the xylan chain is hydrolysed by the successive removal of xylobiose units from one end of the chain and not by random cleavage. In contrast Howard *et al.* (1960) detected xylose oligosaccharides of a high degree of polymerization during all but the last stages of the hydrolysis, which was evidently by random cleavage of the chains.

In addition to hydrolysing the relatively simple xylans and arabino-xylans, *Epidinium* hemicellulase also hydrolysed the more complex mixture of polymers in clover or wheat-straw hemicellulose. It is not clear how sugars other than xylose and arabinose are released from these polymers; they were, however, detected only in the later stages of the hydrolysis. Probably the hydrolysis of these hemicellulose preparations by the epidinial extracts represents the action of a mixture of several enzymes, and the collective term 'hemicellulase' has been used throughout the present paper in view of this possibility. Presumably the xylobiase is a separate enzyme, although it might be a less specific β-1,4-glycosidase responsible also for the hydrolysis of cellobiose. The β-glucosidase and β-fructofuranosidase activity may have come from the few intact holotrichs remaining in the epidinial preparations.

So far as we are aware, the results reported in the present paper provide the first indication that at least one species of rumen ciliate, *Epidinium ecaudatum*, contains enzymes capable of hydrolysing plant hemicellulose and is thus able to contribute to the fermentation of this plant fraction in the rumen.

SUMMARY

1. Extracts prepared from the rumen protozoon, *Epidinium ecaudatum* (Crawley), under conditions which would not be expected to disrupt any associated bacteria, readily hydrolysed various plant hemicelluloses and starch. Xylobiose, cellobiose, sucrose, isomaltose and melibiose were also hydrolysed, but not cellulose.

2. The hemicelluloses were hydrolysed to their constituent monosaccharides by a mechanism which involved the initial liberation of arabinose followed by the release of xylobiose and finally of xylose, glucose, galactose and uronic acid.

3. The hydrolysis appeared to involve cleavage of xylobiose from the end of the xylan chain rather than random cleavage of the chain.

4. The hemicellulase and the xylobiase both possessed optimum activity in the pH range 5·8–6·2, and the hemicellulase activity was almost constant over the temperature range 37–51°.

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The Metabolism of Isolated Rat-Kidney Nuclei

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Rees & Rowland (1961 a) described the metabolism and chemical composition of isolated rat-liver nuclei. The nuclei incorporated amino acids into nuclear protein and adenine and orotic acid into ribonucleic acid. These processes were inhibited by anoxia, 2,4-dinitrophenol, chlorpromazine and azide. An enzyme system capable of oxidizing reduced diphosphopyridine nucleotide was detected and it was suggested that the liver nuclei possessed an oxidative energy-producing

Table 1. *Recovery of nuclei from rat-kidney homogenates*

For nuclei the results given are the mean values of 30 preparations; for homogenates the results are the mean values for four preparations. Results are expressed as μg./100 mg. wet wt. of kidney ($\pm$s.e.).

	Homogenate	Nuclear preparation	Recovery (%)
Nitrogen	2940 ($\pm$115)	264 ($\pm$9·5)	9·0
DNA phosphorus	45·2 ($\pm$0·3)	17·98 ($\pm$0·8)	39·8

pathway. In this paper the results of a similar investigation into the metabolism of isolated rat-kidney nuclei are presented.

MATERIALS AND METHODS

Animals. Male albino rats, weight 200–250 g., were used.

Reagents. These were as described by Rees & Rowland (1961a) and [1-^{14}C]valine was obtained from The Radiochemical Centre, Amersham, Bucks.

Preparation of nuclei. Each rat was anaesthetized with ether and the kidneys, in turn, were perfused through the renal veins with 50 ml. of ice-cold 0·25M-sucrose containing calcium chloride to a final concentration of 1·8 mM. This removed most of the blood. The kidneys were removed, weighed and homogenized in 25 ml. of ice-cold 0·25M-sucrose containing calcium chloride to a final concentration of 5 mM. The procedure then followed was as described for the isolation of rat-liver nuclei (Rees & Rowland, 1961a), except that the initial centrifuging for kidney nuclei was at 4800*g* (7000 rev./min.) instead of 8200*g* (9000 rev./min.). The final residue was suspended in 14 ml. of ice-cold 0·25M-sucrose. Microscopic examination showed that such preparations were not contaminated with cellular debris and erythrocytes. Counts of nuclear numbers were made as described by Grant & Rees (1957).

Analytical methods. Breakdown of ATP, determination of DNA, RNA, phospholipid, nitrogen, DPNH-cytochrome reductase, DPNH-neotetrazolium reductase, cytochrome oxidase and succinoxidase were as described by Rees & Rowland (1961a). Lactic dehydrogenase was determined by the method of Kornberg (1955).

The methods of incorporation of radioactivity *in vitro* were as described previously by Rees & Rowland (1961a).

RESULTS

Chemical composition. The recovery of nuclei from kidney homogenates was estimated by determining the nitrogen and DNA contents of both whole homogenates and nuclear preparations (Table 1). The DNA, RNA, phospholipid and nitrogen contents of the nuclear preparations are given in Table 2.

Synthetic reactions. Kidney nuclei *in vitro* incorporated [2-^{14}C]glycine and [1-^{14}C]valine into nuclear protein, and [6-^{14}C]orotic acid and [8-^{14}C]-adenine into nucleic acids, the rate of incorporation being approximately linear over 2 hr. (Table 3). In fact, the incorporation of both amino acids and RNA precursors would continue for at least 5 hr.,

Table 2. *Chemical composition of rat-kidney nuclei isolated in 0·25M-sucrose*

Results are the means of values, $\pm$s.e., from 30 preparations.

	$\mu\mu$g./nucleus	μg./mg. of nuclear nitrogen
Nitrogen	14·0$\pm$0·43	—
Phosphorus		
DNA	0·94$\pm$0·007	68·6$\pm$1·95
RNA	0·44$\pm$0·015	32·4$\pm$1·34
Phospholipid	0·48$\pm$0·015	34·5$\pm$1·16

Table 3. *Incorporation of [2-^{14}C]glycine and [^{14}C]valine into nuclear protein and of [6-^{14}C]orotic acid and [8-^{14}C]adenine into nuclear ribonucleic acid*

Systems used were as described in the Materials and Methods section.

Time of incubation (min.)	Glycine	Valine	Adenine	Orotic acid
	(Counts/min. at infinite thickness)			
30	59	36	—	—
40	—	—	120	130
60	136	97	—	—
80	—	—	305	186
90	—	—	—	—
120	262	231	505	323

and after 4 hr. the rate appeared to increase. The addition of ATP to the incubation medium had no effect on the rate of incorporation.

To confirm that the uptake of amino acids represented true incorporation into protein, and not exchange, the method of Allfrey, Mirsky & Osawa (1957) was applied. Nuclear suspensions were allowed to incorporate radioactive amino acids for 1 hr., after which the nuclei were centrifuged down. The radioactive supernatant was discarded, and the nuclei were resuspended in an incubation medium in which the radioactive amino acid has been replaced by 200 times the quantity of non-labelled amino acid. Incubation was continued for another hour and the final radioactivity compared with a nuclear preparation allowed to incorporate radioactive amino acid for 2 hr. The results (Table 4) indicate that most of the incorporation was irreversible.

Rees & Rowland (1961a) demonstrated that the replacement of Na$^+$ ion by K$^+$ ion in the incubation media containing labelled constituents was without

effect on the incorporation of these constituents into isolated rat-liver nuclei. This finding was in contrast with those of Allfrey, Mirsky & Osawa (1955) and Breitman & Webster (1959) with

Table 4. *Effect of resuspension of nuclei which have incorporated labelled amino acid in a medium containing excess of non-radioactive amino acid*

Systems used were as described in the Materials and Methods section.

Time of incubation (hr.)	Glycine	Valine
	(Counts/min. at infinite thickness)	
0	112	0
1	242	154
2	454 (226*)	226 (107†)

* With unlabelled glycine added.
† With unlabelled valine added.

Table 5. *Effect of anoxia, 2,4-dinitrophenol and chlorpromazine on the incorporation in vitro of* [14C]*valine into nuclear protein and* [14C]*orotic acid into nuclear ribonucleic acid*

Systems were as described in the Materials and Methods section. Additions are recorded as final concentration. Incubation time was 90 min.

Treatment	Valine	Orotic acid
	(Counts/min. at infinite thickness)	
None	329	425
Anoxia	112	118
Dinitrophenol		
0·1 mM	152	510
0·3 mM	124	403
0·6 mM	79	374
0·8 mM	74	230
Chlorpromazine		
0·15 mM	82	433
0·3 mM	50	458

thymus nuclei. With kidney nuclei, replacement of Na^+ ion by K^+ ion in media containing [14C]valine increased incorporation from 340 to 442 counts/min.; the corresponding increase with [14C]orotic acid was from 280 to 393 counts/min.

The effects of anoxia and 2,4-dinitrophenol on the incorporation of [14C]valine into nuclear protein, and of [14C]orotic acid into RNA, were tested (Table 5). With valine, the inhibition by anoxia was about 70 % and at the higher concentrations dinitrophenol inhibition was about 80 %. The dinitrophenol effect could not be reversed by addition of ATP. With orotic acid, anoxia inhibited the incorporation by about 75 %; 0·8 mM-dinitrophenol caused about 50 % inhibition.

Chlorpromazine inhibits oxidative phosphorylation (Dawkins, Judah & Rees, 1959) and, in liver nuclei, it inhibited both protein and nucleic acid synthesis (Rees & Rowland, 1961 *a*). The incorporation of valine by kidney nuclei was inhibited by about 80 % with chlorpromazine even at a low concentration (0·15 mM), but the incorporation of orotic acid was not affected (Table 5).

Investigation of enzymes associated with energy mechanisms. As in liver nuclei, the inhibition of the synthetic processes in kidney nuclei by anoxia, dinitrophenol and chlorpromazine suggests the existence of an oxidative phosphorylation system. Acid-soluble nucleotides could not be detected in ice-cold 2 % (w/v) perchloric acid extracts of kidney nuclei. However, like liver nuclei (Rees & Rowland, 1961 *a*), kidney nuclei contain a Mg^{2+} ion-activated adenosine triphosphatase which is stimulated by dinitrophenol (Table 6). The isolated kidney nuclei also contain succinoxidase, cytochrome oxidase, DPNH–cytochrome *c* reductase, DPNH–neotetrazolium reductase and

Table 6. *Activity of enzymes in isolated rat-kidney nuclei and mitochondria*

Enzyme-assay systems were as referred to in the Materials and Methods section. Results and ranges are obtained from at least four experiments. In all cases enzyme activity is linearly related to enzyme concentration.

	Nuclei			
Enzyme system studied	Complete system	Without tissue	Without substrate	Mitochondria (complete system)
Succinoxidase (μl. of O_2/hr./mg. of N)	226 (120–355)	0	0	1640 (1470–1765)
DPNH–cytochrome *c* reductase (μmoles of cytochrome *c* reduced/hr./mg. of N)	12·9 (11·3–15·9)	0	0·15	39 (38–40)
DPNH–neotetrazolium reductase (mg. of formazan formed/hr./mg. of N)	2·85 (2·44–3·75)	0·11	0·10	9·4 (8·2–9·9)
Lactic dehydrogenase (unit/mg. of protein)	0·70 (0·46–0·91)	0	0	0
Cytochrome oxidase (μl. of O_2/hr./mg. of N)	1082 (830–1270)	0	0	3380 (2900–3760)
Mg^{2+} ion-stimulated adenosine triphosphatase (μmoles of phosphate/20 min./mg. of N)	3·7 (3·5–3·8)	—	—	12·1 (11·8–12·6)
Dinitrophenol-stimulated adenosine triphosphatase (μmoles of phosphate/20 min./mg. of N)	6·45 (6·2–6·7)	—	—	17·8 (17·2–18·8)

lactic dehydrogenase (Table 6). The DPNH-neotetrazolium-reductase activity was markedly inhibited by dicoumarin. Both cytochrome oxidase and succinoxidase are considered to be solely localized in the mitochondria (Schneider, 1946), but our findings suggest that there is a nuclear cytochrome oxidase. Succinoxidase activity varies considerably in different nuclear preparations and is sometimes undetectable, which suggests that this enzymic activity is due to variable degrees of mitochondrial contamination. However, even in the apparent absence of succinoxidase, cytochrome-oxidase activity is invariably found. In rat liver Rees & Rowland (1961a) showed that the ratio of cytochrome oxidase to succinoxidase was appreciably higher in nuclei (4·8) than in mitochondria (2·5), and similar results have been obtained in kidney preparations [nuclei 4·8, (3·5–6·8) and mitochondria 2·1 (2·0–2·2)]. These findings suggest that there is a cytochrome oxidase in nuclei which cannot be ascribed to mitochondrial contamination. In order to test this, the total nitrogen content, the succinoxidase and cytochrome-oxidase activities of mitochondria and nuclei prepared in parallel from the same kidney homogenate were determined. By assuming succinoxidase activity to be due entirely to mitochondria, the percentage of mitochondrial nitrogen in the nuclear preparations was calculated. In different nuclear preparations this ranged from zero to 24% of nuclear nitrogen, but in each case the amount of oxygen consumed by the nuclei for cytochrome-oxidase activity well exceeded the amount attributable to the calculated mitochondrial contamination (Table 7). Similar corrections can be applied for the enzyme values in Table 6. Based on the mean succinoxidase values (Table 6), a mitochondrial contamination level of 14% has been calculated and the nuclear-enzyme activities adjusted to give the following values: DPNH–cytochrome c reductase, 7·5 μmoles of cytochrome c reduced/hr./mg. of N; DPNH–neotetrazolium reductase, 1·55 mg. of formazan formed/hr./mg. of N; lactic dehydrogenase, as in Table 6; Mg^{2+} ion-stimulated adenosine triphosphatase, 1·9 μmoles of

phosphate/20 min./mg. of N, which was increased 53% by the addition of dinitrophenol (final concentration 0·03 mM).

DISCUSSION

The method of isolation and purification of nuclei from rat kidney was essentially the same as the procedure described by Rees & Rowland (1961a) for rat liver. After perfusion kidney tissue is almost free of blood and microscopic examination showed that the nuclear preparations were practically free of erythrocytes and cellular debris. It was impossible to obtain a reliable count of numbers of nuclei in the kidney homogenate. An estimate of recovery of nuclei therefore can only be based on the recoveries of nitrogen and DNA. Recoveries of nitrogen were 9% with kidney nuclei and about 5% with liver nuclei. The number of nuclei/100 mg. wet wt. of tissue was also higher for kidney [19·1 ($\pm$0·5) $\times 10^6$] than for liver (8 $\times 10^6$).

The recoveries of DNA from rat liver and rat kidney were about 40% in both cases. The RNA/DNA ratio in kidney nuclei was 0·47, which is of the same order as recent values obtained for liver nuclei (Rees & Rowland, 1961b).

The results of incorporation experiments and inhibitor studies show a marked similarity between rat-liver and rat-kidney nuclei apart from the absence of an effect of chlorpromazine on the incorporation of orotic acid by kidney nuclei. Chlorpromazine acts on flavoproteins (Dawkins et al. 1959, 1960). DPNH–Cytochrome c reductase is a flavoprotein enzyme and was shown to be active in kidney nuclei. If this enzyme is involved in a system of electron-transferring enzymes producing energy for protein and RNA synthesis, it is difficult to explain the lack of effect of chlorpromazine on the incorporation of orotic acid. However, in support of an electron-transferring and energy-producing system is the presence of a Mg^{2+} ion-activated adenosine triphosphatase, which, in mitochondria, is thought to be a reversal of some of the oxidative phosphorylation reactions, and also the presence of the oxidation–reduction enzymes cytochrome oxidase and DPNH–cytochrome c reductase. Since kidney nuclei contain DPNH–neotetrazolium reductase it is possible that a quinone is involved in the pathway for the oxidation of DPNH, as suggested for liver nuclei (Rees & Rowland, 1961a). In addition the presence of lactic dehydrogenase offers a means of producing DPNH in kidney nuclei.

SUMMARY

1. The isolation of rat-kidney nuclei in 0·25 M-sucrose is described and the nucleic acid and phospholipid contents are reported.

Table 7. *Cytochrome-oxidase activity due to mitochondrial contamination of nuclear preparations*

System was as described in Materials and Methods section. The results presented are for three different nuclear preparations.

	Oxygen consumed (μl./hr./ml. of nuclear preparation)		
Nuclear preparations ...	1	2	3
Total consumption	413	593	510
Consumption attributable to mitochondrial contamination	0	290	276

2. The nuclei *in vitro* incorporate [2-^{14}C]glycine and [1-^{14}C]valine into nuclear protein and [6-^{14}C]-orotic acid and [8-^{14}C]adenine into nucleic acids.

3. Anoxia and 2,4-dinitrophenol inhibit the incorporation of the radioactive precursors into nuclear proteins and nucleic acids. Chlorpromazine inhibits the incorporation of valine, but has no effect on the incorporation of orotic acid.

4. The specific activities of adenosine triphosphatase, succinoxidase, cytochrome oxidase, DPNH–cytochrome *c* reductase, DPNH–neotetrazolium reductase and lactic dehydrogenase are reported. Evidence suggesting that succinoxidase activity is due to mitochondrial contamination whereas cytochrome oxidase is a true nuclear component is presented.

5. The chemical composition and metabolism of rat-kidney nuclei and rat-liver nuclei are compared.

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Adenosine Triphosphatase in the Microsomal Fraction from Rat Brain

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Skou (1957) isolated a microsomal fraction from an homogenate of the leg nerves of the shore crab which contained an adenosine triphosphatase requiring magnesium; to obtain maximum activity both Na$^+$ and K$^+$ ions were necessary (Skou, 1957). From the results of detailed studies of the ionic requirements for this adenosine triphosphatase, Skou (1957, 1960) suggested that this enzyme was involved in the active extrusion of sodium from the nerve fibre.

Later work on the hydrolysis of ATP by erythrocyte membranes has also suggested that this process is involved in the maintenance of the ionic composition of the mammalian erythrocyte. The main basis for this conclusion is the quantitative similarity of the ionic requirements for both systems (Mg^{2+}, Na$^+$ and K$^+$ ions) and their inhibition by similar concentrations of cardiac glycosides (Post 1959; Post, Merritt, Kinsolving & Albright, 1960; Dunham & Glynn, 1960, 1961; Glynn, 1957*a*).

Adenosine triphosphatase has been found in mammalian nervous tissue: Abood & Gerard (1954) found it in a microsomal fraction from an homogenate of rat nerve, and in disrupted rat brain it is

stimulated by magnesium, sodium and potassium (Utter, 1950; Hess & Pope, 1957). Järnefelt (1961) obtained a stimulation of 15–30 % by Na$^+$ ions alone with a microsomal fraction from rat brain. In a preliminary note Deul & McIlwain (1961) showed also that, in the presence of potassium, sodium stimulated (2·5-fold) the adenosine triphosphatase of a microsomal fraction from guinea-pig cerebral cortex.

The present paper describes the isolation of a microsomal fraction from whole rat brain and the properties of the adenosine triphosphatase present in it.

METHODS

Homogenization. In initial experiments the Potter–Elvehjem homogenizer with a Perspex pestle (total difference in diameter between tube and pestle, 0·010 in.; speed of rotation, 1100 rev./min.) was used (Aldridge, Emery & Street, 1960). Although good preparations could be obtained in this way, more reproducible preparations were obtained with a M.S.E. homogenizer (Measuring and Scientific Equipment Ltd.) fitted with a tachometer. With the 10 ml. vortex flask, one rat brain was homogenized in 7·5 ml. of ice-cold 0·3M-sucrose for 2 min. at 9000 rev./min.