretory work, that of sodium absorption, is done (the brush border of the proximal tubule cells) do not oxidize acetoacetate whereas other cells do. This problem requires further investigation.

Glucose consumption by the kidney. Although the kidney produces glucose in the presence of excess of gluconeogenic precursor, it consumes glucose if this is present in the medium at physiological concentrations (5 mM) and if glucogenic precursors are absent (see also Newsholme & Underwood,

1965). The rate observed (54 μmoles/hr./g. dry wt.) was low in relation to the oxygen uptake (1620 μmoles when no substrate was added; Table 5). Since about half the glucose consumed appeared as lactate, the contribution of glucose to the fuel of respiration was less than 10%. The formation of lactate was probably due to the metabolism of the white medulla. Slices of white medulla form lactate aerobically at the rate of 700 μmoles/hr./g. dry wt. (György *et al.* 1928), and on the assumption that the medulla contributes 12% of the total tissue weight, a rate of 84 μmoles of lactate/hr./g. dry wt. would be expected. The observed rate (about 50 μmoles) is somewhat lower: the difference may have been due to a reconversion into glucose in the cortex of part of the lactate formed in the medulla. This reconversion would be expected to be slow at the concentration of lactate arising in the experiments. The formation of lactate in the perfused kidney suggests that the aerobic glycolysis observed in medullary slices is not an artifact, as has been suspected by György *et al.* (1928).

In slices of rat kidney cortex starved for 48hr., E. A. Newsholme & A. H. Underwood (personal communication) found a glucose uptake of 5 μmoles/hr./g. wet wt. or 20 μmoles/hr./g. dry wt. Assuming that the cortex contributes 88% to the total weight of the kidney the glucose consumption of the cortex was 18 μmoles/hr./g. of dry kidney. The aerobic glucose consumption in the white medulla in the experiments of György *et al.* (1928) was 350 μmoles/hr./g. dry wt. If the medulla contributes 12% to the total dry weight, the glucose uptake of the medulla, calculated per g. of whole kidney, is 42 μmoles/hr. Thus the total calculated glucose consumption of cortex plus medulla slices would be 18+42=60 μmoles, which is in agreement with the observed value of 54 μmoles in the perfused kidney. It follows that the absolute rates of glucose consumption are about the same in slices and in the perfused kidney, and this suggests that under the test conditions (48hr. starvation) glucose makes no significant contribution to the oxygen consumption connected with the secretory work. Fatty acid is presumably the main fuel under normal physiological conditions.

Interconversion of lactate and pyruvate. Added L-lactate formed considerable amounts of pyruvate, and added pyruvate formed lactate. This also occurs in kidney-cortex slices (Krebs, Gascoyne & Notton, 1967) and the perfused liver (Schimassek, 1963). The formation of lactate from pyruvate implies that under the conditions of perfusion large amounts of $NADH_2$ are available in the cytoplasm. This finding has been discussed by Krebs *et al.* (1967) in relation to the provision of cytoplasmic reducing power for gluconeogenesis in liver and kidney.

Comparison of rates of gluconeogenesis in liver and kidney. For comparison with the present results, the data on the perfused liver given in Tables 1 and 2 by Ross *et al.* (1967) have been multiplied by 215 to convert μmoles/min./g. wet wt. into μmoles/hr./g. dry wt., the wet wt./dry wt. of the perfused

Table 9. *Comparison of maximal rates of gluconeogenesis in the perfused liver and kidney of rat*

Rates refer to tissues from rats starved for 48hr. For comparison the units of the values reported for rat liver by Ross *et al.* (1967, Tables 1 and 2) have been changed from μmoles/min./g. fresh wt. to μmoles/hr./g. dry wt. A conversion factor of 215, based on a wet wt./dry wt. ratio of 3·59, was used. The maximal rates for lactate and pyruvate are those obtained on addition of glucagon to the perfusion medium. All figures are μmoles/hr./g. dry wt. The rates given for the liver were obtained with initial substrate concentrations of 10mM; those of kidney apply to initial substrate concentrations of 5mM. The liver showed no differences between 5 and 10mM. With lactate higher rates were obtained in the kidney at 10mM (126±9 μmoles/hr./g. dry wt.) (4).

	Liver			Kidney		
	Glucose synthesis		Substrate removed	Glucose synthesis		Substrate removed
Precursor added	Total	Corrected for endogenous		Total	Corrected for endogenous	
None	30	—	—	3	—	
L-Lactate	400	370	−800	82	79	−405
Pyruvate	310	280	−1115	304	301	−1246
Glycerol	103	73	−219	95	92	−282
Succinate	52	22	−22	310	307	−1054
L-Glutamate	67	37	−32	107	104	−518
L-Aspartate	50	20		72	69	−696
L-Malate	50	20	−50	206	203	−892
Fumarate	41	11	−34	269	266	−865

liver being 3·59. The maximal rates of glucose formation from lactate (i.e. those obtained in the presence of glucagon) were about five times higher in the liver; those of pyruvate and glycerol were about equal in both tissues (Table 9). Succinate, malate and fumarate formed glucose 14–20 times as rapidly in the kidney and glutamate and aspartate about three times as rapidly. These calculations are based on values from which the endogenous glucose formation has been deducted.

With the dicarboxylic acids and glutamate still greater differences exist in the rates of the removal of the added substrates. That of succinate was 50 times, that of malate 26 times and that of glutamate 15 times higher in the kidney. These figures illustrate the limited permeability of rat liver for these substrates because the activities for key enzymes such as glutamate dehydrogenase, glutamate–oxaloacetate transaminase and malate dehydrogenase are not significantly lower in liver than in kidney. The only relevant enzyme whose activity is higher in the kidney, by a factor of 2·5 in the starved rat, is phosphopyruvate carboxylase (Henning, Stumpf, Ohly & Seubert, 1966). As this is one of the rate-limiting enzymes of gluconeogenesis the difference is important but it does not explain the 14–20-fold difference in the rates of gluconeogenesis from succinate, malate and fumarate.

Glutamine synthetase in rat kidney. Glutamine has long been known to be synthesized in the kidneys of certain (though apparently not in all) mammalian species (Krebs, 1935), but the high rate of the discharge of glutamine into the circulation by the perfused rat kidney is nevertheless surprising. The perfused rat liver releases hardly any glutamine under the same conditions (J. M. Nishiitsutsuji-Uwo, B. D. Ross & H. A. Krebs, unpublished work), nor do rat liver slices form major quantities of glutamine from added glutamate. In view of the presence of glutamine synthetase in rat liver (Wu, 1963, 1964) this negative result must be due to the slow rate of penetration of glutamate into the liver. Although it is not yet possible to assess the contribution of liver to the glutamine supply, it is evident that in the rat the kidney is a major source of the tissue and blood glutamine.

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