Biochem. J. (1972) **128**, 869–877
Printed in Great Britain

The Control of Nucleic Acid and Nicotinamide Nucleotide Synthesis in Regenerating Rat Liver

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(*Received* 9 *December* 1971)

1. The effect of injecting nicotinamide on the incorporation of [14C]orotate into the hepatic nucleic acids of rats after partial hepatectomy was investigated. 2. At 3h after partial hepatectomy the rapid incorporation of [14C]orotate into RNA, and at 20h after partial hepatectomy the incorporation of [14C]orotate into both RNA and DNA, were inhibited in a dose-dependent fashion by the previous injection of nicotinamide. 3. The injection of nicotinamide at various times before the injection of [14C]orotate at 20h after partial hepatectomy revealed an inhibition of the incorporation of orotate into RNA and DNA which was non-linear with respect to the duration of nicotinamide pretreatment. 4. The induction of a hepatic ATP depletion by ethionine demonstrated that the synthesis of hepatic NAD and NADP in partially hepatectomized rats was more susceptible to an ATP deficiency than in control rats. 5. The total hepatic activity of ribose phosphate pyrophosphokinase (EC 2.7.6.1) was assayed at various times after partial hepatectomy and found to be only marginally greater than the maximum rate of hepatic NAD synthesis induced *in vivo* by nicotinamide injection between 12 and 24h after partial hepatectomy. 6. It is suggested that a competition exists between NAD synthesis and purine and pyrimidine nucleotide synthesis for available ATP and particularly 5-phosphoribosyl 1-pyrophosphate. In regenerating liver the competition is normally in favour of the synthesis of nucleic acid precursors, at the expense of NAD synthesis. This situation may be reversed by the injection of nicotinamide with a subsequent inhibition of nucleic acid synthesis.

The regenerating liver provides a useful area of study for investigation of the biochemical events leading up to and after mitosis. Two facets of this process are of particular interest: (*a*) the initiation of the pre-mitotic state and (*b*) the order in which the metabolic processes occur before mitosis itself. Much more is known about the metabolic processes occurring during the pre-mitotic period than about the process (or processes) that initiate these events. Indeed, with respect to RNA and DNA synthesis the general outline of the sequence of events before cell division is well established (see Bucher, 1963, 1967*a*,*b*). However, as regards the process (or processes) involved in the initiation of these events, although no lack of hypotheses exist, little substantive evidence is available to support any one more than another. One such hypothesis is that of Morton (1958, 1961), who suggested that the concentration of NAD is the critical factor in the instigation of cell division. This hypothesis was based on the observations that rapidly dividing cells exhibit low NAD concentrations and that one of the enzymes involved in NAD synthesis, NMN adenylyltransferase (EC 2.7.7.1), is located in the nucleus.

Investigations in this laboratory (Ferris & Clark, 1971) have confirmed that regenerating liver, in common with other rapidly dividing cells, exhibits a low nicotinamide nucleotide concentration. However, measurements of the activities of key enzymes involved in hepatic NAD formation, including NMN adenylyltransferase, and of the maximum potential rate of hepatic NAD synthesis as realized by nicotinamide injection, indicated that the synthetic potential of regenerating rat liver for NAD was not impaired. It was proposed that the low nicotinamide nucleotide concentrations observed in regenerating rat liver were a consequence of the restricted availability of certain precursors and cofactors. An additional observation, that the NAD and DNA content of regenerating liver were inversely proportional to each other, suggested that the metabolites concerned were common to both nucleic acid and nicotinamide nucleotide synthesis, i.e. ATP and 5-phosphoribosyl 1-pyrophosphate. Accordingly, experiments are reported in the present paper on the effects of a cellular ATP deficiency on nicotinamide nucleotide synthesis in regenerating liver as caused by ethionine. Also the potential rate of 5-phosphoribosyl 1-pyrophosphate synthesis has been assessed and compared with the rate of NAD and nucleic acid formation.

Further support for the involvement of the nicotinamide nucleotides in the regulation of cell division has been invoked from the observations of Oide (1958) and Kanatani (1960) that nicotinamide has an anti-mitotic effect on regenerating liver and planaria respectively, nicotinamide being a known precursor of the nicotinamide nucleotides (Kaplan *et al.*, 1957). Burzio & Koide (1970) have proposed a hypothesis to explain the anti-mitotic action of nicotinamide, based on their observation that the preincubation of isolated rat liver nuclei with NAD^+ decreased the subsequent template activity of the chromatin for DNA synthesis. They suggested that increased concentrations of nicotinamide lead to the accumulation of elevated concentrations of NAD which in turn favour the poly(ADP-ribose) polymerase reaction. This leads to the inhibition of DNA synthesis by the ADP-ribosylation of nucleoproteins required for DNA formation. However, the template activity of chromatin required for RNA synthesis does not appear to be affected (Burzio & Koide, 1970, 1971). This would suggest, therefore, that if nicotinamide is acting directly in this way, it would have an inhibitory effect on DNA synthesis but not on RNA synthesis. If, however, there was a competition between the NAD synthesis induced by nicotinamide and the RNA/DNA synthesis for common precursors, as suggested by Ferris & Clark (1971), an effect on both RNA and DNA synthesis would be expected. Accordingly, experiments have been performed to assess the effects of NAD synthesis induced by nicotinamide on both the concentrations of the nucleic acids and their turnover as assessed by incorporation of [^{14}C]orotate into hepatic RNA and DNA after partial hepatectomy.

The experiments reported here suggest that the low nicotinamide nucleotide content of regenerating liver is a result of competition between nicotinamide nucleotide synthesis and nucleic acid synthesis, i.e. purine and pyrimidine nucleotide formation, for the common precursors ATP and in particular 5-phosphoribosyl 1-pyrophosphate. It is suggested that the action of nicotinamide on mitosis and nucleic acid synthesis reflects the increased competition instigated by the increased NAD synthesis rather than an effect mediated via poly(ADP-ribose) polymerase activity as suggested by Burzio & Koide (1970).

Methods

Animals

Female albino rats of the Wistar strain (approx. 150g body wt.) were used in all experiments and were fed on stock diet 41B *ad lib.*

Partial hepatectomy was performed under diethyl ether anaesthesia by the method of Higgins & Anderson (1931), resulting in the ablation of 64±4% of the liver. All substances to be injected were dissolved in 0.9% NaCl and the intravenous injections were carried out via the tail vein, under very light ether anaesthesia.

All animals were killed by cervical dislocation.

Reagents

ATP, NAD^+, $NADP^+$, glucose 6-phosphate, yeast alcohol dehydrogenase (EC 1.1.1.1), glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and yeast RNA were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K. DL-Ethionine, phenazine methosulphate, ribose 5-phosphate and calf thymus DNA (type V) were purchased from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. BBOT scintillant [2,5-bis-(5-t-butylbenzoxazol-2-yl)thiophen] was from CIBA Ltd. (Basle, Switzerland). Nicotinamide was purchased from Hopkin and Williams (Chadwell Heath, Essex, U.K.). [8-^{14}C]-Adenine and [6-^{14}C]orotic acid were purchased from The Radiochemical Centre (Amersham, Bucks., U.K.). All other reagents were of the AnalaR grade where possible.

Determination of NAD, NADP, DNA and RNA

Liver samples were extracted and assayed for their content of NAD^+, NADH, $NADP^+$ and NADPH by the method of Greenbaum *et al.* (1965).

DNA was determined by the method of Burton (1955) with calf thymus DNA type V (Sigma) as a standard.

RNA was determined by the method of Ceriotti (1955) with yeast RNA (Boehringer) as a standard.

Assay of ribose phosphate pyrophosphokinase (EC 2.7.6.1)

The assay of ribose phosphate pyrophosphokinase was based on that of Henderson & Khoo (1965*a*), in which the synthesis of 5-phosphoribosyl 1-pyrophosphate is coupled with its utilization for the conversion of adenine into AMP by adenine phosphoribosyltransferase (EC 2.4.2.7). The equilibrium of the adenine phosphoribosyl transferase reaction is strongly in favour of AMP formation (Flaks *et al.*, 1957) and the activity of this enzyme in rat liver is at least fivefold greater than the activity of ribose phosphate pyrophosphokinase as assayed by this method (Murray, 1966). Thus, the supernatant preparation of rat liver used to assay ribose phosphate pyrophosphokinase activity will itself contain a sufficient excess of adenine phosphoribosyltransferase activity. Rat liver was homogenized in 0.25M-sucrose (1g of liver/4ml of 0.25M-sucrose at 4°C) and centrifuged at 100000g for 1h at 4°C. The supernatant was used immediately for the enzyme

determinations. The incubation mixture contained 7.5 μmol of $MgCl_2$, 5 μmol of potassium phosphate buffer, pH 7.2, 0.5 μmol of ATP, 3 μmol of ribose 5-phosphate, 0.2 μmol of [8-^{14}C]adenine (0.1 μCi) and 0.1 ml of supernatant containing both enzymes in a final volume of 1.5 ml. The reaction was found to proceed linearly with respect to time for up to 40 min and with respect to volume of supernatant up to 0.3 ml. Incubation was carried out as a routine for 30 min at 37°C and the reaction was terminated by boiling at 100°C for 3 min. The protein was removed by centrifugation, and 100 μl portions of the supernatant were spotted on Whatman no. 1 chromatography paper, together with carrier AMP. The chromatographic separation was carried out by the descending technique with 5% (w/v) Na_2HPO_4 as solvent. This chromatographic procedure results in a good separation of the adenine base from its nucleotides but poor separation of the nucleotides from each other. The adenine nucleotide spot was located under u.v. light, cut out and counted for radioactivity in 15 ml of BBOT scintillation fluid (4 g of BBOT in 1 litre of toluene).

Incorporation of precursors into nucleic acids

Rapid incorporation of [6-^{14}C]*orotate into hepatic RNA 3h after partial hepatectomy.* The rate of incorporation of intravenously injected [6-^{14}C]-orotate into RNA at 3h, after partial hepatectomy was measured by the method of Fujioka *et al.* (1963). [6-^{14}C]Orotate (0.5 μCi, 8.2 nmol) in 0.9% NaCl was injected into the tail vein of rats 3h after partial hepatectomy. The rate of incorporation of [^{14}C]-orotate into hepatic RNA was linear for approx. 8 min, and so, as a routine, rats were killed 7 min after the orotate injection.

Incorporation of [6-^{14}C]*orotate into hepatic RNA and DNA 20h after partial hepatectomy.* The incorporation of [6-^{14}C]orotate into hepatic RNA and DNA at 20h after partial hepatectomy was assessed by using the method of Fleck & Munro (1962) to separate the hepatic RNA and DNA. Rats were injected intraperitoneally with 1 μCi of [6-^{14}C]-orotate in 1 ml of 0.9% NaCl 20h after partial hepatectomy. The rate of incorporation of ^{14}C into the hepatic RNA and DNA was found to be linear for the first 45 min after injection, so rats were killed as a routine 30 min after the injection of orotate. Samples of the RNA ribonucleotides and DNA were dried on glass-fibre discs (Whatman GF/A) and counted for radioactivity in 5 ml of BBOT scintillation fluid.

Results

Effect of nicotinamide on nucleic acid content and turnover

During the first 3–6h after partial hepatectomy, RNA, but no appreciable DNA, synthesis occurs, whereas at 18–20h after the operation substantial DNA synthesis has been initiated (Bucher, 1963, 1967*a*,*b*). Thus at 3h after partial hepatectomy intravenously injected [6-^{14}C]orotate was incorporated into RNA alone (cf. Fujioka *et al.*, 1963), but at 20h it was incorporated into both RNA and DNA (G. M. Ferris & J. B. Clark, unpublished work).

If instead of measuring the incorporation of [^{14}C]-orotate the total RNA and DNA content of the liver was measured, at both 3 and 20h after partial hepatectomy the RNA content was not influenced by nicotinamide (or by the NAD produced from it) (Table 1), whereas at 20h the DNA content showed an inverse relationship with NAD (Ferris & Clark, 1971). However, measurement of the total RNA takes no account of the fact that 85–90% of the total rat liver RNA is in the relatively stable ribosomal fraction (Hirsch, 1967). Thus any changes in the remaining 10–15% which is actively turning over are unlikely to be detected by this method.

Table 1. *Hepatic RNA content after partial hepatectomy*

Animals in group I were injected with either nicotinamide (500 mg/kg) or an equivalent vol. of 0.9% NaCl (control) at 3h after partial hepatectomy and were killed at 6h. Animals in group II were similarly treated at 18h after partial hepatectomy and were killed at 21h. RNA was determined as described in the Methods section. The results are expressed as mg of RNA, and are the means ± S.E.M. for at least five animals.

		Hepatic RNA content	
		(mg/g)	(mg/100 g body wt.)
Group I	Treated	11.6 ± 0.9	16.9 ± 0.9
	Control	10.8 ± 0.2	17.8 ± 0.6
Group II	Treated	11.4 ± 0.3	23.0 ± 1.4
	Control	11.2 ± 0.3	21.5 ± 1.0

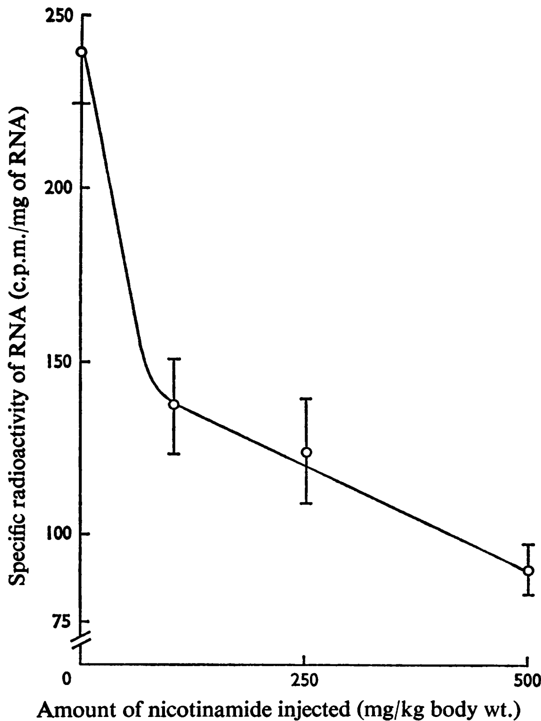

Fig. 1. *Effect of nicotinamide on the incorporation of [^{14}C]orotate into hepatic RNA 3h after partial hepatectomy*

Female albino rats were injected intraperitoneally with nicotinamide in 0.9% NaCl at the various doses shown, 1.5h after partial hepatectomy. [6-^{14}C]-Orotate (0.5μCi) was injected into the tail vein of these rats 1.5h after the nicotinamide (i.e. 3h after partial hepatectomy) and they were killed 7min later. The specific radioactivity of the hepatic RNA was determined as indicated in the Methods section. The control (zero nicotinamide) rats were injected intraperitoneally with 0.9% NaCl alone. Each point represents the average for five animals and the vertical bars represent twice the S.E.M. The control (zero nicotinamide) value for RNA specific radioactivity was 239±17c.p.m./mg of RNA (five animals).

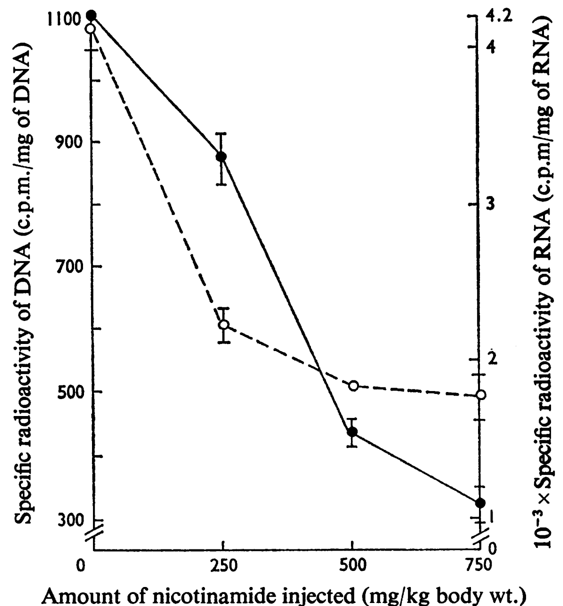

Fig. 2. *Effect of various doses of nicotinamide on the incorporation of [^{14}C]orotate into hepatic RNA and DNA 20h after partial hepatectomy*

Female albino rats were injected intraperitoneally with nicotinamide in 0.9% NaCl at the various doses shown, 18h after partial hepatectomy, and with 1μCi of [6-^{14}C]orotate in 0.9% NaCl 20h after partial hepatectomy and were killed 30min later. The specific radioactivity of the hepatic RNA (○) and DNA (●) was determined as indicated in the Methods section. The control (zero nicotinamide) rats were injected with 0.9% NaCl alone. Each point represents the average for five animals and the vertical bars represent twice the S.E.M. The control (zero nicotinamide) values were: RNA specific radioactivity, 4137±130c.p.m./mg of RNA; DNA specific radioactivity, 1097±54c.p.m./mg of DNA (five animals).

Effects of nicotinamide dose on incorporation of [6-^{14}C]orotate

The effects of various doses of nicotinamide on the specific radioactivity of the hepatic RNA after [6-^{14}C]orotate injection at 3h after partial hepatectomy are shown in Fig. 1. It is clear that there was a dose-dependent inhibition of the incorporation of [^{14}C]orotate into hepatic RNA by nicotinamide. A dose of 500mg of nicotinamide/kg body wt., which has been shown to result in the greatest rate of hepatic NAD synthesis in both normal (Greengard *et al.*, 1964) and partially hepatectomized rats (Ferris & Clark, 1971), inhibited the incorporation of [^{14}C]-orotate into hepatic RNA by 62%.

A similar dose-dependent inhibition by nicotinamide of the incorporation of [6-^{14}C]orotate into the hepatic nucleic acids was observed at 20h after partial hepatectomy (Fig. 2). The previous injection of 500mg of nicotinamide/kg lowered the final specific radioactivity of RNA by 55% and that of DNA by 60%. Increasing the nicotinamide dose to 750mg of nicotinamide/kg caused a further depression of the specific radioactivity of DNA to 71%, but no additional effect on RNA.

Effect of time of pretreatment of nicotinamide on incorporation of [6-^{14}*C*]*orotate*

Rats were injected intraperitoneally with 500mg of nicotinamide/kg at the various times before the injection of [6-^{14}C]orotate at 20h after partial hepatectomy. The specific radioactivity of the hepatic RNA and DNA determined 30min later is shown in Fig. 3. This shows a non-linear response with time of pretreatment, showing an almost negligible incorporation of [6-^{14}C]orotate into hepatic RNA and DNA when nicotinamide is injected 1.5h before the orotate, but a value approaching control amounts when the nicotinamide is injected 3h before the orotate. This non-linear response with time may be contrasted with the results of Clark & Pinder (1969), who showed that after the intraperitoneal injection of 500mg of nicotinamide/kg in starved unoperated rats, the hepatic NAD concentration increased linearly for up to 5h. Further, preliminary experiments indicate that the rate of NAD synthesis induced by nicotinamide during the period 17–21h after hepatectomy also occurs in linear fashion.

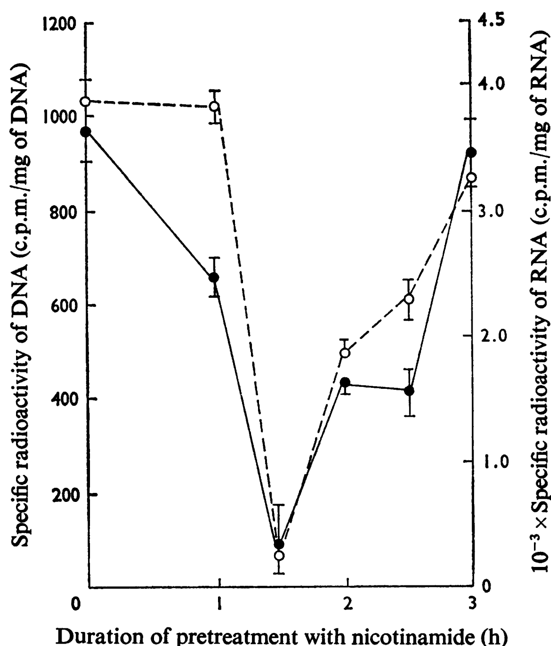

Fig. 3. *Effect of the duration of nicotinamide pretreatment on the incorporation of* [^{14}*C*]*orotate into hepatic RNA and DNA 20h after partial hepatectomy*

Female albino rats were injected intraperitoneally with 500mg of nicotinamide/kg at the various times shown, before the intraperitoneal injection of 1μCi of [6-^{14}C]orotate at 20h after partial hepatectomy. The rats were killed 30min after the orotate injection and the specific radioactivity of the hepatic RNA (○) and DNA (●) was determined as indicated in the Methods section. The control (no nicotinamide pretreatment) received an intraperitoneal injection of nicotinamide and [6-^{14}C]orotate simultaneously 20h after partial hepatectomy. Each point represents the average for five animals and the vertical bars represent twice the S.E.M. Control (no nicotinamide pretreatment) values were: RNA specific radioactivity, 3870±160c.p.m./mg of RNA (five animals); DNA specific radioactivity, 970±75 c.p.m./mg of DNA (five animals).

Effect of ethionine on the hepatic nicotinamide nucleotides during regeneration

The effect of an ethionine-induced hepatic ATP depletion at various times after partial hepatectomy on the steady-state content and the rate of nicotinamide-induced synthesis of the nicotinamide nucleotides during regeneration was investigated (Figs. 4 and 5).

The injection of ethionine alone caused little change in the total hepatic NAD after partial hepatectomy, a response also reported in control unoperated rats (Pinder & Clark, 1968; Clark & Pinder, 1969). However, after partial hepatectomy, when ethionine and nicotinamide were injected together there was a marked decrease in the NAD synthesis induced by nicotinamide (Fig. 4), whereas no such effect was observed in control animals (Pinder & Clark, 1968; Clark & Pinder, 1969).

Ethionine alone caused a decrease in the total hepatic NADP in regenerating liver (Fig. 5), although the decrease was less (18%) than the decrease observed on ethionine administration in a normal control liver (40–50%) (Pinder & Clark, 1968; Clark & Pinder, 1969). The injection of nicotinamide alone, however, caused a similar increase in total hepatic NADP (30%) in regenerating liver to that observed in normal livers (Pinder & Clark, 1968). Administration of nicotinamide and ethionine together partially prevented the loss of hepatic NADP observed on the injection of ethionine alone, the extent and time-course of the inhibition being similar in regenerating and normal liver.

Hepatic ribose phosphate pyrophosphokinase activity during regeneration

The total hepatic activity of ribose phosphate pyrophosphokinase was assayed *in vitro* in tissue extracts of regenerating liver at various times after partial hepatectomy (Fig. 6). Fig. 6 also shows, for comparison, the rates of synthesis of total hepatic NAD induced by nicotinamide injection (Ferris & Clark, 1971). The control zero-time value for fed, unoperated rats of 0.74μmol/h per g of liver (Fig. 6)

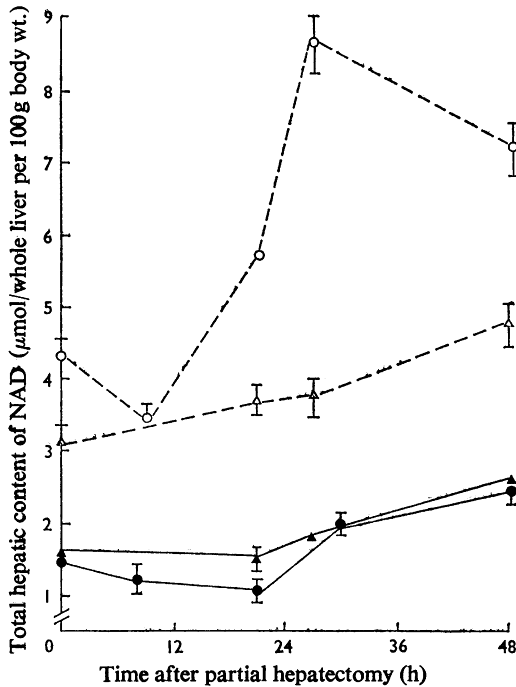

Fig. 4. *Effect of ethionine (▲) and ethionine+nicotinamide (△) on the total liver content of NAD at intervals after partial hepatectomy*

The results are compared with the effects of nicotinamide (○) or no injection (●) on the total liver content of NAD at intervals after partial hepatectomy. Female albino rats were injected intraperitoneally with 1 g of ethionine/kg body wt. and 500mg of nicotinamide/kg, together or alone in 0.9% NaCl, at various times after partial hepatectomy and were killed 3h later. The whole liver content of NAD was determined as indicated in the Methods section and is plotted at a time 3h after injection. Each point represents the average for five animals and the vertical bars represent twice the S.E.M. The total liver content of NAD at zero-time was calculated from the amount of liver remaining immediately after partial hepatectomy and the total hepatic NAD content of fed unoperated rats injected with ethionine, ethionine+nicotinamide, nicotinamide or untreated. The control (zero-time) values were: untreated, 1.48±0.06μmol of NAD/whole liver per 100g body wt. (five animals); ethionine injected, 1.58±0.05μmol of NAD/whole liver per 100g body wt. (five animals); nicotinamide injected, 4.27±0.23μmol of NAD/whole liver per 100g body wt. (five animals); nicotinamide+ethionine injected, 3.10±0.25μmol of NAD/whole liver per 100g body wt. (five animals). The average weight of the liver remnant immediately after partial hepatectomy was 1.53±0.11g/100g body wt.

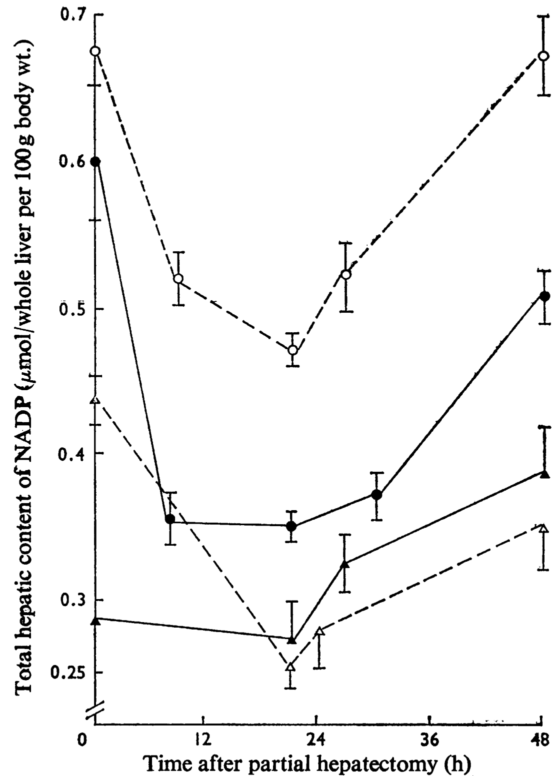

Fig. 5. *Effect of ethionine (▲) and ethionine+nicotinamide (△) on the total liver content of NADP at intervals after partial hepatectomy*

The results are compared with the effects of nicotinamide (○) or no injection (●) on the total liver content of NADP at intervals after partial hepatectomy. Female albino rats were injected intraperitoneally with 1g of ethionine/kg body wt. and 500mg of nicotinamide/kg, together or alone in 0.1% NaCl, at various times after partial hepatectomy and were killed 3h later. The whole liver content of NADP was determined as indicated in the Methods section and is plotted at a time 3h after injection. Each point represents the average of five animals and the vertical bars represent twice the S.E.M. The total liver content of NADP at zero-time was calculated from the amount of liver remaining immediately after partial hepatectomy and the total hepatic NADP content of fed unoperated rats injected with ethionine, ethionine+nicotinamide, nicotinamide or untreated. The control (zero-time) values were: untreated, 0.58±0.022μmol of NADP/whole liver per 100g body wt. (five animals); ethionine injected, 0.29±0.005μmol of NADP/whole liver per 100g body wt. (five animals); nicotinamide injected, 0.68±0.023μmol of NADP/whole liver per 100g body wt. (five animals); nicotinamide+ethionine injected,

may be compared with a rate of 5-phosphoribosyl 1-pyrophosphate synthesis by liver slices of 0.4 μmol/h per g of liver (Rajalakshmi & Handschumacher, 1968).

The total hepatic activity of ribose phosphate pyrophosphokinase increased very rapidly between 12 and 24h after partial hepatectomy, by 150%. This is the period of liver regeneration when there is a rapid synthesis of DNA and an increase in the total hepatic activity of NMN adenylyltransferase, NMN pyrophosphorylase (Ferris & Clark, 1971) and a number of other enzymes of nucleotide and nucleic acid metabolism (Bucher, 1963, 1967*a*,*b*). It is also clear that during the period 12–24h after partial hepatectomy, the potential rate of hepatic 5-phosphoribosyl 1-pyrophosphate synthesis assessed by the activity *in vitro* of ribose phosphate pyrophosphokinase only marginally exceeds the potential rate of synthesis of hepatic NAD as realized *in vivo* by nicotinamide injection. The stoicheiometry of NAD synthesis, regardless of which pathway is operative, is such that 1 mol of 5-phosphoribosyl 1-pyrophosphate is required for each mol of NAD synthesized.

Discussion

The relative equilibrium between different metabolic pathways requiring common precursors undoubtedly plays an important part in the biochemical control of metabolic events. The regenerating liver provides a situation where during the pre-mitotic period both RNA and DNA synthesis are maximally active. In addition, nicotinamide injection engenders a state in regenerating as well as normal liver, where nicotinamide nucleotide synthesis is maximally stimulated. Superimposing both these activities simultaneously leads to the extreme situation where common precursors become rate-limiting and the normal cellular priorities break down. Such a situation allows considerable insight into the normal metabolic balance between these pathways.

From the incorporation studies (Figs. 1 and 2) it is obvious that NAD synthesis induced by nicotinamide competes successfully with both RNA and DNA synthesis, whereas no relationships could be established between total liver RNA and the amounts of NAD induced by nicotinamide (Table 1) analogous to those that have been previously reported for DNA (Ferris & Clark, 1971). This is in accord with other workers, who have reported that net gains in cellular RNA do not occur until 9–15h after partial hepatectomy and only then to a relatively small

Fig. 5 *Caption continued*

0.44±0.015 μmol of NADP/whole liver per 100g body wt. (five animals). The average weight of the liver remnant immediately after partial hepatectomy was 1.53±0.11 g/100g body wt.

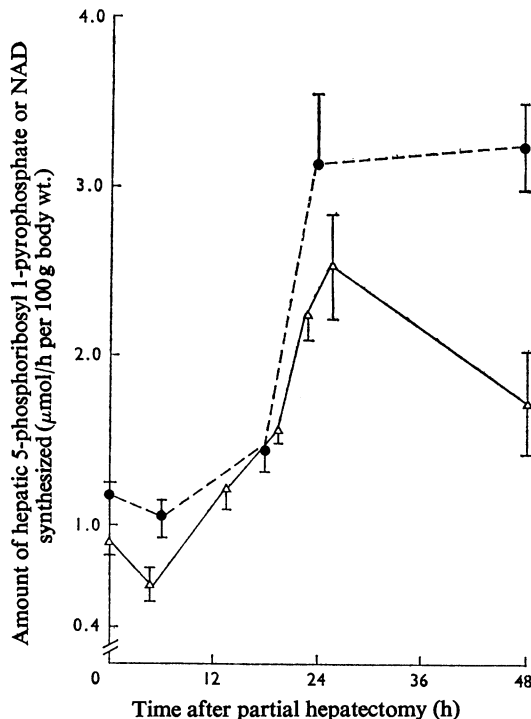

Fig. 6. *Total hepatic ribose phosphate pyrophosphokinase activity (●) and the rate of NAD synthesis induced by nicotinamide NAD (△) at intervals after partial hepatectomy*

The results for NAD synthesis induced by nicotinamide are those of Ferris & Clark (1971). Female albino rats were killed at intervals after partial hepatectomy and the activity of ribose phosphate pyrophosphokinase was determined in supernatant preparations of the liver as indicated in the Methods section. Both the whole liver enzyme activity and the rate of hepatic NAD synthesis induced by nicotinamide are expressed as μmol/h per 100g body wt. The zero-time values were calculated from the weight of liver remaining after partial hepatectomy and the values determined in normal fed female rats. Each point represents the average for five animals and the vertical bars represent twice the S.E.M. The control (zero-time) values were: NAD synthesis, 0.93±0.08 μmol of NAD synthesized/h per whole liver per 100g body wt. (five animals); ribose phosphate pyrophosphokinase activity, 1.16±0.06 μmol of 5-phosphoribosyl 1-pyrophosphate synthesized/h per whole liver per 100g body wt. (five animals). The average weight of the liver remnant immediately after partial hepatectomy was 1.53±0.11 g/100g body wt.

extent (20–30%) (Lieberman & Kane, 1965; Bucher, 1967*b*). The observed dose-dependent nicotinamide-induced inhibition of [^{14}C]orotate incorporation (Figs. 1 and 2) may be explained by a progressive inhibition of one or more steps between orotate and the nucleic acids or by a progressive expansion of one of the intermediate pools. There is no evidence to support the latter; on the contrary, nicotinamide injection is known to lower the hepatic ATP content in a non-linear fashion (with respect to time) (Clark & Pinder, 1969) and may also lower the hepatic free glutamine pool. This could lead to a lower rate of carbamoyl phosphate synthesis and hence a smaller endogenous orotate pool. Such a situation might provide some explanation for the very marked biphasic effect of different durations of nicotinamide pretreatment (Fig. 3).

Both ATP and 5-phosphoribosyl 1-pyrophosphate are involved in the synthesis of both the nicotinamide nucleotides and the nucleic acids. Ethionine, which is known to cause an extensive hepatic ATP depletion (Villa-Trevino *et al.*, 1966), appears to decrease the ability of nicotinamide to induce increased NAD and NADP concentrations in the regenerating liver (Fig. 4 and 5), as compared with the normal liver (Clark & Pinder, 1969). In the case of NAD this may be explained in part, by the report (Stirpe & Della Corte, 1968) that the nuclei of regenerating rat liver exhibit a less discrete compartmentalization of metabolites and a ready loss of NMN adenyltransferase on isolation, the latter suggesting a loss of integrity of the nuclear membrane. The effects of ethionine on the NADP concentration in regenerating liver may result primarily from a decreased availability of cytoplasmic ATP, since the K_m value of NAD-kinase for ATP is very close to the normal physiological concentration (Slater & Sawyer, 1966) and the rate of turnover of hepatic ATP is greatly increased during regeneration (Ove *et al.*, 1967).

The stoicheiometry of NAD synthesis is such that whichever pathway is operative, 1 mol of 5-phosphoribosyl 1-pyrophosphate is required for the synthesis of 1 mol of NAD (Chaykin, 1967). Measurement of the maximum potential rate of hepatic 5-phosphoribosyl 1-pyrophosphate synthesis after partial hepatectomy revealed that it was of a similar order of magnitude to the rate of NAD synthesis induced by nicotinamide *in vivo*, particularly during the period 12–24h after partial hepatectomy. Since the concentration of 5-phosphoribosyl 1-pyrophosphate in rat liver is only of the order of 40nmol/g wet wt. (Pinder *et al.*, 1971), the similarity between the rates of 5-phosphoribosyl 1-pyrophosphate and nicotinamide-induced NAD synthesis would imply that other reactions requiring 5-phosphoribosyl 1-pyrophosphate would be severely restricted in the presence of nicotinamide. Thus, the inhibition of the incorporation of [6-^{14}C]orotate into nucleic acids after partial hepatectomy by nicotinamide could be the result of competition between NAD and nucleic acid synthesis for available 5-phosphoribosyl 1-pyrophosphate. Analogous situations have been reported in which the utilization of large amounts of 5-phosphoribosyl 1-pyrophosphate in one reaction inhibits the occurrence of another 5-phosphoribosyl 1-pyrophosphate-requiring reaction; namely in Ehrlich Ascites cells the rate of 5-phosphoribosyl 1-pyrophosphate synthesis is insufficient to support maximal synthesis of the ribonucleotides from two purine bases simultaneously (Henderson & Khoo, 1965*b*), and the inhibitory effect of a diet containing 1% orotate on hepatic NAD synthesis induced by nicotinamide (Windmueller, 1965).

It is proposed therefore in rapidly dividing cells that nicotinamide induces a stimulated NAD synthesis which leads to a decreased availability of 5-phosphoribosyl 1-pyrophosphate which in turn leads to a decreased RNA and DNA synthesis. Such a mechanism would explain the reports that nicotinamide inhibits (*a*) mitosis in regenerating rat liver (Oide, 1958), bean root tips and regenerating planaria (Kanatani, 1960) and (*b*) ^{32}P incorporation into RNA of hypertrophying rat kidney (Revel & Mandel, 1962). The hypothesis of Burzio & Koide (1970) that nicotinamide inhibits mitosis via poly-(ADP-ribose) inhibition of DNA polymerase offers no explanation of the effect of nicotinamide on RNA turnover reported here. Apart from this it also ignores the fact that nicotinamide has been clearly demonstrated to be a potent inhibitor of poly(ADP-ribose) polymerase activity (Nishizuka *et al.*, 1968; Clark *et al.*, 1971; Preiss *et al.*, 1971).

G. M. F. is grateful to the Cancer Research Campaign for a research assistantship and running costs.

References

Bucher, N. L. R. (1963) *Int. Rev. Cytol.* **15**, 245–300
Bucher, N. L. R. (1967*a*) *N. Engl. J. Med.* **277**, 686–696
Bucher, N. L. R. (1967*b*) *N. Engl. J. Med.* **277**, 738–746
Burton, K. (1955) *Biochem. J.* **61**, 473–483
Burzio, L. & Koide, S. S. (1970) *Biochem. Biophys. Res. Commun.* **40**, 1013–1020
Burzio, L. & Koide, S. S. (1971) *Biochem. Biophys. Res. Commun.* **42**, 1185–1190
Ceriotti, G. (1955) *J. Biol. Chem.* **214**, 59–70
Chaykin, S. (1967) *Annu. Rev. Biochem.* **36**, 149–170
Clark, J. B. & Pinder, S. (1969) *Biochem. J.* **114**, 321–330
Clark, J. B., Ferris, G. M. & Pinder, S. (1971) *Biochim. Biophys. Acta* **238**, 82–85
Ferris, G. M. & Clark, J. B. (1971) *Biochem. J.* **121**, 655–662
Flaks, J. G., Erwin, M. J. & Buchanan, J. M. (1957) *J. Biol. Chem.* **229**, 603–612
Fleck, A. & Munro, H. N. (1962) *Biochim. Biophys. Acta* **55**, 571–583
Fujioka, M., Koga, M. & Lieberman, I. (1963) *J. Biol. Chem.* **238**, 3401–3406

Greenbaum, A. L., Clark, J. B. & McLean, P. (1965) *Biochem. J.* **95**, 161–166
Greengard, P., Quinn, G. P. & Reid, M. B. (1964) *J. Biol. Chem.* **239**, 1887–1892
Henderson, J. F. & Khoo, M. K. (1965*a*) *J. Biol. Chem.* **240**, 2349–2357
Henderson, J. F. & Khoo, M. K. (1965*b*) *J. Biol. Chem.* **240**, 2358–2362
Higgins, G. M. & Anderson, R. M. (1931) *Arch. Pathol.* **12**, 186–202
Hirsch, C. A. (1967) *J. Biol. Chem.* **242**, 2822–2827
Kanatani, H. (1960) *J. Fac. Sci. Univ. Tokyo* **9**, 387–395
Kaplan, N. O., Goldin, A., Humphreys, S. R. & Stolzenbach, F. E. (1957) *J. Biol. Chem.* **226**, 365–371
Lieberman, I. & Kane, P. (1965) *J. Biol. Chem.* **240**, 1737–1741
Morton, R. K. (1958) *Nature (London)* **181**, 540–542
Morton, R. K. (1961) *Aust. J. Sci.* **24**, 260–278
Murray, A. W. (1966) *Biochem. J.* **100**, 671–674
Nishizuka, Y., Ueda, K., Nakazawa, K., Reeder, R. H., Honjo, T. & Hayaishi, O. (1968) *J. Vitaminol. (Kyoto)* **14**, 143–152
Oide, H. (1958) *Gann* **49**, 49–56
Ove, P., Takai, S., Umeda, T. & Lieberman, I. (1967) *J. Biol. Chem.* **242**, 4963–4971
Pinder, S. & Clark, J. B. (1968) *FEBS Lett.* **1**, 206–208
Pinder, S., Clark, J. B. & Greenbaum, A. L. (1971) *Methods Enzymol.* **18B**, 20–46
Preiss, J., Schlaeger, R. & Hilz, H. (1971) *FEBS Lett.* **19**, 244–246
Rajalakshmi, S. & Handschumacher, R. E. (1968) *Biochim. Biophys. Acta* **155**, 317–325
Revel, M. & Mandel, P. (1962) *Cancer Res.* **22**, 456–462
Slater, T. F. & Sawyer, B. C. (1966) *Biochem. Pharmacol.* **15**, 1267–1271
Stirpe, F. & Della Corte, E. (1968) *Biochem. J.* **108**, 89–93
Villa-Trevino, S., Shull, K. H. & Farber, E. (1966) *J. Biol. Chem.* **241**, 4670–4674
Windmueller, H. G. (1965) *J. Nutr.* **85**, 221–229