THE EFFECT OF ATP ON THE SYNTHESIS OF THE NICOTINAMIDE NUCLEOTIDES

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The synthesis of NAD and NADP presents an interesting problem in cellular activity in that whilst NADP formation from NAD by NAD-kinase (E. C. 2. 7. 1. 23) is exclusively cytoplasmic [1], the formation of NAD from its precursors includes a step involving the enzyme NAD pyrophosphorylase (E. C. 2. 7. 7. 1) which is located exclusively in the nucleus [2]. Thus the nucleus exerts a measure of control over NAD synthesis and hence to some extent over NADP formation. The other compound common to both biosynthetic systems is ATP and therefore study of the formation of NAD and NADP should reflect to some extent the availability of ATP to reactions occurring in different cell compartments.

It has been reported that injection of ethionine causes a marked depletion of total hepatic ATP after only 2 hours [3]. We have observed a differential effect on NAD and NADP synthesis in the livers of animals treated with ethionine where the concentration of NAD remained virtually unchanged after 5 hr [4,5] although a transient decrease may occur, but the concentration of NADP decreased by some 30% over the same time interval. We have confirmed and extended these findings and we are able to report that the ethionine dependent ATP deficiency does not inhibit the ability of the hepatic NAD synthesising systems to form elevated NAD levels under the appropriate conditions. Also under such conditions of elevated NAD levels there is a positive correlation between the ATP concentration present and the hepatic NADP concentration.

Female albino rats, body weight approximately 130-140 g, were starved overnight and injected intraperitoneally with 0.86 mmole DL ethionine in 6 ml of 0.9% NaCl (1 g/kg body weight) or 0.57 mmole of nicotinamide similarly dissolved (500 mg/kg body weight) or with a mixture of the two, 0.86 mmole DL ethionine and 0.57 mmole nicotinamide, also dissolved in 6 ml of 0.9% NaCl. The animals were killed by cervical dislocation at hourly intervals for the first five hours after injection and the liver was extracted and assayed for nicotinamide nucleotides [6] and ATP [7].

The results of these experiments are given as the total hepatic nicotinamide nucleotides (oxidized and reduced) or total hepatic ATP, as this is probably the most significant as far as the whole animal is concerned and they are expressed as a % of the zero time values as they involved several series of experiments. Each point plotted in fig. 1 represents the average of at least 4 animals.

Fig. 1A shows the change in total hepatic NAD during the first five hours after injection of ethionine, nicotinamide or ethionine + nicotinamide. In the case of ethionine the total hepatic NAD decreases slightly but then returns to the zero time value at 5 hours. Nicotinamide injection, however, leads to the well established [8,9] increase in total hepatic NAD, in these experiments an increase greater than 3 times the zero time values. Injection of a mixture of ethionine and nicotinamide leads to an increase in the hepatic NAD similar to that of nicotinamide alone over a period of 5 hours. Fig. 1B depicts the change in total hepatic NADP that occurs under the same conditions as in fig. 1A. Ethionine causes a progressive fall in hepatic NADP to a level approximately one half of the zero time value at 5 hours (see [4,6]) and a similar effect is seen when a mixture of nicotin-

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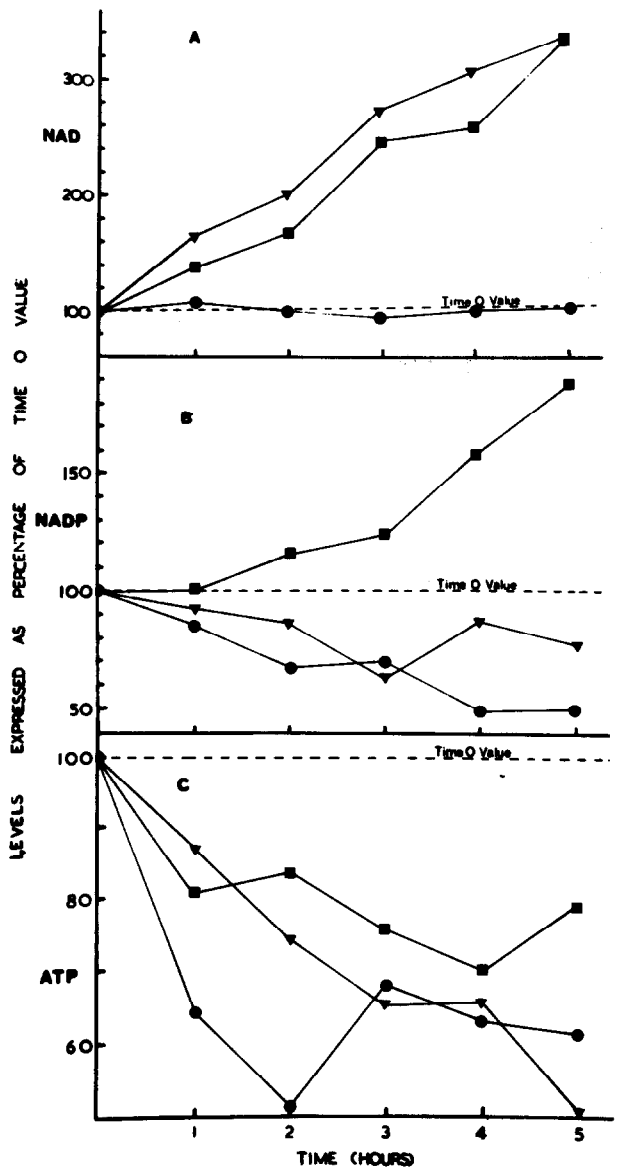

Fig. 1. Time course of the effect of DL ethionine, nicotinamide or DL ethionine + nicotinamide on total hepatic content of NAD, NADP and ATP. The amounts of each agent injected and the methods of assay are as in the text. The results are expressed as percentages of time 0 values calculated from the μmoles of nucleotide (oxidized and reduced) per total liver. Each point represents the average of at least 4 rats and ●—● represents the injection of ethionine alone, ■—■ nicotinamide alone, and ▼—▼ the mixture of the two. The time 0 values were for NAD, 3.89 ± 0.023 μmoles/total liver; NADP, 1.44 ± 0.17 μmoles/total liver; ATP, 3.37 ± 0.037 μmoles/total liver.

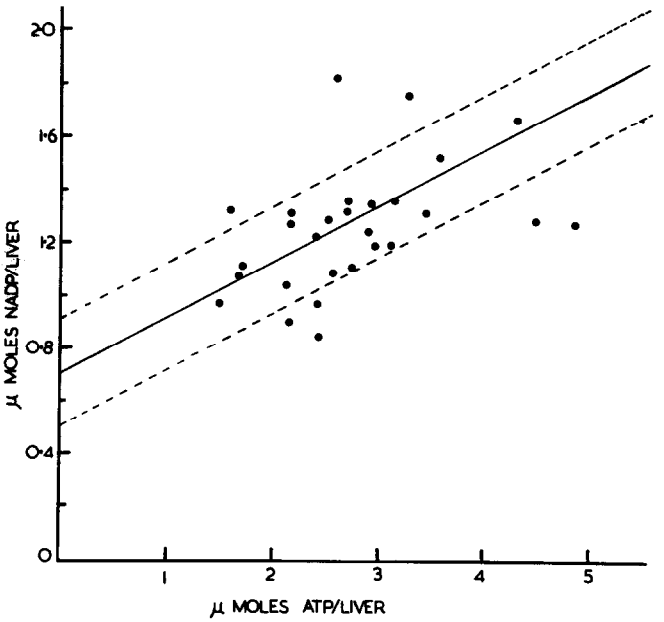

Fig. 2. NADP concentration plotted as a function of ATP concentration in the liver of rats treated with a mixture of ethionine and nicotinamide. The amounts of each agent injected and the methods of assay are as in the text. The results are plotted as μmoles of NADP per total liver against the corresponding ATP concentration expressed as μmoles/total liver. Each point represents a single animal and the correlation coefficient of NADP concentration with ATP concentration was found to be 0.8596 which has a P value of < 0.001. The distance between the dotted line and the regression line represents twice the standard error of the estimate of the regression line.

amide and ethionine is injected, except that the total hepatic NADP level falls only to 63% of the zero time value. When nicotinamide alone is injected, a doubling of the total hepatic NADP is apparent. Fig. 1C shows the changes of total hepatic ATP under these three different treatments. As expected [3] ethionine alone causes a marked fall in total hepatic ATP to a value of about one half. Likewise, injection of nicotinamide alone causes a significant but smaller decrease in total hepatic ATP to a level of about 70% of the zero time value (cf. [10]). The administration of a mixture of the two, ethionine and nicotinamide, also causes a fall in hepatic ATP to approximately one half.

These results demonstrate several important points regarding the availability of ATP for biosynthetic reactions in different cell compartments and in parti-

cular for nicotinamide nucleotide synthesis. It appears that the injection of ethionine in no way impairs the ability of the NAD synthesising systems to form elevated levels of NAD on injection of nicotinamide; at least for the first five hours after injection there is no difference between the increase in hepatic NAD after nicotinamide injection in the presence or absence of ethionine (fig. 1A). Also from fig. 1B it is apparent that ethionine prevents any increase in hepatic NADP even in the presence of elevated levels of NAD after nicotinamide injection. It appears, therefore, that ethionine may interfere only with cytoplasmic ATP requiring systems such as the NAD^+-kinase step, whereas systems involving a nuclear ATP requiring step may not be affected. This may be explained by the suggestion that ethionine causes its effects by forming s-adenosyl ethionine through the agency of the methionine activating system (methionine adenosyl transferase E. C. 2. 4. 2. 13) which is located in the cytoplasm [3,11]. However, the fact still remains that NAD synthesis is able to continue unimpeded by the fall in total hepatic ATP. This suggests that cellular ATP is either strictly compartmentalised in the sense that the compartment from which the NAD synthesising systems draw their ATP is unaffected by ethionine unlike the main pool of cellular ATP or that the NAD synthesising systems are able to maintain their ATP levels most efficiently even in the presence of a 50% fall in the overall cellular ATP. A possible explanation for this latter alternative is that the ATP is derived from a separate system to that providing the vast bulk of the cellular ATP, e.g. nuclear phosphorylation (although whether liver nuclei possess a significant nuclear phosphorylation system is subject to doubt [12]). It is worth noting that Siebert [13] has reported, after preparing nuclei by a non-aqueous technique from the livers of animals treated with ethionine, that whilst cytoplasmic ATP falls drastically the nuclear ATP remains constant.

From these experiments we have also been able to demonstrate a positive correlation between NADP concentration and ATP concentration (fig. 2) under conditions in which there are elevated levels of NAD. This was derived from the results of the experiment in which nicotinamide and ethionine were injected together. In these circumstances the NAD^+-kinase reaction becomes zero order with respect to NAD and a positive statistically significant correlation coefficient was obtained for the relationship between NADP and ATP. This is an *in vivo* demonstration that an enzyme reaction is proceeding in a fashion which would be predicted from *in vitro* considerations.

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