# Adenosinetriphosphatase and Hexokinase in the Epithelium of the Small Intestine in Normal and Adrenalectomized Rats

By E. LÜTHY and F. VERZÁR
*Physiological Laboratory, University of Basel, Switzerland*

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The so-called selective absorption of certain hexoses has been explained as a phosphorylation which increases the diffusion gradient, followed by a dephosphorylation inside the cell (Verzár & McDougall, 1936). This theory was supported by the results of later studies which showed that the intestinal mucosa is the organ richest in alkaline phosphatase. Histochemical investigations have shown that alkaline phosphatase is present in a very high concentration in the striated border, where it can be demonstrated after only 1 or 2 sec. of incubation with $\beta$-glycerophosphate. The alkaline phosphatase which lies under the striated border, inside the cells, is seen histologically only after a somewhat longer incubation (Verzár, Sailer & Richterich, 1952). The selective absorption of glucose is diminished after adrenalectomy, and it was shown that at the same time the alkaline phosphatase activity also decreases considerably (Verzár & Sailer, 1952). These observations supported the view based at one time mainly on experiments with inhibitors, that selective absorption of glucose is connected with alkaline phosphatase activity.

Hele (1950) showed that the intestinal mucosa contains hexokinase, which transfers phosphoric acid from adenosine triphosphoric acid (ATP) to glucose and other sugars, in proportion to the velocity of their absorption. If this is the mechanism, then the alkaline phosphatase may be presumed to act by facilitating the dephosphorylation of the hexose phosphoric acid.

It is, however, difficult to understand why the alkaline phosphatase should occur in the highest concentration in the striated border if it is only involved in the breakdown of the phosphorylated sugar. One possibility is suggested by the observation first indicated by Roche, Laromiguière & Laurens (1943), and studied by Meyerhof & Green (1949*a–c*), that under certain conditions the alkaline phosphatase may act by transferring phosphate groups from energy-rich phosphorus compounds, such as creatine phosphoric acid, to a substrate such as glucose or glycerol. If conditions were suitable for such an activity in the intestinal mucosa, the same enzyme would phosphorylate and dephosphorylate the substrate. Such conditions are, however, not proven for the intestinal mucosa.

Hele also showed that the mucosa contains an active ATPase. We have already studied the changes in alkaline phosphatase activity after adrenalectomy (Verzár & Sailer, 1952) and now describe the changes in ATPase and hexokinase activity which follow after adrenalectomy.

## EXPERIMENTAL

White rats of our own laboratory stock were used. They were kept on the usual stock diet and fasted for 18 hr. before the experiment. Adrenalectomy was carried out through the lumbar region, and the insufficiency assessed by daily measurement of the body weight: in Fig. 1 '+' signifies that only the body weight decreased, '++' that besides this decrease the animals were very quiet, and '+++' that they were unable to climb on to an almost vertical fence. The animals were killed by pithing and were immediately bled. The upper part of the small intestine was quickly removed and a portion 20 cm. long (measured from the pylorus) was cut open, washed with ice-cold water, laid on a glass plate, and the mucosa carefully scraped off with a slide. This material was mixed with 3 ml. of ice-cold distilled water in a glass homogenizer and ground for 2·5 min.

ATPase activity was estimated according to Hele (1953) using a veronal:acetate buffer of pH 7·8 (0·05 M), containing 0·01 M-$MgCl_2$ and 0·015 M ATP. To 1 ml. of this solution 0·5 ml. dispersion was added and kept at 30° for 10 min., or in other experiments at 20° for 20 min. The mixture was then deproteinized with 5 ml. 5% trichloroacetic acid and diluted with 10 ml. distilled water. Phosphoric acid was estimated according to Lohmann & Jendrassik (1926).

ATP was prepared from rabbit muscle as described by LePage (1949). The Ba salt was converted into the Na salt.

Hexokinase activity was estimated by the method of Hele (1953). The following solution was used: veronal acetate buffer 0·05 M, pH 7·8, $MgCl_2$ 0·01 M, ATP 0·025 M, NaF 0·06 M and glucose 0·0055 M (100 mg./100 ml.). To 1 ml. of this solution 0·5 ml. dispersion was added and kept at 30° for 10 min. It was then deproteinized with $ZnSO_4$ and NaOH and glucose estimated by the Hagedorn-Jensen method.

## RESULTS

### *Decrease of adenosinetriphosphatase activity after adrenalectomy*

ATPase activity was estimated in the intestinal mucosa of twenty normal and twenty-three adrenalectomized animals. The latter were used between the 6th and 10th day after adrenalectomy.

Summarizing all results on normal and adrenalectomized animals, the mean values in $\mu$g. P after 10 min. incubation were $660 \pm 18{\cdot}9$ in normal rats and $438 \pm 18{\cdot}0$ in adrenalectomized rats; $P < 0{\cdot}01$. In Fig. 1 all values are plotted in groups according to the degree of insufficiency. It is obvious that ATPase activity decreases after adrenalectomy the more so the greater the degree of insufficiency. In Fig. 1 we have not included five normal and five adrenalectomized animals which were all tested on the same day and gave such low ATPase activity that we had to assume a technical error.

*Restoration of adenosinetriphosphatase activity by treatment with corticosteroids*

Thirteen adrenalectomized animals which showed distinct signs of insufficiency were treated from the 8th day onwards with daily intramuscular injections of 1·5 mg. deoxycorticosterone acetate (DCA) and 0·5 mg. cortisone acetate dissolved in arachis oil. The grade of insufficiency was judged by the fall of body weight, which decreased before the treatment and increased again after. The results are shown in Table 1. The first two animals (nos. 57 and 58) were killed 12 hr. after the injection of corticoids. The ATPase activity was found to be equal to that of unoperated animals. The other animals were injected once daily for either 1, 2, 3 or 4 days and killed 24 hr. after the last injection. If only one injection was given, at least in two cases, ATPase was not restored after 24 hr. If, however, the treatment lasted several days, the restoration was usually complete.

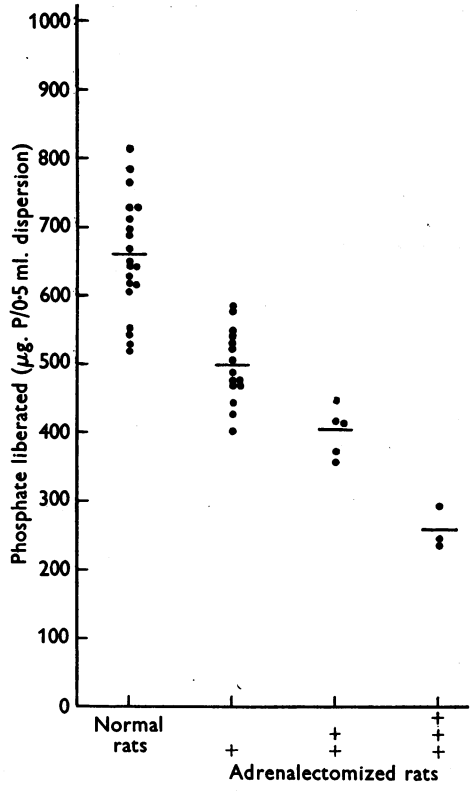


Fig. 1. Decrease of ATPase activity in the intestinal mucosa after adrenalectomy. Crosses indicate the degree of insufficiency in adrenalectomized animals; mean values are indicated by horizontal lines.

Table 1. *Restoration of ATPase activity of intestinal mucosa in adrenalectomized rats by daily injections of 1·5 mg. DCA and 0·5 mg. cortisone*

| Experimental animal (no.) | Body weight (g.) | | | Days after adrenalectomy on which injections were made | Time interval between last injection and determination of ATPase activity (hr.) | Inorganic phosphate in incubation mixture ($\mu$g. P) | | |
|---|---|---|---|---|---|---|---|---|
| | Before adrenalectomy | Before treatment | After treatment | | | Initial | After 10 min. incubation | Difference |
| 57 | 126 | 117 | 120 | 8 | 12 | 91 | 736 | 645 |
| 58 | 113 | 109 | 111 | 8 | 12 | 88 | 809 | 721 |
| 59 | 148 | 146 | 151 | 8 | 24 | 100 | 720 | 620 |
| 60 | 136 | 134 | 140 | 8 | 24 | 100 | 737 | 637 |
| 50 | 118 | 111 | 111 | 9 | 24 | 61 | 478 | 417 |
| 55 | 96 | 87 | 89 | 9 | 24 | 113 | 657 | 544 |
| 51 | 103 | 98 | 105 | 8 and 9 | 24 | 82 | 833 | 751 |
| 52 | 149 | 134 | 141 | 8 and 9 | 24 | 93 | 695 | 602 |
| 56 | 78 | 73 | 83 | 9 and 10 | 24 | 93 | 734 | 641 |
| 61 | 89 | 87 | 106 | 8 and 9 | 24 | 90 | 630 | 540 |
| 62 | 97 | 98 | 113 | 8 and 9 | 24 | 90 | 650 | 560 |
| 54 | 146 | 113 | 144 | 8, 9 and 10 | 24 | 79 | 752 | 673 |
| 53 | 130 | 112 | 120 | 7, 8, 9 and 10 | 24 | 80 | 708 | 628 |

Table 2. *Effect of deoxycorticosterone (DC) on the ATPase activity of dispersions of intestinal mucosa* in vitro

The amount of added DC was 10 mg./100 ml.

| Rats | Number of rats | Mean values (μg. P) Initial | After 10 min. incubation in the absence of DC | After 10 min. incubation with DC | *t** | *P* |
|---|---|---|---|---|---|---|
| Normal | 12 | 94·0±4·6 | 691·0±36·1 | 679·0±30·9 | 0·252 | 0·8<0·9 |
| Adrenalectomized | 11 | 77·0±5·0 | 477·0±35·7 | 475·0±35·5 | 0·039 | 0·9<1·0 |

* Student's *t* test.

We have also examined whether it is possible to restore the decreased ATPase activity of adrenalectomized animals by addition of deoxycorticosterone to the tissue dispersion *in vitro*. Tissues from twelve normal and eleven adrenalectomized animals were compared. Deoxycorticosterone was added in an ethanolic solution, 10 mg./100 ml. 0·5 % ethanol. Controls were made each time with the ethanolic solvent alone. Table 2 gives the results which show that ATPase activity was not restored *in vitro* by the corticoid.

*Some characteristics of the intestinal adenosinetriphosphatase and alkaline phosphatase*

Fig. 2 shows the ATPase activity at 20 and 30°. The curves are based on the mean values from seven normal and eight adrenalectomized animals. The ATPase activity is very high. The difference between normal and adrenalectomized animals is obvious.

Fig. 3 illustrates the influence of pH on ATPase and on alkaline phosphatase activity. Each curve represents mean values from three experiments on different intestinal mucosa dispersions. ATPase has a pH optimum at 8·95 and alkaline phosphatase is equally active at pH's 9 and 9·4. This is in agreement with the findings of Thoai, Roche & Bernhard (1950).

We compared the inhibitory action of sodium fluoride on ATPase and alkaline phosphatase. To 0·5 ml. dispersion of intestinal mucosa, 1 ml. of buffer solution and sufficient ATP or β-glycerophosphate was added so that the solution contained 1·2 mg. P. This mixture was incubated for 10 min. at 30°. Under these conditions ATPase (twenty experiments) produced without sodium fluoride 596 ± 18 μg. P, and in the presence of 0·06 M sodium fluoride 384 ± 20 μg. P as inorganic phosphate; the difference is 212 ± 9 μg. P. Under the same conditions, in fifteen experiments, alkaline phosphatase produced without sodium fluoride 476 ± 29 μg. P and in the presence of 0·06 M sodium fluoride 490 ± 30 μg. P in the form of phosphate; the difference is 14 ± 6 μg. P. This difference in activity between ATPase and alkaline phosphatase has a *P* value of < 0·01. It is obvious that sodium fluoride inhibited the ATPase and not the alkaline phosphatase. This is in agreement with the work of Roche *et al.* (1943).

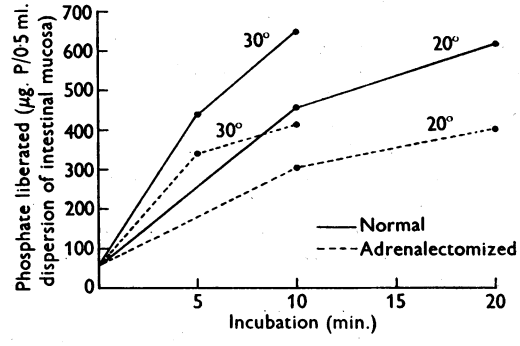


Fig. 2. ATPase activity in intestinal mucosa from normal and adrenalectomized rats, at 30° and at 20°. ●—●, normal animals; ●- - -●, adrenalectomized animals.

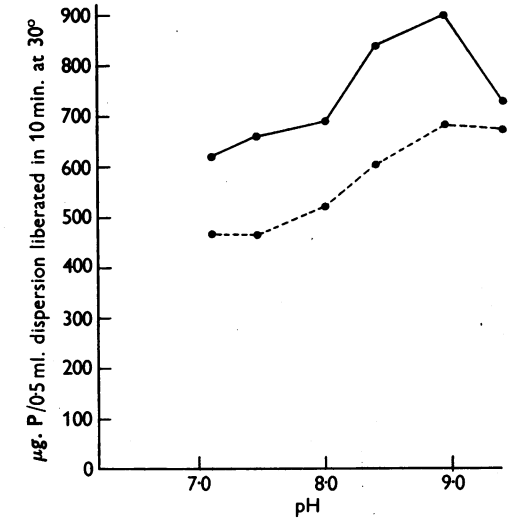


Fig. 3. Influence of pH on ATPase and alkaline phosphatase activity in the intestinal mucosa. ●—●, ATPase; ●- - -●, alkaline phosphatase. Each curve represents mean values of three experiments.

The difference in the pH optimum and the different sensitivity towards sodium fluoride strengthen the view that the ATPase and the alkaline phosphatase activities of the intestinal mucosa are due to different enzymes.

The reaction rates of alkaline phosphatase and of ATPase under the conditions used in our assays are directly proportional to the enzyme concentrations. This is shown in Fig. 4. The dispersion of intestinal mucosa of normal rats was used in quantities from 0·1 to 0·5 ml. and always brought up to 0·5 ml. with distilled water. To one series ATP and to the other sodium β-glycerophosphate was added in such quantities that after complete splitting 1·2 mg. P would be liberated. Alkaline phosphatase splits β-glycerophosphate completely; ATPase splits from ATP one molecule of phosphoric acid. The mucosa of four animals was assayed. In each case the activity was proportional to the concentration of enzyme. The values in Fig. 4 are means from four experiments.

*Hexokinase activity in the intestinal mucosa from adrenalectomized rats*

The results of hexokinase activity measurements are summarized in Table 3. The table shows that there is no difference in hexokinase activity between twenty-one normal and twenty-five adrenalectomized animals. One or more adrenalectomized animals were always compared on the same day with one or more normal animals.

We tried to relate the hexokinase activity to the grade of insufficiency caused by adrenalectomy. The mean value for the decrease of glucose in normal rats was 28·2 ± 1·4 mg. in 10 min. at 30°; after adrenalectomy, in eleven animals with insufficiency graded +, 26·9 ± 0·9 mg.; in ten animals with insufficiency graded + +, 27·0 ± 1·6 mg.; in four animals with insufficiency graded + + +, 24·6 ± 1·5 mg. The differences are small and not significant.

Hexokinase activity was largely dependent on the concentration of ATP, as shown by Fig. 5. From Fig. 5 it is obvious that only with 15 and 28 mg. ATP was it possible to show hexokinase activity over a period of 10 or 15 min., respectively. Inhibition of ATPase with sodium fluoride furthers hexokinase reaction as Long (1952) and Hele (1950) have found. Sodium fluoride inhibits ATPase, and ATP is then available for the hexokinase reaction. We have confirmed this. Hexokinase activity of normal intestinal mucosa dispersion gave in five experiments, without sodium fluoride, a mean value of 16·1 ± 0·94 mg. glucose disappearing and with sodium fluoride 25·6 ± 0·97 mg. glucose disappearing ($P < 0·01$).

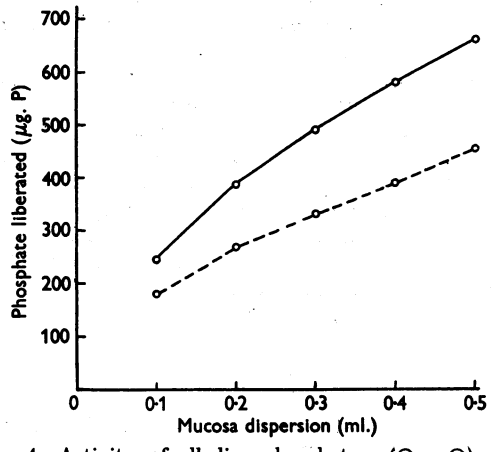


Fig. 4. Activity of alkaline phosphatase (O- - -O) and ATPase (O—O) in dispersions of intestinal mucosa from normal rats; phosphate liberated in 10 min. at 30°, by different quantities of dispersion from the mucosa.

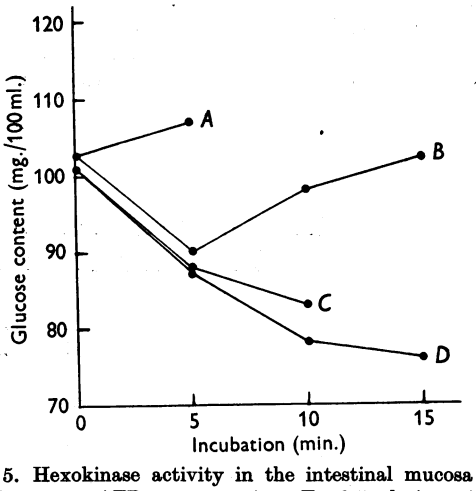


Fig. 5. Hexokinase activity in the intestinal mucosa in relation to ATP concentration. To 0·5 ml. intestinal mucosa dispersion 1 ml. buffered solution was added containing the following quantities of ATP, 5 mg. (*A*), 10 mg. (*B*), 15 mg. (*C*), and 28 mg. (*D*), respectively.

Table 3. *The hexokinase activity of intestinal mucosa from normal and adrenalectomized rats*

Disappearance of glucose in a system containing 0·5 ml. dispersion of intestinal mucosa, ATP and NaF, after 10 min. incubation at 30°. Number of animals in brackets.

| Rats | | Glucose content (mg./100 ml.) Initial | After incubation | Difference | *t* | *P* |
|---|---|---|---|---|---|---|
| Normal | (21) | 109·5 ± 1·5 | 81·3 ± 1·6 | 28·2 ± 1·4 | — | — |
| Adrenalectomized | (25) | 110·4 ± 1·0 | 84·7 ± 1·2 | 26·7 ± 0·7 | 0·94 | 0·3 < 0·4 |

## DISCUSSION

It was found that ATPase activity decreases after adrenalectomy similarly to alkaline phosphatase. Both enzymic activities can be restored by treatment of the animals with corticosteroids, within a short time. We were, however, unable to find a difference in hexokinase activity between normal and adrenalectomized animals.

Intestinal ATPase is obviously a different enzyme from the alkaline phosphatase. The two enzymes have a different pH optimum and behave differently towards fluoride. In spite of the decrease of ATPase activity caused by adrenalectomy, there was no change in the hexokinase activity, as might have been supposed on the basis of the observation that ATPase inhibition by fluoride increases the hexokinase reaction (Hele, 1950; Long, 1952) in the intestinal mucosa.

## SUMMARY

1. The adenosinetriphosphatase (ATPase) activity of the intestinal mucosa of rats decreased considerably after adrenalectomy, roughly in proportion to the degree of insufficiency.

2. Injections of corticosteroids into adrenalectomized rats restored the ATPase activity. It was, however, impossible to restore the ATPase activity *in vitro* by the addition of deoxycorticosterone to the dispersion of the intestinal mucosa.

3. The hexokinase activity of the intestinal mucosa did not decrease after adrenalectomy.

4. Hexokinase activity in dispersions of intestinal mucosa was dependent on ATP concentration.

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# The Oligosaccharides Produced by the Action of Yeast Invertase Preparations on Sucrose

By J. S. D. BACON
*Department of Biochemistry, The University of Sheffield*

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Yeast invertase, which was first prepared from the yeast cell by Berthelot (1860), has been investigated extensively since that time, both because of its use in industrial processes and because it provided a simple model for the investigation of enzyme kinetics. Its action was regarded as bringing about the hydrolysis of sucrose and other substances having an unsubstituted β-D-fructofuranose residue.

However, in 1950 it was discovered independently by Blanchard & Albon (1950) and Bacon & Edelman (1950) that carbohydrates other than sucrose, glucose and fructose could be detected while sucrose was being attacked by various preparations of the enzyme.

By the use of chromatography on cellulose powder and on charcoal, we have isolated one disaccharide and three trisaccharides from the reaction mixture in amounts sufficient to determine their general composition, and to measure their specific rotations. In addition, evidence has been obtained for the presence of two other disaccharides with similar properties and composition. These six substances are the major additional products of the action of yeast invertase on sucrose. The account of their properties given here, taken with the results of a number of other investigations (Fischer, Kohtès & Fellig, 1951; White & Secor, 1952; White, 1952; Albon, Bell, Blanchard, Gross & Rundell, 1953;