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Very early effects of aminonucleoside on some rat liver enzymes

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ALTHOUGH it is well known that aminonucleoside causes nephrosis in rats after 5 days, very little information is available on the "prenephrotic" period, i.e. before the onset of nephrosis. Since in the livers of nephrotic rats increased protein synthesis, accelerated turnover of energy-rich phosphorus compounds, increase in RNA and DNA synthesis, prolongation of r-RNA half-life, as well as rise in blood glucose level and proteinuria have been observed,[1-5] the liver seems to be basically involved in the pathology of nephrosis. Many other amino-purine derivatives produce partly similar metabolic changes in the hepatocytes, aminonucleoside-puromycin (AMN) is known to result in experimental nephrosis. Our aim was to find any early influences of this compound on some enzymes in the glycogen–glucose pathway, Krebs cycle and in the nucleic acid metabolism as possible sources of metabolic changes which may be involved in the ultimate cell damage leading to nephrosis. For this study, we selected enzymes which are known to be disturbed in the nephrotic kidney or which may be involved in the prenephrotic changes in the liver.[4-10]

Male Wistar rats, weighing 200–250 g, were supplied Purina lab chow and tap water *ad lib.* The test rats were anaesthetized with ether, and 1·5 ml of a 1% solution of aminonucleoside-puromycin (AMN, Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A.) in 0·9% NaCl, pH adjusted to 7·4, was injected. Because rats excrete 25–50 per cent of the AMN as unchanged compound and 90 per cent of the remainder as breakdown products within the first 8 hr,[1] we administered the compound rapidly by i v.—into the jugular vein—rather than the commonly used i.m. or s.c. route. The same number of rats used as controls against each test group received 1·5 ml physiological NaCl only. Groups of animals treated with AMN or saline were sacrificed by decapitation after different time intervals of 1–96 hr. Food was removed from each group 14–16 hr before they were killed The livers were removed immediately and placed in ice-cold 0·5 M sucrose solution containing 37·5 mM *tris* maleate, 1% Dextran-250, 5 mM $MgCl_2$, 5 mM mercaptoethanol, at pH 6·4, and homogenized. Samples of the homogenate were frozen and stored at −20 pending enzyme assay

Enzyme assays. Glucose 6-phosphate phosphohydrolase (EC 3.1.3.9) activity was determined according to the method of Swanson.[11] The amount of *p*-nitrophenol released from *p*-nitrophenyl-phosphate was used to indicate acid phosphatase (EC 3.1.3.2) activity (Sadowski and Steiner).[12] NAD-malic dehydrogenase (EC 1 1.1.37) was determined by measuring the nicotinamide adenine dinucleotide, reduced from (NADH), as described by Strominger and Lowry.[13] Urate-oxidase (EC 1.7.3.3) activity was measured by the method of Schneider and Hogeboom.[14] Protein content of the liver homogenate was determined by the method of Lowry *et al.*[15]

Results of the enzyme assays are summarized in Tables 1 and 2. Although the applied low dose of AMN does not seem to change significantly the liver protein content, it has significant influence on the activities of some enzymes shortly after its injection

TABLE 1. ACTIVITIES OF ENZYMES AFTER AMN IN RAT LIVER HOMOGENATES*

Hr after AMN	No. of rats†	Protein (AMN-treated) (mg/100 mg wet liver) Mean (S.E.M.)‡	P§	Acid phosphatase (μmoles librated p-nitrophenol/min/mg protein) Control Mean (S.E.M.)	AMN-treated Mean (S.E.M.)	P	Glucose 6-Phosphatase (μmoles Pi liberated/min/mg protein) Control Mean (S.E.M.)	AMN-treated Mean (S.E.M.)	P
1	6	5·88 (0·16)	NS¶	0·710 (0·120)	0·870 (0·019)	NS	0·200 (0·024)	0 230 (0·006)	NS
2	6	6·16 (0·06)	NS	0·680 (0·080)	0·740 (0 023)	NS	0·170 (0 022)	0·332 (0·006)	<0·001
5	8	6·60 (0·07)	<0·1	0·550 (0·060)	0 560 (0·032)	NS	0·150 (0·016)	0·244 (0 005)	<0·001
10	6	6·58 (0·18)	<0·2	0 630 (0·070)	0·932 (0·022)	<0·01	0 140 (0·012)	0·180 (0·10)	<0·05
18	6	6·15 (0·14)	NS	0·660 (0·082)	1·160 (0·086)	<0·02	0 130 (0·021)	0·130 (0·008)	NS
24	8	6·92 (0·14)	<0·05	0·680 (0·080)	0·730 (0·012)	NS	0·140 (0 016)	0·190 (0·005)	<0·01
48	8	6·30 (0·30)	NS	0·710 (0·092)	0·706 (0·016)	NS	0 150 (0·010)	0·142 (0·007)	NS
96	6	6·09 (0·11)	NS	0·670 (0·096)	0·673 (0·028)	NS	0·170 (0·012)	0 134 (0 004)	<0·02

* AMN = aminonucleoside – Puromycin.
† Same number used for controls.
‡ Arithmetic means and standard error of means.
§ Significance by Student's *t*-test, as compared to corresponding controls.
¶ NS = not significant.

TABLE 2. ACTIVITIES OF ENZYMES AFTER AMN IN RAT LIVER HOMOGENATES*

Hr after AMN	No. of rats†	NAD-malic dehydrogenase (nmoles DPN reduced/min/mg protein) Control Mean (S.E.M.)‡	AMN-treated Mean (S.E.M.)	P§	Urate oxidase (μmoles uric acid destroyed/min/mg protein) Control Mean (S.E.M.)	AMN-treated Mean (S.E.M.)	P
1	6	2·67 (0·22)	1·79 (0·072)	<0·01	0·581 (0·060)	0·512 (0·014)	NS¶
2	6	2·86 (0·21)	1·80 (0·070)	<0·005	0·610 (0·060)	0·570 (0·022)	NS
5	8	5·38 (0·31)	1·00 (0·076)	<0·001	0·610 (0·080)	0·448 (0·015)	<0·1
10	6	3·65 (0·19)	0·95 (0·034)	<0·001	0·620 (0·065)	0·195 (0·016)	<0·001
18	6	3·20 (0·30)	1·31 (0·04)	<0·001	0·606 (0·06)	0·183 (0·018)	<0·001
24	8	2·48 (0·21)	1·66 (0·08)	<0·02	0·806 (0·07)	0·483 (0·025)	<0·005
48	8	2·16 (0·22)	1·21 (0·10)	<0·01	0·570 (0·04)	0·438 (0·016)	<0·02
96	6	2·08 (0·26)	0·92 (0·06)	<0·005	0·533 (0·05)	0·463 (0·014)	NS

* AMN = aminonucleoside-puromycin.
† Same number used for controls.
‡ Arithmetic means and standard error of means.
§ Significance by Student's *t*-test, as compared to corresponding controls.
¶ NS = not significant.

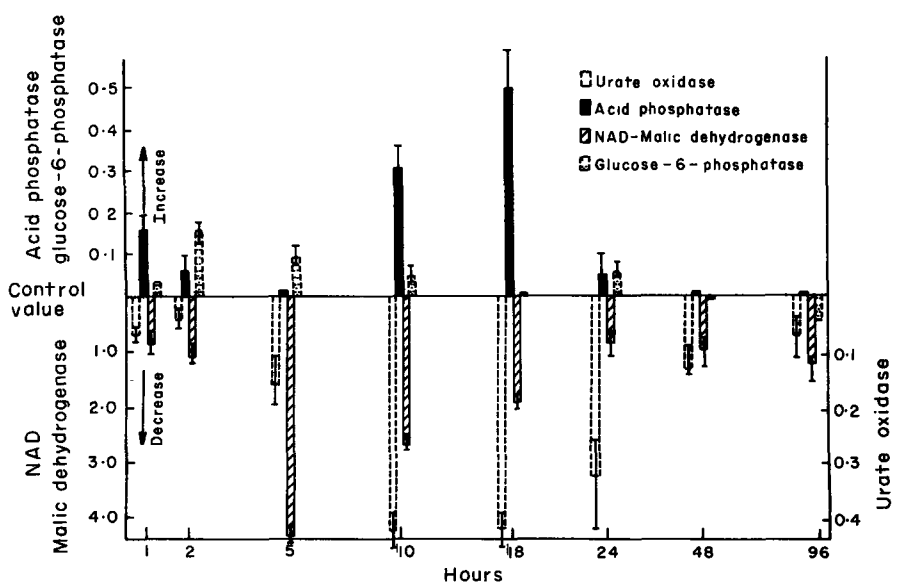

FIG. 1. Changes in enzyme activities (means and maximum–minimum values) between AMN-treated and control animals. Baseline shows the control value. Units used are the same as in Table 1.

Glucose 6-phosphatase showed its highest activity in the first 10 hr, but the other hydrolytic enzyme, acid phosphatase, significantly increased its activity only when glucose 6-phosphatase returned to control level. The most impressive changes were observed in the activity of NAD-malic dehydrogenase. This enzyme was significantly depressed through the whole experimental period (96 hr). Activity of urate oxidase was also decreased from 5 hr after AMN treatment.

It has been reported that 1 hr after AMN injection liver glycogen content decreases[4,16] and serum glucose level increases.[5] In our experiments, an important enzyme for conversion of glycogen to blood glucose, glucose 6-phosphatase, showed the highest activity for approximately 10 hr after AMN. After this time no significant increase in the activity of this enzyme was observed. The other hydrolytic (lysosomal) enzyme, acid phosphatase, increased its activity much more significantly for a short time but only from 10 hr after AMN. Although the acid phosphatase level rises in hepatic cell damage, its exact role is obscure because of its lack of specific action In nephrotic kidney homogenate, the activity of NAD-malic dehydrogenase is decreased, while the amount of oxaloacetate increases.[6-8] This compound has an inhibiting action on NAD-malic dehydrogenase and on other Krebs cycle enzymes.[7-10] We have found that the activity of this enzyme in the liver is also greatly reduced after AMN, at 5 hr, more than a 5-fold activity loss was observed (Fig. 1). Because this lower activity persisted through the whole experimental period, it seems likely that AMN interferes with carbohydrate metabolism in the hepatocytes below the triosephosphate level a short time after its introduction.

Finally, the known increase of nucleic acid content in nephrotic liver[2-4] was considered, and the activity of the purine catabolism-terminating enzyme, urate oxidase, was assayed It has been suggested that not only increased synthesis but also a lower rate of decomposition may be responsible for the higher nucleic acid levels.[3] In our experiments, the activity of urate oxidase significantly decreased from 5 hr after AMN and remained at a lower level for at least 48 hr. If we assume a close correlation between enzyme activity and substrate concentration, this observation would support the suggested lower decomposition rate of nucleic acids. Stimulation of glycoprotein synthesis by AMN was shown to occur progressively from the first to the fifth day after injection of AMN [17] Not only the elevation of protein synthesis well before the onset of proteinuria, but also the very early influence of AMN on liver glycoprotein metabolism was demonstrated. Although in our experiments only a small single dose of AMN was used (approximately 6·65 mg/100 g body wt), its influence on some phases of liver metabolism at a very early prenephrotic stage was observed. The described enzyme activity changes may be partly irreversible and related to the later onset of nephrosis.

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