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CHANGES IN THE ADENINE NUCLEOTIDE CONTENT OF PREPARATIONS OF THE RAT SMALL INTESTINE *IN VITRO*

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SUMMARY

1. The adenine nucleotide content of rat jejunal mucosa has been measured. For fresh tissue the concentrations of the three nucleotides in the total water are approximately: ATP, 1·4 mM; ADP, 1·0 mM; and AMP, 0·5 mM.

2. The adenine nucleotide content of mucosal slices prepared from rat jejunum was about 80 % of that of fresh tissue, but the slices rapidly lost nucleotides when they were incubated *in vitro*. After a 4 min incubation the mucosal tissue contained about 1 μmole/g dry wt. of each of the three nucleotides. This represented only 14 % of the ATP, 20 % of the ADP and 36 % of the AMP originally present. Further incubation had little effect on the ATP and ADP content, but some additional AMP was lost.

3. Additions of glucose, amino acids, phosphate, pyruvate, butyrate or glucose-6-phosphate did not prevent the loss of nucleotides from the mucosal slices. The addition of ADP (1 mM) did restore the ATP content of slices to a value close to that of fresh tissue within 2 min, but the nucleotide content declined again on further incubation.

4. The respiration rate of mucosal slices falls progressively during incubation *in vitro*. This decline was largely prevented by the addition of ADP.

5. Although nucleotide loss occurred most rapidly from mucosal slices, it was also observed with rings of whole intestinal wall and, less rapidly, with everted sacs.

6. ATP added to incubations of mucosal slices disappeared at a rate of about 30 μmole/min.g dry wt.

7. These results suggest that the adenine nucleotide content of preparations of rat small intestine studied *in vitro* is likely to be severely reduced. The addition of nucleotides to the incubation medium produces only a temporary increase in the nucleotide content of the tissue.

INTRODUCTION

The accumulation of amino acids and sugars by the mucosal epithelium of the small intestine is thought to depend ultimately on a supply of energy from cellular metabolism, but the mechanism by which transport is coupled to metabolism has remained obscure. Although the Na^+/K^+ ATPase seems likely to play an important role in this coupling, few measurements of the ATP content of mucosal tissue have been made. The manner in which the nucleotide content of this tissue changes during incubation *in vitro* is also unknown.

In this paper we report measurements of the amounts of ATP, ADP and AMP in fresh mucosal tissue from the jejunum of the rat small intestine. The changes in the levels of these three nucleotides have also been followed during incubation of the tissue *in vitro* under a variety of conditions. Slices of mucosa (Bronk & Parsons, 1965) were chosen for much of this work because they can be incubated under controlled conditions and deproteinized rapidly. Another advantage of mucosal slices is that they lack the underlying smooth muscle layers which might make it difficult to obtain accurate measurements of adenine nucleotide content of the mucosal epithelium. The changes in nucleotide levels found with mucosal slices incubated *in vitro* have been compared with those obtained with rings or everted sacs of small intestine.

METHODS

Male Wistar rats of 200–250 g body weight, that had been allowed free access to food, were used in all the experiments. The preparation of rings and mucosal slices from the jejunum of the small intestine, the incubation of the tissue and the measurement of oxygen consumption were carried out as described by Bronk & Parsons (1965). Everted sacs were made by the method of Wilson & Wiseman (1954). Incubations were made at 37° C in a modified Krebs bicarbonate Ringer (Bronk & Parsons, 1965) gassed with air containing 5% CO_2 v/v. The amino acid mixture added to some incubations was made up as described by Bronk & Parsons (1966). The intestine was rinsed through with ice-cold 0·9% NaCl. In some experiments 0·9% KCl was used as a rinsing solution, but this substitution had no effect on the results obtained. The general procedure for isolating the tissue from the incubation medium before the estimation of metabolites has been described previously (Leese & Bronk, 1972*a*), and some elaborations of this technique are described in the Results section.

Estimation of nucleotides

ATP levels were measured by the method of Leese & Bronk (1972*b*), ADP and AMP by the method of Adam (1965). A new procedure was developed for the estimation of the nucleotide content of fresh tissue. A short segment of the jejunum from a rat anaesthetized with ether was rinsed free of debris with ice-cold 0·9% NaCl. The handle of a tissue extraction device (see Fig. 1) was inserted into the distal end

of the segment until it appeared at the proximal end, and then, with the mesentery still intact, it was rapidly pulled through the loop of intestine. The mucosa which was stripped off by this device was then immediately deproteinized in 6% perchloric acid and subsequently extracted as previously described (Leese & Bronk, 1972*a*). In some experiments, the rats were killed by a blow on the head, without the use of an anaesthetic, in order to determine whether the cessation of the mesenteric circulation affected nucleotide levels.

The release of nucleotides was also monitored by measuring the increase in absorbance at 260 nm. The absorbance of serial samples of incubation medium, taken from a variety of gut preparations was measured at this wave-length, and the absorbance of the protein in the final sample from each incubation was also measured at 280 nm. The ratio of the absorbance at 280 nm to that at 260 nm was then used to calculate the total amount of nucleotides plus nucleic acids in the sample (Dawson, Elliott, Elliott & Jones, 1969). To check the accuracy of the protein estimation, medium samples from a mucosal slice experiment were also assayed for protein by the method of Lowry, Rosebrough, Farr & Randall (1951), using bovine serum albumin as standard. In eight determinations the absorbance method gave values $93 \pm 7\%$ of that of the Lowry *et al.* method.

Expression of results

The results for adenine nucleotide content are expressed as μmole/g dry wt. of tissue rather than in terms of concentration because of the uncertainties associated with the accurate measurement of tissue water. The results for oxygen uptake are expressed in terms of Q_{O_2} (i.e. as μl. O_2 consumed/mg dry wt. of tissue. hr). The oxygen uptake over the first 30 sec after substrate addition is called the 'initial Q_{O_2}' whereas that over the full incubation period after addition of substrate is called the 'average Q_{O_2}'. All results are expressed as means $\pm$ S.E. of the mean followed by the number of observations in parentheses.

RESULTS

Adenine nucleotide content of fresh mucosal tissue

The data in Table 1 show the adenine nucleotide content of mucosal scrapings made directly from segments of rat jejunum immediately after rinsing. It is interesting to note that killing the rat with a blow on the head instead of using ether anaesthesia appeared to reduce the ATP content from 7·3 to 2·9 μmole/g dry wt. When mucosal slices were prepared from intestinal segments from unanaesthetized rats, the ATP content was almost equivalent to the level in fresh tissue. However, during the brief manipulation before incubation of the tissue, the ATP concentration dropped to 3·1 μmole/g dry wt. All of the animals used in the remainder of the work described in this paper were killed by a blow on the head.

Adenine nucleotide content of mucosal slices after incubation in vitro

The amounts of ATP, ADP and AMP in mucosal slices after incubation for various times are shown in Fig. 2. The tissue levels of all three nucleotides declined rapidly in the first two minutes of incubation, and then fell

somewhat more slowly during the remainder of the incubation. At 8 min only 1 μmole/g dry wt. of ATP, 0·9 μmole/g dry wt. of ADP and 0·3 μmole/g dry wt. of AMP remained. Fig. 2 also shows that addition of glucose (11·1 mM) failed to prevent the decline in the levels of any of these nucleotides. Other substrates were also tested in an attempt to arrest the

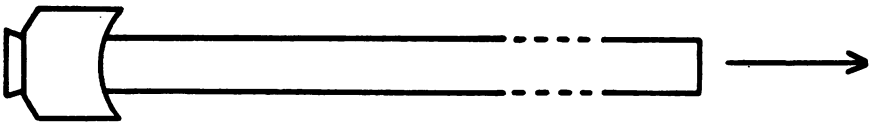

Fig. 1. Tissue extraction device. The device consists of an aluminium rod, 10 cm long, and 1·5 mm in diameter with a chamfered Perspex sleeve, 4·4 mm in diameter, attached to one end of the rod. When it is pulled through a segment of intestine in the direction shown by the arrow, it scrapes off the mucosa, which may then be deproteinized immediately.

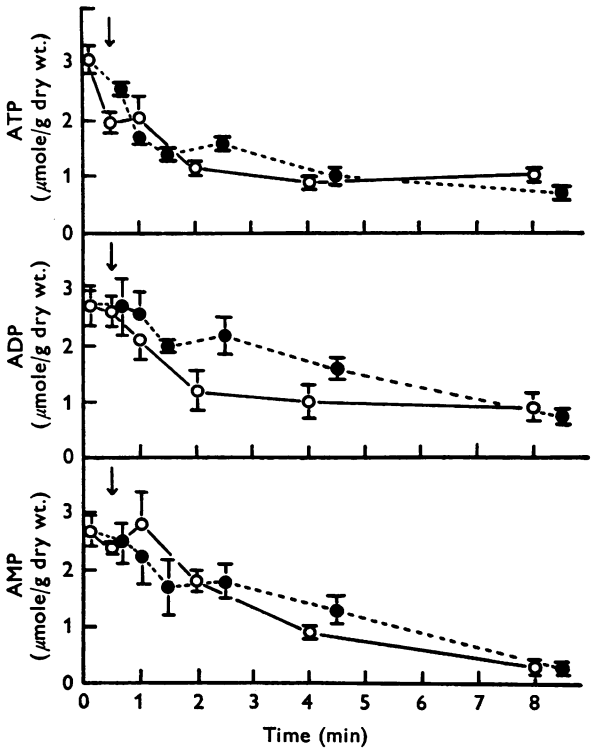

Fig. 2. The ATP, ADP and AMP content of mucosal slices after various times of incubation. ○, control; ●, glucose (11·1 mM) added after 30 sec pre-incubation at the point indicated by the arrow. The vertical lines show the S.E. of the means. Each value is the mean of four observations. Some of the ATP data were published previously (Leese & Bronk, 1972*a*).

loss of nucleotides from the tissue. Additions of pyruvate, butyrate or glucose-6-phosphate at a final concentration of 1 mM had no effect nor did the additions of 10^{-4} M ouabain, a mixture of amino acids, or a medium containing twice the normal phosphate concentration (4 mM). Replacement of the sodium ions in the medium with potassium ions also had no effect.

Respiration of mucosal slices

The decline in nucleotide levels reported in the previous section was associated with a progressive drop in the rate of oxygen uptake by the mucosal slices. The upper curve in Fig. 3 shows how the oxygen content of the incubation medium declined when the slices were incubated in the absence of substrate. When the tissue was added there was a very rapid

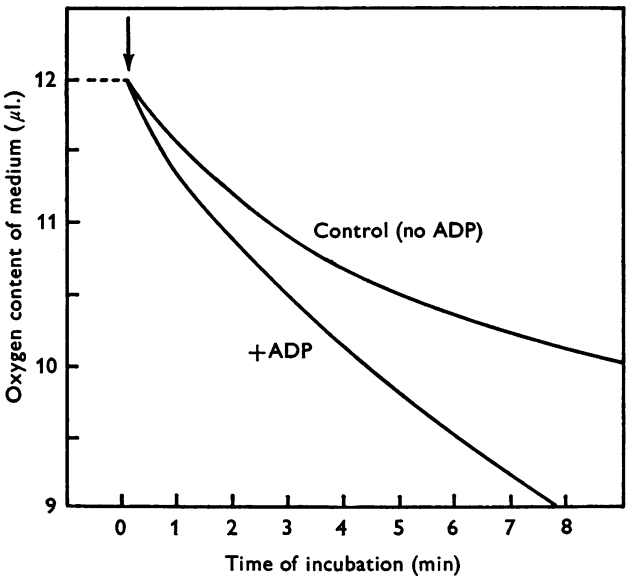

Fig. 3. Oxygen electrode recordings showing oxygen consumption by mucosal slices in the presence and absence of 1 mM-ADP. Each curve shows the oxygen uptake in a single incubation of approximately 5 mg tissue added at the point indicated by the arrow. Volume of incubation vessel 3 ml. The inital Q_{O_2} was calculated by taking the slope of the trace over the first 30 sec after the addition of the tissue.

phase of oxygen uptake with a mean Q_{O_2} of 13·5 ± 0·4 (48) which lasted about 10 sec, the Q_{O_2} then dropping to 5·96 ± 0·63 (10) at 30 sec and continuing to fall until at 4 minutes it was only 14 % of the initial rate (1·9 ± 0·23 (10)). The Q_{O_2} then remained at or below this very low level throughout the second 4-min period. A series of experiments was carried out to determine the effects of adding a mixture of amino acids (Bronk & Parsons,

1966) 30 sec after the start of incubation. The results are given in Table 2. None of the additions tested was able to maintain the initial Q_{O_2} although the amino acids did diminish the extent to which the oxygen consumption declined. Glucose appeared to have little effect on the pattern of oxygen uptake, or on the increased oxygen uptake observed in the presence of amino acids.

TABLE 1. Adenine nucleotide content of mucosa isolated from segments of rat jejunum

Preparation	Nucleotide content (μmole/g dry wt.) ATP	ADP	AMP	$\frac{[ATP][AMP]}{[ADP]^2}$
Mucosal scrapings (from rats anaesthetized with ether)	7·3 ± 0·47 (8)	5·4 ± 0·26 (4)	2·8 ± 0·39 (5)	0·70
Mucosal scrapings (from rats killed by a blow on the head)	2·9 ± 0·25 (5)	5·0 ± 0·8 (4)	3·4 ± 0·26 (5)	0·39
Mucosal slices (from rats killed by a blow on the head)	6·4 × 0·48 (7)	4·5 ± 0·42 (7)	1·7 ± 0·14 (5)	0·66

The effects of the addition of ADP to incubations of mucosal slices

The lower curve in Fig. 3 shows that the oxygen consumption of mucosal slices continues at a high rate in the presence of 1 mM-ADP. This is in marked contrast to the pattern of oxygen uptake in the absence of ADP. The changes in Q_{O_2} brought about by the addition of ADP after 4 min pre-incubation are given in Table 3, and it is apparent that ADP approximately doubled the rate of oxygen consumption. The effects of various substrates added together with ADP are shown in Table 4. The values for the average Q_{O_2} over the entire 8 min period with the various substrates plus ADP are only slightly below the Q_{O_2} values obtained over the first 30 sec with these substrates in the absence of ADP (Table 2).

In addition to stimulating respiration 1 mM-ADP caused a dramatic increase in the ATP content of mucosal slices. In many of the experiments the ADP was added together with 11·1 mM glucose, but the addition of the sugar had no significant effect on the observed rise in ATP.

Initially the assays of ATP following the addition of ADP were carried out on the combined tissue plus incubation medium. Fig. 4 shows the rise in ATP content measured in this way after the addition of 1 mM-ADP plus 11·1 mM glucose after 4 min pre-incubation in substrate-free medium.

About 3 min after the addition of glucose and ADP, the ATP reached a maximum level which was over 10 times that found at the beginning of the experiment and over 30 times the level at 4 min. However, when the medium samples were taken before the addition of perchloric acid they were found to contain approximately 90% of the total ATP. In order to determine the source of this ATP, the tissue and medium were separated by centrifugation after a 4 min pre-incubation and the medium alone was then incubated for 2 min in the presence of 1 mM-ADP. Somewhat surprisingly, the medium was found to produce 99 ± 14 (4) μmole ATP/g dry wt. of the tissue originally present, or more than 3 times the amount produced in the presence of tissue. It seemed possible that adenylate kinase (myokinase) was leaking from the tissue into the medium and catalysing the formation of ATP from ADP by the reaction:

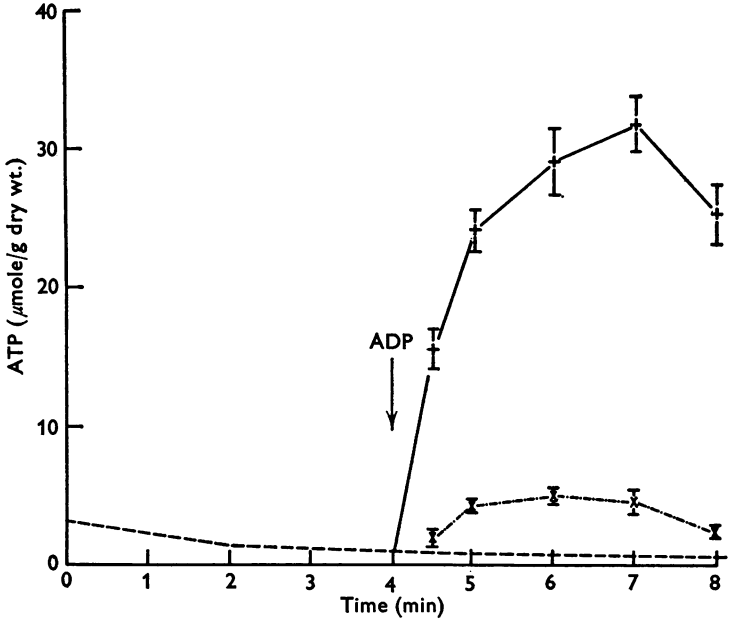

Fig. 4. The influence of 1 mM-ADP on the ATP content of mucosal tissue and on the tissue plus incubation medium. +, ATP content of tissue plus medium; ×, ATP content of tissue alone. Dashed line represents the decline in tissue ATP levels, given in detail in the top part of Fig. 2. The vertical lines show the standard errors of the mean. Each value is the mean of six determinations. The values for the ATP content of the tissue alone were published previously (Leese & Bronk, 1972*a*).

$$2\,\text{ADP} \leftrightharpoons \text{ATP} + \text{AMP}.$$

This possibility was tested by adding AMP to the incubation medium, since this nucleotide is known to inhibit adenylate kinase (Colowick, 1955).

When 1 mM-ADP plus 1 mM-AMP was added to the medium after the removal of the tissue, the amount of ATP formed in 2 min was reduced to $57 \pm 6{\cdot}8$ (4) μmole/g dry wt. and with 1 mM-ADP plus 5 mM or 1 mM-ADP plus 10 mM-AMP the amounts of ATP formed were only $25 \pm 3{\cdot}1$ (4) and $17 \pm 2{\cdot}2$ (4) μmole ATP/g dry wt., respectively. A similar inhibition of ATP formation by AMP was also observed when the mucosal tissue was present in the incubations.

These findings strongly suggested that adenylate kinase was responsible for the production of ATP from ADP in the incubation medium. In order to explain the fact that more ATP was formed in the medium after the tissue had been removed, one may postulate either that ATP is broken down by the tissue as soon as it is formed, or that some of the ADP taken up by the tissue is not available for the adenylate kinase reaction.

In view of these considerations it was important to know the ATP content of the tissue precisely, and a procedure was devised to make this possible (Leese & Bronk, 1972*a*). The ATP content of the tissue was obtained by calculating the difference between the ATP content of the medium plus tissue and that of the medium alone. Both types of samples were deproteinized as rapidly as possible, and subsequently treated identically. Adenylate kinase in the samples was completely destroyed by employing the procedure of Kushmerick, Larson & Davies (1969). The tissue ATP values obtained with this method are shown in Fig. 4. The peak ATP content (5 μmole/g dry wt.) was reached 2 min after ADP addition. This was about 70% of the level found in fresh mucosa (Table 1).

Loss of adenine nucleotides from mucosal slices, rings and everted sacs

Having described in detail the leakage of ATP, ADP and AMP from mucosal slices, it was of interest to determine whether this phenomenon would be observed in other preparations of intestinal tissue. Comparisons were made of the release of 260 nm absorbing material in mucosal slices, isolated rings and everted sacs. The results are shown in Fig. 5 and the proportion of nucleotides plus nucleic acid released in each case is given in the legend.

Adenine-containing compounds are rapidly lost from mucosal slices during the first 8 min of incubation, in confirmation of the data shown in Fig. 2. Rings of small intestine are obviously a more organized type of preparation, but they still show a considerable release of 260 nm absorbing material. By contrast, everted sacs show only a small but steady release of 260 nm absorbing material. The addition of glucose at a concentration of 11·1 mM had no effect on the pattern of release shown in any of the three preparations.

Analysis of the ATP content of freshly prepared rings has given a mean value of 10·7 ± 0·69 (4) μmole/dry wt. If the mucosa contains 7·3 μmole ATP/g dry wt. (Table 1) and accounts for 65% of the dry weight (Bronk & Parsons, 1965) the muscle layers must contain 17·1 μmole ATP/g dry wt. After the rings were incubated for 4 min the ATP content fell to 6·5 ± 0·45 (4) μmole/g dry wt. If the ATP content of the muscle did not change during incubation, the overall nucleotide loss would bring the mucosal ATP content down to 0·8 μmole/g dry wt., which is similar to the value obtained for mucosal slices incubated for a similar period of time.

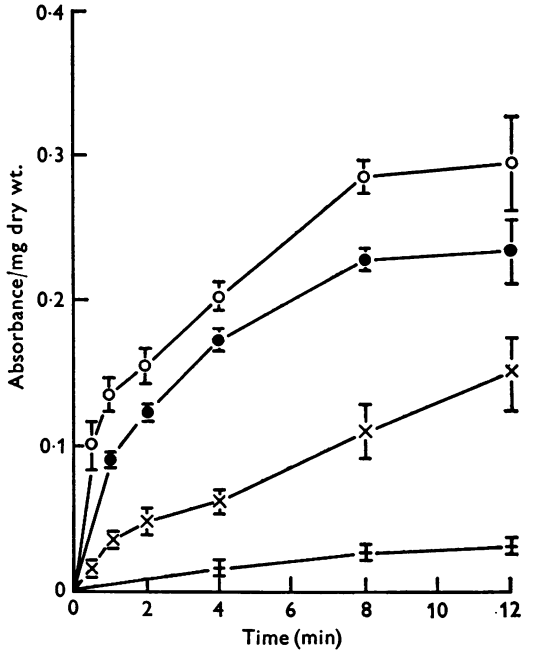

Fig. 5. The time course of the release of material absorbing at 260 nm by intestinal preparations *in vitro*. ○, Absorbance of mucosal slice incubation medium at 260 nm; ●, absorbance of mucosal slice incubation medium at 280 nm; ×, absorbance of intestinal ring incubation medium at 260 nm; +, absorbance of everted sacs incubation medium at 260 nm. All the results are expressed per mg dry wt. of tissue in a volume of 3 ml. Values are means of six determinations and the vertical lines show the S.E. of the means. The percentage of 260 nm absorption due to nucleotides + nucleic acids was calculated for the 12 min value obtained with each preparation and gave the following results: mucosal slices 6·9%; rings, 5·9%; everted sacs 3·9%.

The addition of ATP to preparations of rat jejunum

Several reports have described the effects obtained when ATP was added to preparations of small intestine. For example, Kohn, Newey & Smyth (1967) and Gerencser & Armstrong (1972) have found effects of ATP on the transmural potential, while Reiser & Christiansen (1971) reported that ATP inhibited the uptake of leucine by isolated epithelial cells. None of these investigators determined the fate of the added ATP, and we have examined this problem without preparations. ATP was added to incubations of mucosal slices after 30 sec pre-incubation to give concentrations of 1, 2 and 5 mM, and after a 4 min incubation analyses showed that the ATP had disappeared at the rate of 28, 36 and 44 μmole/min.g dry wt. respectively. Preliminary experiments have shown that 1 mM ATP disappears at a comparable rate when infused into the lumen of an isolated segment of rat jejunum *in vivo*.

DISCUSSION

The highest nucleotide levels we have observed in fresh mucosal tissue were found in mucosal scrapings obtained from rinsed jejunal segments from anaesthetized rats. The use of an anaesthetic allowed the sample of mucosal scrapings to be taken while the mesenteric circulation was still intact. When the animals were killed before obtaining the scraping the ATP content was reduced by more than 50 %. Apparently the preparation of the mucosal slices allows the partial restoration of the mucosal ATP, since the nucleotide content of the slices is more than 80 % of that found in the mucosal scrapings from anaesthetized rats. From the nucleotide content, an approximate figure can be obtained for the concentrations of each of the three nucleotides in the mucosal tissue water. Jejunal mucosa contains approximately 5·25 ml. of tissue water/g dry wt. (Leese & Bronk, 1972*a*) so that in the mucosal scrapings from the anaesthetized rats ATP, ADP and AMP were present at concentrations of 1·4, 1·0 and 0·5 mM, respectively.

As far as we are aware there are no other reports of the ATP, ADP and AMP content of fresh mucosal tissue. Iemhoff, Van Den Berg, De Pijper & Hulsmann (1970) measured the ratios of the three nucleotides in everted segments of rat intestine and in isolated epithelial cells, but they did not report absolute values. These authors found ratios of ATP:ADP:AMP of 11:28:100 in the segments and 67:300:100 in the isolated cells. These results differ considerably from each other and from the data reported in this paper. Our measurements give ratios (ATP:ADP:AMP) of 261:193:100 for fresh tissue and 333:300:100 for mucosal slices incubated for 8 min. In view of the leakage of nucleotides from mucosal tissue which we have

observed it is probably not surprising that the ATP in the preparation used by Iemhoff *et al.* (1970) was largely depleted. Their intestinal segments were everted on a glass rod and incubated for 15 min at 37° C; their epithelial cells were obtained as the result of a 60 min isolation procedure.

Our results are most closely comparable with those obtained by Brosnan, Krebs & Williamson (1970) for rat liver *in vivo*. If their values are expressed on a dry weight basis they give approximately 12·3, 5·8, 1·5 μmole/g dry wt. as the content of ATP, ADP and AMP respectively. Brosnan *et al.* (1970) obtained a value of 0·54 for the ratio [ATP] [AMP]/[ADP]^2 which is close to the value of 0·70 obtained in the present work.

When the mucosal slices were incubated *in vitro* for 4 min the amounts of all three nucleotides fell to about 1 μmole/g dry wt. For ATP, ADP and AMP this meant that the slices contained only 14, 20 and 36 % respectively of the amounts found in fresh tissue. After a further 4 min incubation there was no change in the ATP but the ADP and AMP content had dropped to 17 and 11 % respectively of the amounts in fresh tissue. Krebs (1969) described a similar loss of nucleotides from rat liver slices. Immediately after preparation the slices contained 53 % of the ATP, 109 % of the ADP and 213 % of the AMP found in the liver *in vivo*. After 10 min incubation the content of ATP, ADP and AMP had dropped to 22, 28 and 47 % of the *in vivo* levels. Little further change occurred when the incubation was prolonged except that the AMP level declined still further. In both the liver slices and the mucosal slices the bulk of the endogenous nucleotide had been lost within a relatively short incubation time.

None of the substrates added to the incubation medium had any consistent effect on the rate at which the nucleotides were lost from the mucosal slices. However, it was possible to restore the nucleotide level nearly to those found in fresh tissue by adding 1 mM-ADP to the incubation medium. Although it is clear from our results that the ATP formed in the incubation medium was almost certainly the result of adenylate kinase activity, the ATP formed in tissue was probably produced by oxidative phosphorylation. From Table 3 it is clear that the addition of ADP increased the Q_{O_2} by a factor of about 2. Assuming a phosphorylation efficiency of 3, this would indicate that the increased respiration of the mucosal slices could yield 8 μmole ATP/min.mg dry wt. This is more than sufficient to account for the maximum rate at which ATP appears in the tissue after ADP addition (3·2 μmole/min.mg dry wt.)

The respiration of mucosal slices *in vitro* decreases with time, and this is found in the presence of glucose or amino acids as well as in the absence of substrate. However, when ADP is added to mucosal slices after 4 min when they are already respiring slowly, there is a marked increase in the rate of oxygen uptake. Thus it seems likely that the loss of adenine nucleo-

tides is responsible for much of the decline of respiratory activity of the mucosal slices *in vitro*.

The results obtained with rings of intestine and everted sacs (Fig. 5) suggest that all *in vitro* preparations lose some nucleotides, although the

TABLE 2. The influence of glucose and amino acids on oxygen consumption of mucosal slices of rat jejunum incubated for 8 min

Additions (after 30 sec pre-incubation)	Oxygen consumption (μl./mg dry wt.hr) Initial Q_{O_2} (first 30 sec after addition of substrate)	Average Q_{O_2} (during the full 8 min after addition of substrate)
None	5·96 ± 0·63 (10)	2·96 ± 0·25 (7)
Glucose (5·55 mM)	4·53 ± 0·36 (8)	3·52 ± 0·22 (8)
Glucose (11·1 mM)	5·31 ± 0·44 (26)	3·28 ± 0·33 (17)
Glucose (28 mM)	4·98 ± 0·36 (14)	4·00 ± 0·24 (14)
Amino acid mixture (1 mg/ml.)	8·19 ± 0·86 (8)	5·68 ± 0·44 (10)
Amino acid mixture (1 mg/ml.) + glucose (5·55 mM)	7·23 ± 0·53 (8)	4·96 ± 0·23 (10)
Amino acid mixture (1 mg/ml.) + glucose (11·1 mM)	6·93 ± 0·51 (7)	5·28 ± 0·30 (8)
Amino acid mixture (1 mg/ml.) + glucose (28 mM)	6·85 ± 0·62 (7)	4·96 ± 0·24 (9)

TABLE 3. The influence of ADP on the respiration of mucosal slices of rat jejunum incubated for 8 min

Addition	Oxygen consumption (μl./mg dry wt.hr) During the first 30 sec after 4 min preincubation in substrate-free medium	Average over the period from 4 to 8 min
None	1·90 ± 0·23 (10)	1·77 ± 0·27 (7)
ADP (1 mM)	3·75 ± 0·25 (6)	3·53 ± 0·24 (6)

TABLE 4. The influence of 1 mM-ADP on the average oxygen consumption of mucosal slices during 8-min incubations

Additions (1 mM-ADP present throughout)	Average oxygen consumption over 8 min (μl./mg dry wt.hr)
None	4·08 ± 0·18 (8)
Glucose (11·1 mM)	5·11 ± 0·67 (8)
Amino acid mixture (1 mg/ml.)	6·73 ± 0·59 (8)
Amino acid mixture (1 mg/ml.) + glucose (11·1 mM)	6·56 ± 0·57 (8)

rate of leakage is greater in the less intact preparations. However, even the everted sacs are likely to be depleted of mucosal nucleotides if they are incubated for long periods. Consequently it seems unwise to assume that the mucosal tissue of any *in vitro* preparation retains sufficient ATP for ATP-requiring processes, unless direct measurements of ATP levels are made. Our results also show that externally added ATP is rapidly broken down, and it appears likely that the addition of ADP is the most effective way of increasing intracellular ATP.

Preliminary investigations have indicated that there was no measurable leakage of nucleotides into the lumen of an isolated jejunal segment *in vivo*. This suggests that the depletion of nucleotides from less intact preparations is unphysiological. However, it should be pointed out that the loss of nucleotides does not impair the integrity of the epithelial cells as shown by their appearance in the phase contrast microscope or the electron microscope (Jasper & Bronk, 1968). We have also shown that the accumulation of glucose by mucosal slices is unaffected by depletion or restoration of the ATP of mucosal slices (Leese & Bronk, 1972*a*).

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