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EXPERIMENTAL BOWEL SHOCK IN RATS*—II
LIVER NUCLEOTIDE CHANGES

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Abstract—Rats were subjected to a standardized bowel strangulation procedure, and their livers removed, frozen and homogenized in cold perchloric acid. An aliquot of neutralized acid-supernatant fluid was adjusted to pH 8·4 and chromatographed on Dowex-1 chloride. Nucleotide fractions were identified by their ultraviolet absorption spectra.

As compared to control animals, the amount of adenosine triphosphate was found to be decreased in liver homogenates of untreated shocked rats, and, in addition, three unusual peaks were detected. One peak was shown to be hypoxanthine, whereas the other two were chromatographically similar to inosine monophosphate. Medication with neomycin sulfate prevented the formation of these products of catabolism in shocked animals. Unshocked animals medicated with neomycin sulfate had significantly greater quantities of liver adenosine triphosphate than did similarly treated controls.

ALTERATIONS in some chemical constituents of plasma and tissues of shocked animals have been extensively investigated. Biochemical and physiological changes associated with traumatic,[1] endotoxin[2] and bowel obstruction[3] shock, including changes in protein[4, 5] and nitrogenous substances[6, 7] have been reported.

One marked change which occurs following trauma is an increase in inorganic phosphate and a decrease in adenosine triphosphate (ATP‡) in tissue.[8] Animals premedicated with antibiotics prior to experimental bowel obstruction exhibit reduced necrosis of the strangulated segment[9, 10] and increased survival.[11, 12] Rosenbaum *et al.*[13] have shown that medication with aureomycin prior to induction of hemorrhagic shock resulted in the maintenance of control levels of "high energy phosphate" compounds in the liver. Quantitative changes in nucleotide metabolism in the livers of shocked animals have not been reported.

An attempt has been made to quantitate the changes in certain nucleotide concentrations in liver homogenates following bowel shock, and to determine the influence of neomycin premedication. Because chemical and enzymic analyses of various nucleotides yields only limited and indirect information, ion exchange chromatography was applied to the problem.

MATERIALS AND METHODS

Female rats of the Holtzman strain, weighing from 200 to 275 g, were used. All animals were maintained *ad libitum* on Purina laboratory chow and water; those

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‡ AMP, ADP and ATP refer to adenosine mono-, di- and tri-phosphate, respectively. IMP, IDP and ITP refer to inosine mono-, di- and tri-phosphate, respectively.

animals medicated with antibiotic received 2 g of neomycin sulfate* per kg of body weight by stomach tube daily for 5 days prior to obstructing the bowel segment.

The method for inducing a standardized bowel obstruction which leads to a state of shock, was that described by Wendel *et al.*[3] Control animals were sham operated. For maximum measurable effects the occlusion time was 4 hr. At the time of obstruction release, or 2 hr thereafter, the animals were anesthesized with ether, and the livers removed and dropped into a dry ice–acetone mixture. Tissues from control animals were obtained and treated in a similar manner. The tissues were removed from the freezing mixture, blotted, weighed and pulverized in a chilled steel mortar. The powdered tissue was homogenized in a Waring blender with 3 vols. (30 ml acid to 10 g of tissue) of cold 0·6 N perchloric acid. The mixture was centrifuged in the cold, the supernatant fraction removed, and the residue re-extracted with 1 vol. of cold 0·6 N perchloric acid. The combined supernatant fractions were neutralized in the cold with 5 N KOH to pH 6·5, using phenol red as an internal indicator. The neutralized extract was then cooled to near freezing and the precipitated potassium perchlorate removed by centrifugation. The resulting supernatant material was brought to volume with water and an aliquot (adjusted to pH 8·4 with ammonium hydroxide) taken for chromatographic analysis.

The adenine nucleotides were resolved and separated quantitatively on columns of Dowex-1 in the chloride cycle by the method of Cohn and Carter[14]. In order to resolve the IMP and ADP which come off the column together, the volume of the first eluting solvent (0·003 N HCl) was increased from 100 to 350 ml. To detect IMP, an eluant composed of 0·05 M NaCl–0·01 N HCl was used instead of 0·2 M NaCl–0·01 N HCl. The fractions were identified and quantified by means of their ultraviolet absorption spectra.[15]

RESULTS AND DISCUSSION

In Table 1 are summarized the results of analyses of liver homogenate extracts from control and shocked rats, medicated and unmedicated, at the time the occluded bowel segment was released, and 2 hr thereafter. After 4 hr of occlusion the ATP concentration in the liver homogenate had declined to one-eighth of the control value, and remained depressed for at least 2 hr. Animals which received neomycin prior to occlusion had ATP concentrations which were consistently higher than those observed in the unmedicated groups.

The ADP analysis was complicated by the fact that even though the optical density at 260 mμ remained about the same, the absorption maximum had shifted to 250 mμ. To determine the cause of this shift the elution volume was increased. It then became apparent (Fig. 1) that this component was composed of three separate peaks.

No AMP was detected after 4 hr of occlusion, however it was detected 2 hr following release of occlusion. A peak with an absorption maximum of 250 mμ, eluted at the same point as AMP, was shown to be hypoxanthine (detected by paper chromatography).

Kleinzeller,[16] Krebs and Hems,[17] and Green *et al.*[18] have shown that the enzymic breakdown of ATP in muscle eventually leads to the formation of IMP. Webster,[19] working with washed myofibrils, detected a direct deamination of ADP to IDP. Although unable to detect IMP in the liver extracts chromatographed, two peaks

* The trade name of The Upjohn Company for neomycin sulfate is Mycifradin.

TABLE 1. ADENINE NUCLEOTIDE CONCENTRATIONS IN RAT LIVER HOMOGENATE

Group*	No. of animals	AMP (μmoles/g)	ADP (μmoles/g)	ATP† (μmoles/g)
1	10	0·78 ± 0·21‡	1·19 ± 0·17	2·54 ± 0·42
2	10	1·12 ± 0·24	1·47 ± 0·22	3·17 ± 0·56
3	10		1·36 ± 0·18	0·31 ± 0·14
4	10	0·85 ± 0·16	1·16 ± 0·21	1·86 ± 0·26
5	8	0·36 ± 0·17	0·48 ± 0·15	0·38 ± 0·24
6	9	0·84 ± 0·12	0·82 ± 0·11	1·09 ± 0·30

* Group 1: unmedicated control; group 2: medicated (neomycin) control; group 3: unmedicated, obstructed bowel, sacrificed at time of occlusion release; group 4: medicated, obstructed bowel, sacrificed at time of occlusion release; group 5: unmedicated, obstructed bowel, sacrificed 2 hr after occlusion release; group 6: medicated, obstructed bowel, sacrificed 2 hr after release of occlusion.

† In all instances, medicated vs. control, differences were significant at $P > 0{\cdot}01$.

‡ Standard deviation.

were found to have absorption spectra similar to those of the inosine nucleotides. The data presented agree with the findings of Hoffman *et al.*,[20] in which a substance from blood of patients in shock was detected which exhibited an absorption spectra similar to that of the inosine nucleotides. When the two peaks similar to IMP were chromatographed, using the solvent system of Webster,[19] one peak was found to be identical with IDP, whereas the other lay between IMP and IDP. Employing the solvent system of Krebs and Hems,[17] both peaks exhibited the same R_f as that of IMP. The two peaks similar to those of IMP may be either unstable cyclic isomers of IMP, similar

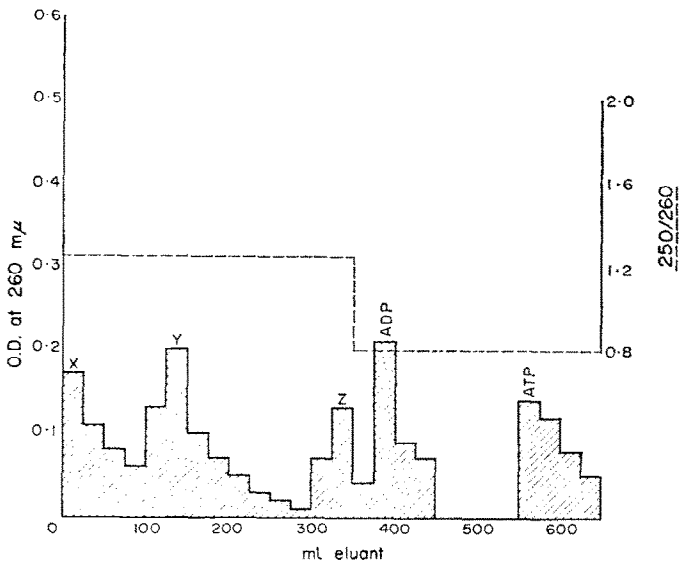

FIG. 1. Chromatographic separation of adenine and inosine nucleotides in homogenate of liver from unmedicated bowel-shocked animals, at time of occlusion release. Peak *X* was shown to be hypoxanthine; peaks *Y* and *Z* were chromatographically similar to IMP. Initial 350 ml of eluant, 0·003 N HCl; next 100 ml, 0·01 N HCl and 0·02 M NaCl; final 200 ml, 0·01 N HCl and 0·05 M NaCl.

to those reported for adenosine-2- and adenosine-3-phosphoric acids[21] with a ring from the 5 to 3, or 5 to 2 position, or substances readily degraded to IMP.

Extracts of liver from animals medicated with neomycin and subjected to bowel shock did not exhibit the hypoxanthine or IMP-like peaks, but instead maintained characteristic nucleotide peaks.

Rosenbaum *et al.*[13] have shown that a marked increase in the rate of phosphorylation occurs in the liver during hemorrhagic shock, leading to a depletion of the high-energy phosphate pool. Under conditions in which aerobic metabolism is sub-optimal, re-phosphorylation of ADP to ATP is depressed,[22] and degradation takes place.

The role of the inosine nucleotides detected in liver homogenates prepared from rats subjected to the bowel strangulation procedure remains unresolved.

SUMMARY

Adenine nucleotides have been quantitatively determined in homogenates of liver from control and bowel-shocked rats, with and without neomycin premedication. The ATP concentration was decreased to approximately one-eighth that of the control value in shocked animals; 2 hr after release of the occluded segment ATP remained depressed.

Homogenates of livers from unshocked animals which had received neomycin had significantly higher ATP concentrations than did unmedicated controls. Likewise, shocked animals which had received neomycin had ATP concentrations consistently higher than those of unmedicated rats.

Three unusual peaks were detected by chromatographic procedures in homogenates of liver from unmedicated, bowel-shocked animals. One peak was shown to be hypoxanthine, the other two were chromatographically similar to IMP.

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