Preparation and some Properties of a Suspension of Fragmented Tubules from Rat Kidney

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A method is described whereby short fragments of rat kidney tubule were obtained when kidney slices were gently dispersed by exposure to collagenase and hyaluronidase. When suspended in buffered saline the fragmented tubules respired actively over a period of several hours, the rate of oxygen consumption being proportional to the amount of cell protein. Oxygen uptake was stimulated by the addition of glucose, lactate, butyrate, α -oxoglutarate and other substrates and was decreased by the omission of Ca^{2+} from the suspending medium. With α -oxoglutarate as the added substrate, dinitrophenol strongly stimulated oxygen uptake. Dinitrophenol had a less-marked stimulatory effect when glucose was the added substrate, and inhibited respiration in the absence of added substrate. Oligomycin inhibited respiration and this inhibition was partially reversed by dinitrophenol. Fragmented tubules synthesized glucose from lactate at a high rate but this capacity for gluconeogenesis was abolished by dinitrophenol and by physically damaging the cells.

One of the important objectives of investigators who study the metabolic activities of mammalian cells is that of obtaining a preparation of functionally intact cells which suffers neither the manipulative disadvantages of whole organs nor the diffusion limitations of tissue slices. A preparation that might fulfil this aim would be a suspension of cells which are totally, or almost totally, detached from each other and yet remain intact in the sense that they exhibit normal biochemical activity.

During the past two decades, workers attempting to obtain suspensions of isolated kidney cells have employed a variety of methods for the disruption of the tissue. These methods include treatment with trypsin (Dulbecco & Vogt, 1954; Youngner, 1954; Fenje, 1960; Wallace *et al.*, 1961; Phillips, 1967), Aspergillin-O (Sabina *et al.*, 1963), tetraphenylboron (Rappaport & Howze, 1966) and various chelating agents (Kalant & Young, 1957; Rappaport & Howze, 1966), as well as the purely mechanical methods of forcing kidney tissue through progressively smaller needles (Zaroff *et al.*, 1961) or a stainless-steel screen and pipette (Phillips, 1967, 1968). Thimmappayya *et al.* (1970) used a modified Potter–Elvehjem homogenizer with a soft rubber pestle in their mechanical method for isolating kidney cells. However, in most of the above studies the isolated cells were used for tissue culture and their metabolic activities received little attention. In the cases where respiratory activity was investigated, the cells prepared by Kalant & Young (1957) exhibited negligible endogenous respiration but those isolated by Phillips (1967, 1968) and by Thimmappayya *et al.* (1970) respired actively.

Despite the few successful mechanical methods of isolating apparently viable kidney cells, a new approach to the technical problem of cell isolation has been taken during recent years. Techniques have been developed in which the intercellular connective tissue of solid organs may be digested by enzymes such as collagenase and hyaluronidase, thus permitting gentle disaggregation of the cells. These methods have been successfully applied to liver (Howard *et al.*, 1967; Howard & Pesch, 1968; Berry & Friend, 1969), small intestine (Perris, 1966; Kimmich, 1970), heart (Cavanaugh *et al.*, 1963; Berry *et al.*, 1970), tumours (Yamada & Ambrose, 1966) and kidney (Burg & Orloff, 1962; Thimmappayya *et al.*, 1970; Ahn & Strausser, 1971), and have largely superseded the more vigorous mechanical methods of disruption used in earlier studies of cell isolation.

It is suggested that the degree of intactness of cells isolated by any method may be judged on the basis of four main criteria, namely: (1) cell structure as determined by phase-contrast and electron microscopy; (2) the ability of the cells to take up or exclude certain 'vital' stains; (3) respiratory activity in appropriate balanced salt solutions under a variety of conditions; (4) the ability of the cells to perform integrated metabolic functions normally performed by the intact organ, e.g. the synthesis of glucose from lactate or pyruvate in liver and kidney.

However, it is better to judge cell viability with reference to criteria (3) and (4) and to use the first two only as confirmatory evidence, since cells which retain a normal microscopic appearance sometimes fail to function as normal cellular entities (Kalant &

Young, 1957; Iype & Bhargava, 1965; Ontko, 1967), and there are considerable interpretative difficulties associated with vital staining (Black & Berenbaum, 1964).

The present paper describes the preparation of a suspension of fragmented tubules from rat kidney by an adaptation of the enzymic procedure detailed by Howard & Pesch (1968) for the isolation of liver parenchymal cells. The results are presented of investigations into the respiratory and gluconeogenic capacities of the cells that comprise the fragmented tubules. Some activities are compared with those of kidney slices and of fragmented tubules that were damaged by freezing and thawing.

Materials and Methods

Materials

Reagents. Hyaluronidase (type I), collagenase (type I), glucose oxidase (type II) and oligomycin were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A.; peroxidase (pure), *o*-dianisidine, sodium *n*-butyrate, sodium succinate, L-glutamic acid and α -oxoglutaric acid were from BDH Ltd., Poole, Dorset, U.K.; sodium pyruvate was from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.; bovine serum albumin powder (fraction V) was from Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K.; 2,4-dinitrophenol was from Hopkin and Williams Ltd., Chadwell Heath, Essex, U.K.; D-glucose and sodium DL-lactate, both A.R. grade, were obtained from Ajax Chemicals Ltd., Sydney, Australia. All other reagents were A.R. grade.

Suspending medium. The buffered saline used for suspending kidney tissue preparations was that devised by Robinson (1949) but with glucose omitted. This glucose-free saline is referred to throughout as the buffered saline. Any modifications to it are indicated in the text.

Tissue preparations

Fragmented tubule suspensions. Adult albino rats (Wistar strain) were killed by a blow to the head and then bled. Kidneys were excised and immediately immersed in cold buffered saline. Each kidney was halved longitudinally and the pelvis and inner region of the medulla were trimmed away. The remaining tissue was sliced by the method of Deutsch (1936). Pooled slices (total weight 2–5 g) were placed in a 250 ml Erlenmeyer flask containing 10 ml of ice-cold 0.05% (w/v) collagenase and 0.1% (w/v) hyaluronidase in buffered saline. The flask was incubated at 37°C with continuous shaking (approx. 120 oscillations/min). After 70 min the contents of the flask were poured on a wetted filter consisting of one layer of surgical gauze overlying two layers of stocking

nylon. Filtration was usually facilitated by gently stirring the suspension on the filter with a blunt glass rod. The incubation flask was rinsed with 10 ml of cold buffered saline and the rinsings were run through the filter to be pooled with the original filtrate. The filtered suspension was transferred to a plastic centrifuge tube and centrifuged at 100g for 1 min. The supernatant fluid was withdrawn by suction and the cell pellet was gently resuspended in 10 ml of cold buffered saline and centrifuged again at 100g for 1 min. The washing procedure was repeated twice with buffered saline. The washed pellet was finally suspended in buffered saline, generally by using 4 ml/g fresh wt. of tissue present at the start of the enzyme treatment. Such suspensions usually contained 4.5–6.5 mg of cell protein/ml of suspension.

Slices. When required, slices were cut as described above. Usually a few slices were retained intact and the remainder were used in the preparation of tubule suspensions. In order that the slices should be comparable with tubule suspensions, they were incubated in buffered saline at 37°C for 70 min but not exposed to collagenase or hyaluronidase.

Damaged tubule suspensions. A normal tubule suspension was rapidly frozen in an acetone–solid CO₂ bath. The frozen suspension was then quickly thawed at 37°C. This procedure was repeated twice to ensure breakage of cells.

Incubations

Tubule suspensions or other preparations, total volume 2.0 ml, were incubated at 37°C in conventional Warburg manometric apparatus shaken at 110 oscillations/min. The gas phase was air and, except in certain experiments in which gluconeogenesis was being studied, the centre well of each flask contained 0.2 ml of 2M-NaOH. Substrates and other agents to be added to the incubation systems were dissolved in Ca²⁺-free buffered saline and all systems were pre-incubated for 10 min to allow equilibration.

Glassware used in incubations was coated with a layer of water-repellant silicone compound to prevent the adherence of cells to it.

Measurements

Oxygen uptake was measured manometrically as described by Umbreit (1957).

Protein was determined by the method of Lowry *et al.* (1951) with a solution of bovine serum albumin being used as a standard.

Glucose was determined by the glucose oxidase method described by Krebs *et al.* (1963). At the end of the incubation period the cells were sedimented by centrifugation and a sample of the supernatant fluid was taken for glucose determination. Preliminary experiments indicated that the cell pellet,

which comprised approx. 4% of the total volume, contained approx. 6% of the total glucose and therefore little error was introduced by using samples of supernatant fluid only for determination of the total glucose present in each system.

Results

Microscopy

Suspensions were examined under phase contrast by using a Zeiss photomicroscope. They were seen to consist mainly of short fragments of tubule with some isolated cells and a few small clumps of cells also present. There was little or no non-cellular material to be seen and few large fragments of tubule.

Although the cells appeared to be undamaged it was impossible to ascertain the precise degree of structural integrity.

Cell yield

Measurements of the protein contents of slices and of fragmented tubule suspensions showed that 1 g of fresh slices contained 121 ± 1.4 mg of protein (mean $\pm$ S.E.M. for four determinations) and, of this, 24.2 ± 1.14 mg of protein (mean $\pm$ S.E.M. for ten experiments) was recovered in the tubule suspension. This represents an average yield of 20%.

Uptake of oxygen by fragmented tubules

Experiments were conducted in which the consumption of oxygen by fragmented tubules and by kidney slices, in the absence and the presence of various added substrates, was measured during incubations of up to 4 h duration. The results shown in Fig. 1 indicate that with no exogenous substrate added the tubules displayed a much higher respiratory activity than slices ($P < 0.001$). The addition of glucose brought about a marked increase in the rate of oxygen uptake by tubule suspensions ($P < 0.001$) and this higher rate was maintained throughout the incubation period. The respiratory rate in the presence of DL-lactate was greater than that with glucose during the first 2 h of incubation ($P < 0.05$) but was not as well maintained subsequently. This was probably due to the progressive lowering of the substrate concentration since, as an oxidizable fuel, 20 μ mol of DL-lactate is equivalent to only 5 μ mol of D-glucose. With kidney slices the uptake of oxygen proceeded at a lower but steady rate in both the absence and the presence of added substrate, indicating that endogenous substrate was better conserved in this preparation than in tubules. In each independent preparation of slices both glucose and lactate appeared to stimulate respiration but, on average, such effects were not statistically significant.

Results of experiments shown in Fig. 2 indicate

Fig. 1. Effect of D-glucose (10mM) and DL-lactate (10mM) on uptake of oxygen by kidney slices and fragmented tubules

Slices (9–19mg of protein) or tubules (5–9mg of protein) were incubated at 37°C in 2ml of the buffered saline. Each curve is the mean of several independent experiments. S.E.M. values are shown as vertical displacement limits of the 2h and 4h points. The number of experiments is given in parentheses adjacent to the 4h point of each curve. o, Slices with no added substrate; Δ , slices with added glucose; $\square$, slices with added lactate; $\bullet$, tubules with no added substrate; $\blacktriangle$, tubules with added glucose; $\blacksquare$, tubules with added lactate.

that butyrate was able to stimulate the respiration of tubule suspensions ($P < 0.001$) and promoted the maintenance of a steady respiratory rate. Commercial bovine serum albumin (1%, w/v) did not significantly enhance the effect of butyrate though it caused a slight increase in the endogenous respiratory rate ($P < 0.02$).

In other experiments, not presented here, it was found that L-glutamate, α -oxoglutarate, pyruvate and succinate also stimulated oxygen uptake by suspensions of fragmented tubules.

It was observed that in systems containing between 2mg and 16mg of cell protein the rate of oxygen uptake was proportional to the amount of cell protein present. This relationship could be demonstrated

Fig. 2. Effect of butyrate (10mM) and bovine serum albumin (1%, w/v) on uptake of oxygen by kidney tubules

Tubules (4–9mg of protein) were incubated at 37°C in 2ml of the buffered saline. Each curve is the mean of several independent experiments. S.E.M. values are shown as vertical displacement limits of the 3h points. The number of experiments is given in parentheses adjacent to the 3h point of each curve. ○, No additions; ●, plus albumin; △, plus butyrate; ▲, plus butyrate and albumin.

for incubations up to 4h long, with or without added glucose.

Effect of Ca^{2+} on oxygen uptake. Fig. 3 again shows that fragmented tubules took up oxygen at a linear rate over a period of several hours when suspended in the buffered saline with added glucose. Omission of Ca^{2+} from the buffered saline caused a small decrease in oxygen consumption during the latter half of the incubation period ($P=0.05$). A much greater decrease was observed when the medium was devoid of added glucose ($P<0.001$), and when both Ca^{2+} and glucose were absent the oxygen consumption over a period of 3h was significantly lower than when only glucose was omitted ($P<0.002$).

The two lower curves in Fig. 3 show the oxygen uptake by kidney slices in the absence of added substrate. In each of three separate experiments the omission of Ca^{2+} from the buffered saline led to a small decrease in the respiratory rate, though this

Fig. 3. Effect of Ca^{2+} and glucose on uptake of oxygen by kidney slices and fragmented tubules

Slices (8–13mg of protein) or tubules (5–11mg of protein) were incubated at 37°C in 2ml of normal or Ca^{2+} -free buffered saline with or without added D-glucose (10mM). Each curve is the mean of several independent experiments. S.E.M. values are shown as vertical displacement limits of the 3h points. The number of experiments is given in parentheses adjacent to the 3h point of each curve. ○, Slices in normal saline without added glucose; ▽, slices in Ca^{2+} -free saline without added glucose; ▲, tubules in normal saline with added glucose; ■, tubules in Ca^{2+} -free saline with added glucose; ●, tubules in normal saline without added glucose; ▼, tubules in Ca^{2+} -free saline without added glucose.

effect was not statistically significant. Similar observations were made with slices incubated in the presence of added glucose. It appears that the effects of Ca^{2+} on the respiratory activities of fragmented tubules and kidney slices are qualitatively similar.

Effects of dinitrophenol and oligomycin on oxygen uptake. The effects of dinitrophenol and oligomycin on the uptake of oxygen by kidney tubules incubated in the absence of added substrate and in the presence of glucose or α -oxoglutarate are shown in Table 1. When no substrate was added, dinitrophenol (0.05mM) had no significant effect on respiration during the first 30min of incubation but inhibited oxygen uptake during the subsequent 30min ($P<0.01$). With glucose present, dinitrophenol

Table 1. *Effect of 2,4-dinitrophenol and oligomycin on oxygen uptake by fragmented kidney tubules in the absence and in the presence of added substrate*

Tubules (9–15 mg of protein) were suspended in 2 ml of the buffered saline either without added substrate or with added D-glucose (10 mM) or α -oxoglutarate (10 mM). Suspensions were equilibrated at 37°C for 10 min. Dinitrophenol (0.05 mM) and oligomycin (5 μ g/ml) were then added as indicated and oxygen uptake was measured during the subsequent 60 min incubation period. The results are given as means $\pm$ S.E.M. and the number of observations with independent preparations of tubules is given in parentheses.

Substrate added ... Time of incubation Additions (min) ...	Uptake of O ₂ (μ mol/10 mg of protein)					
	None		Glucose		α -Oxoglutarate	
	0–30	30–60	0–30	30–60	0–30	30–60
None	9.3 $\pm$ 0.4 (4)	7.2 $\pm$ 0.1 (4)	10.4 $\pm$ 0.5 (6)	9.1 $\pm$ 0.5 (6)	11.4 $\pm$ 0.9 (4)	9.2 $\pm$ 0.6 (4)
Dinitrophenol	8.0 $\pm$ 0.4 (4)	4.9 $\pm$ 0.3 (4)	12.4 $\pm$ 0.5 (6)	8.5 $\pm$ 0.9 (6)	17.4 $\pm$ 1.0 (4)	12.4 $\pm$ 0.6 (4)
Oligomycin	4.8 $\pm$ 0.2 (4)	2.5 $\pm$ 0.2 (4)	6.1 $\pm$ 0.4 (6)	3.0 $\pm$ 0.2 (6)	7.2 $\pm$ 0.5 (4)	4.9 $\pm$ 0.3 (4)
Oligomycin + dinitrophenol	6.5 $\pm$ 0.2 (4)	2.1 $\pm$ 0.2 (4)	10.6 $\pm$ 0.4 (6)	5.7 $\pm$ 0.1 (6)	13.8 $\pm$ 0.4 (4)	8.1 $\pm$ 0.5 (4)

stimulated oxygen uptake slightly during the first 30 min only ($P < 0.05$), whereas when α -oxoglutarate was added as substrate, dinitrophenol markedly stimulated oxygen consumption throughout the 60 min incubation period ($P < 0.01$).

In contrast, oligomycin (5 μ g/ml) inhibited oxygen uptake throughout the incubation in both the presence and the absence of added substrate. When dinitrophenol was added with oligomycin, the amount of oxygen taken up in the presence of glucose or α -oxoglutarate was much greater than when only oligomycin was added ($P < 0.001$). However, it was found that when incubations were continued for a further 2 h, the respiratory rates in the presence of both oligomycin and dinitrophenol decreased to values near those that occurred with oligomycin alone. With tubule suspensions to which no substrate was added, dinitrophenol brought about a significant reversal of the oligomycin inhibition during the first 30 min ($P < 0.002$), but the rate of oxygen uptake remained significantly lower than that which occurred in the absence of both metabolic inhibitors.

Gluconeogenesis in fragmented tubules

An important indication of the functional integrity of kidney tissue is its ability to synthesize glucose from lactate or pyruvate. Table 2 contains the results of four experiments in which the synthesis of glucose in tubule suspensions containing lactate was measured. Expt. 1 (Table 2) indicates that undamaged tubules synthesized a significant amount of glucose with lactate as substrate, though little or no glucose was formed by tubules which had been damaged by freezing and thawing. Dinitrophenol (0.1 mM) abol-

ished gluconeogenesis, although only partially inhibiting respiration. Gluconeogenesis was also decreased by washing the fragmented tubules more than the three times involved in the normal preparative procedure (Table 2, Expt. 2). With three extra washes only 6% of the activity was lost but with nine extra washes the synthesis of glucose was decreased by 25%. This decrease was not accompanied by a depression of the respiratory rate and this suggests that the cells suffered relatively minor structural damage.

In Expts. 1 and 2 in Table 2 the oxygen uptake was also measured manometrically and this necessitated the absorption of respiratory CO₂ by alkali. However, since CO₂ is required for the pyruvate carboxylase reaction of the gluconeogenic pathway it was considered that glucose synthesis should also be determined under conditions where CO₂ was not absorbed. The results of two such determinations are given in Expts. 3 and 4 of Table 2. Under 'high pCO₂' conditions, created when the CO₂ was not absorbed, glucose synthesis was approximately double that occurring in systems where CO₂ was absorbed to maintain 'low pCO₂' conditions. These results strongly suggest that the concentration of CO₂ was the limiting factor in the synthesis of glucose in Expts. 1 and 2. They also indicate that fragmented tubules have a high gluconeogenic capacity.

Discussion

Studies of mammalian cell function have been partially restricted by the limitations of the tissue preparations available. Whole organs, whether isolated or *in situ*, are difficult to manipulate and

Table 2. *Synthesis of glucose from lactate in fragmented kidney tubules*

Suspensions of tubules, treated as shown and containing 8–13 mg of cell protein in a total volume of 2 ml of the buffered saline, were incubated for 1 h at 37°C with or without added DL-lactate (10 mM) and dinitrophenol (0.1 mM). In 'low pCO₂' systems the metabolic CO₂ was absorbed by 0.2 ml of 2 M-NaOH, whereas in 'high pCO₂' systems no alkali was present.

Expt. no.	Treatment	pCO ₂	Glucose synthesized (μmol/h per mg of protein)			Uptake of O ₂ (μmol/h per mg of protein)		
			No added substrate	Lactate	Lactate + dinitrophenol	No added substrate	Lactate	Lactate + dinitrophenol
1	Normal	Low	0.010	0.070	0.007	1.38	1.91	1.28
	Damaged	Low	0.000	0.003	0.008	0.28	0.37	0.31
2	Normal	Low	0.004	0.072	—	1.45	2.01	—
	Extra washes (3)	Low	0.006	0.069	—	1.39	1.99	—
	Extra washes (9)	Low	0.006	0.054	—	1.34	2.10	—
3	Normal	Low	0.002	0.082	—	1.46	2.38	—
	Normal	High	0.002	0.151	—	—	—	—
4	Normal	Low	0.002	0.109	—	1.49	2.11	—
	Normal	High	0.008	0.219	—	—	—	—

investigations with them may be limited by uncontrollable factors such as variations in the flow of perfusate to different regions of the organ. With tissue slices most of the cells are not directly exposed to the bulk of the suspending medium and the immediate environments of many cells are affected by the rates of diffusion of substances through the extracellular space of the slice. However, a suspension of undamaged detached cells, or small groups of cells, which has none of the above disadvantages, might be a suitable preparation for the study of metabolism and other cell functions, though it should be recognized that cells in isolation might behave differently from cells which are held together in an organized tissue structure (Jacob & Bhargava, 1962).

In the study described in the present paper, suspensions of fragmented tubules were obtained from rat kidney by exposing slices of cortex and outer medulla to collagenase and hyaluronidase. The method used was a modification of that employed by Howard & Pesch (1968) for the isolation of liver parenchymal cells. Although Phillips (1967) has warned of the possible deleterious effects of exposing cells to proteolytic enzymes, his experimental observations related only to trypsin and not to collagenase. The collagenase used in the present studies contained some protease and peptidase activities but, at low concentrations, these would not be expected to damage cell membranes severely. The cells of the fragmented tubules appeared structurally intact when examined by phase-contrast microscopy.

The tubules respired actively and respiration was

found to be partially dependent on Ca²⁺, an observation that has been made with other preparations of isolated kidney cells (Thimmappayya *et al.*, 1970; Ahn & Strausser, 1971) and with kidney cortex slices (Robinson, 1949). Oxygen uptake was stimulated by several added substrates known to stimulate respiration in kidney cortex slices (Kratzing, 1961; Weidemann & Krebs, 1969). The small stimulation of oxygen consumption by bovine serum albumin was possibly due to contaminating fatty acids in the commercial albumin (Garland *et al.*, 1964; Weidemann & Krebs, 1969). Serum albumin has been shown to stimulate strongly the respiration of isolated liver cells (Howard & Pesch, 1968) and intestinal cells (Perris, 1966).

The rate of oxygen uptake by tubules was close to that achieved by the isolated perfused rat kidney (Nishiitsutsuji-Uwo *et al.*, 1967) and was more than double that which occurred in kidney slices. Thimmappayya *et al.* (1970) also found that tubules, prepared by an enzymic method, exhibited much higher respiratory activity than either slices or detached cells isolated by mechanical or enzymic procedures. They attributed the higher respiratory rate to a loss of respiratory control, an interpretation which implies that, under normal conditions, the ADP concentration in kidney cells limits the respiratory rate. A loss of respiratory control that is accompanied by an increase in the rate of oxygen consumption could be brought about either by an increase in the ADP concentration to a non-limiting level, or by an uncoupling of respiratory-chain

phosphorylation. In the present study it appears unlikely that the high rate of oxygen uptake was due to the uncoupling of respiratory-chain phosphorylation. The inhibition of respiration by oligomycin and the reversal of that inhibition by dinitrophenol are consistent with a significant degree of coupling between respiration and phosphorylation. However, oligomycin has also been shown to inhibit the $(\text{Na}^+ + \text{K}^+)$ -stimulated adenosine triphosphatase of kidney cell membranes and to restrict severely the active uptake of K^+ and the extrusion of Na^+ (Whittam *et al.*, 1964). The consequent changes in the ionic composition of the intracellular compartments which might occur during long incubations could lead to a decrease in respiratory activity. Dinitrophenol would not be expected to relieve this type of inhibition.

The marked stimulation of oxygen uptake by dinitrophenol alone when α -oxoglutarate was the substrate suggests that, in the absence of dinitrophenol, respiration was limited by the ADP available. This probably applies also to the first 30 min of incubation with glucose as substrate. In contrast, the failure of dinitrophenol to stimulate either endogenous respiration or that which occurred during the second 30 min of incubation with glucose, might indicate that under those conditions respiration was limited by the concentration of oxidizable substrate in the mitochondria. The inhibition of endogenous respiration by dinitrophenol during the latter part of the incubation might be attributable to a fall in the ATP concentration leading to a decrease in the rate of activation and oxidation of fatty acids, which form the main endogenous substrates of kidney (Weidemann & Krebs, 1969). Such an effect has been observed in mitochondria isolated from liver (Van den Bergh, 1966) and kidney (Rossi *et al.*, 1968).

Another observation which indicates that there is strong coupling of respiratory-chain phosphorylation in the fragmented tubules is that they are capable of synthesizing glucose from lactate at a high rate. Hems *et al.* (1966) have emphasized that gluconeogenesis is extremely exacting in terms of ATP requirements and, moreover, when lactate is the substrate, the metabolic pathway requires the participation of mitochondrial and cytoplasmic enzyme systems working in unison. Therefore gluconeogenesis might be expected to be one of the first cellular activities to be adversely affected by structural damage or by impairment of energy conservation in the cell. The observed rate of $0.2 \mu\text{mol}$ of glucose synthesized/h per mg of cell protein is higher than that reported for kidney cortex slices (Krebs *et al.*, 1963) and for isolated perfused kidney (Nishiitsutsuji-Uwo *et al.*, 1967). The ATP required for this synthesis could not be stored in the cells and could be provided only by coupled respiratory-chain phosphorylation. This view is confirmed by the observation that

dinitrophenol abolished glucose synthesis from lactate.

It is concluded therefore that fragmented tubules, prepared by the method described in the present paper, exhibit a high rate of respiration with strong energy coupling and also retain a high degree of cellular integrity. It is expected that such preparations might be used to gain further insights into kidney metabolism and cell function.

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