Nicotinamide Nucleotide Synthesis in Regenerating Rat Liver

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1. The concentrations and total content of the nicotinamide nucleotides were measured in the livers of rats at various times after partial hepatectomy and laparotomy (sham hepatectomy) and correlated with other events in the regeneration process. 2. The NAD content and concentration in rat liver were relatively unaffected by laparotomy, but fell to a minimum, 25 and 33% below control values respectively, 24 h after partial hepatectomy. NADP content and concentration were affected similarly by both laparotomy and partial hepatectomy, falling rapidly and remaining depressed for up to 48 h. 3. The effect of injecting various doses of nicotinamide on the liver DNA and NAD 18 h after partial hepatectomy was studied and revealed an inverse correlation between NAD content and DNA content. 4. Injections of nicotinamide at various times after partial hepatectomy revealed that the ability to synthesize NAD from nicotinamide was impaired during the first 12 h, rose to a peak at 26 h and fell again by 48 h after partial hepatectomy. 5. The total liver activity of NAD pyrophosphorylase (EC 2.7.7.1) remained at or slightly above the initial value for 12 h after partial hepatectomy and then rose continuously until 48 h after operation. The activity of NMN pyrophosphorylase (EC 2.4.2.12) showed a similar pattern of change after partial hepatectomy, but was at no time greater than 5% of the activity of NAD pyrophosphorylase. 6. The results are discussed with reference to the control of NAD synthesis in rapidly dividing tissue. It is suggested that the availability of cofactors and substrates for NAD synthesis is more important as a controlling factor than the maximum enzyme activities. It is concluded that the low concentrations of nicotinamide nucleotides in rapidly dividing tissues are the result of competition between NAD synthesis and nucleic acid synthesis for common precursor and cofactors.

It has been demonstrated that the concentrations of nicotinamide nucleotides (NAD and NADP) are low in a variety of transplantable and induced tumours (Jedeikin & Weinhouse, 1955; Briggs, 1960; Clark, Greenbaum & McLean, 1966) as compared with the host tissue. Similarly the NAD and NADP content of foetal tissue is low and increases to adult values during maturation (Dawkins, 1959; Nemeth & Dickerman, 1960; Caiger, Morton, Filsell & Jarrett, 1962; Burch & Von Dippe, 1964). Preliminary results have also indicated that the NAD content of regenerating liver may be lower than normal (de Burgh, 1957). Evidence of this nature led to the hypothesis that NAD is important in the control of cell division (Morton, 1958). It was suggested that this control occurs as a result of the intranuclear location of one enzyme of NAD synthesis, NAD pyrophosphorylase (ATP-NMN adenyltransferase, EC 2.7.7.1) (Hogeboom & Schneider, 1952; Branster & Morton, 1956). The activity of this enzyme was shown to be depressed

in mammary carcinoma (Branster & Morton, 1956) and foetal liver (Dawkins, 1959) when compared with the respective normal tissues. However, a positive correlation has been reported between the activity of this enzyme and mitosis during the lactation cycle of rat mammary glands (Greenbaum & Pinder, 1968), i.e. an increased activity of NAD pyrophosphorylase occurred at the same time as increased mitosis. Initial reports of a decreased activity of NAD pyrophosphorylase per nucleus from regenerating liver have been demonstrated to be an artifact of the method of isolation of nuclei (Stirpe & Della Corte, 1968), and it has been shown that when rat liver nuclei of various classes are separated by zonal centrifugation a positive correlation exists between DNA synthesis and NAD pyrophosphorylase activity (Haines, Johnson, Mathias & Ridge, 1969).

There exists a large body of evidence as to the sequence of events, both biochemical and anatomical, that follow partial hepatectomy in the rat (for

review see Bucher, 1963), and in particular the rapid synthesis of nucleic acids that precedes the first mitotic division of regeneration. The action of injected nicotinamide, in inducing rapid synthesis of hepatic NAD, has been shown to be primarily a result of the increased substrate available for synthesis (Clark & Pinder, 1969). (It should be stressed here that the nicotinamide-induced synthesis of NAD refers to net synthesis, i.e. the balance between the rates of synthesis and degradation.) This technique therefore provides a rapid method for assessing the capacity of the liver to synthesize NAD, in the presence of saturating amounts of substrate, in various experimental states.

In the present study the hepatic concentrations of the oxidized and reduced nicotinamide nucleotides were measured after partial hepatectomy and laparotomy and correlated with other events of the regenerative process. The potential NAD-synthesizing capacity of the regenerating liver was assessed by the technique of nicotinamide-induced NAD synthesis. In addition, the activities of NAD pyrophosphorylase and NMN pyrophosphorylase (NMN-pyrophosphate phosphoribosyltransferase, EC 2.4.2.12), as determined by assays *in vitro*, were measured at various stages of early regeneration.

Results are presented to indicate that the concentrations of NAD in regenerating rat liver do not generally correlate significantly with the potential NAD-synthesizing capacity of the liver. This conclusion is independent of whether the maximal activity is assessed on the basis of optimum activities of the synthetic enzyme *in vitro* or by the rate of nicotinamide-induced NAD synthesis. The low concentrations of NAD characteristic of regenerating liver appear to be due to a competition that exists between NAD and nucleic acid synthesis, probably for precursors common to both synthetic systems.

METHODS

Animals. Female albino rats of the Wistar strain (body wt. approx. 150 g) were used in all experiments and fed on stock diet 41B (Bruce & Parkes, 1949) *ad libitum*.

Partial hepatectomy was performed under ether anaesthesia by the method of Higgins & Anderson (1931), resulting in ablation of $64 \pm 4\%$ of the liver. The livers of laparotomized animals were exposed and handled and the abdomens closed as for partial hepatectomy.

Animals were injected intraperitoneally with nicotinamide (500 mg/kg body wt., unless otherwise stated) in 0.5 ml of sterile 0.9% NaCl.

All animals were killed by cervical dislocation.

Reagents. Phenazine methosulphate was obtained from Sigma Chemical Co. (St Louis, Mo., U.S.A.). NAD⁺, NADP⁺, ATP, glucose 6-phosphate, yeast alcohol dehydrogenase (EC 1.1.1.1) and glucose 6-phosphate

dehydrogenase (EC 1.1.1.49) were obtained from C. F. Boehringer und Soehne G.m.b.H. (Mannheim, Germany). Nicotinamide was purchased from Hopkin and Williams (Chadwell Heath, Essex, U.K.). Ribose 5-phosphate 1-pyrophosphate and NMN were supplied by P-L Biochemicals Inc. (Milwaukee, Wis., U.S.A.). [*carboxyl*-¹⁴C]-Nicotinamide was purchased from The Radiochemical Centre (Amersham, Bucks., U.K.). All other chemicals were of A.R. grade where possible.

Determination of NAD, NADP and DNA. Liver samples were extracted and assayed for the content of NAD⁺, NADH, NADP⁺ and NADPH by the method of Greenbaum, Clark & McLean (1965a).

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

Enzymic procedures. NAD pyrophosphorylase activity was assayed essentially by the method of Kornberg (1950) as modified by Greenbaum, Clark & McLean (1965b).

NMN pyrophosphorylase activity was assayed by the method of Dietrich, Fuller, Yero & Martinez (1966).

Nuclei. Nuclei were isolated and counted in a clinical haemocytometer (improved Neubauer counting chamber) by the method of Branster & Morton (1956).

RESULTS

Expression of results. During liver regeneration the mass of the liver may change without any alteration in the number of cells present in the liver, e.g. during the first 20 h after partial hepatectomy, when the wet weight is increasing, but no mitoses occur (Harkness, 1957; Bucher, 1963). Thus considerations of metabolite concentrations or enzyme activities expressed per unit wet weight of liver are complicated by these changes of liver mass. Accordingly most of the results are recorded on the basis of whole liver and refer to the total amount of a given metabolite or enzyme activity present in the whole regenerating liver remnant at the time indicated. The total liver weight of a rat is dependent on its body size (Higgins & Anderson, 1931) and all the results for whole liver are therefore expressed per 100 g body wt. Zero-time values are calculated from the weight of liver remaining in partially hepatectomized rats immediately after operation and the value of the parameter measured in fed unoperated rats.

Effect of partial hepatectomy and laparotomy. Fig. 1 shows the changes in total NAD and NADP concentrations (expressed as $\mu\text{mol/g}$ wet wt.) in the liver during the first 48 h after operation. It is apparent that the NAD concentration was altered to a much greater extent by partial hepatectomy than by laparotomy, decreasing by 33% as opposed to 6% during the first 24 h after operation. The concentration of NAD fell consistently during the first 24 h after partial hepatectomy and then returned to the original value ($0.85 \mu\text{mol/g}$ wet wt.) by 48 h. Both treatments, however, had similar effects

Fig. 1. Effect of partial hepatectomy and laparotomy on hepatic concentrations of NAD and NADP. Operations were carried out on female albino rats at zero time and the NAD and NADP concentrations in the liver were determined as indicated in the Methods section at intervals after operation. The results are expressed as the sum of $\text{NAD}^+ + \text{NADH}$ and the sum of $\text{NADP}^+ + \text{NADPH}$ in $\mu\text{mol/g}$ wet wt., each point being the average of five animals. ●, NAD concentration after partial hepatectomy; ○, NAD concentration after laparotomy; ▲, NADP concentration after partial hepatectomy; △, NADP concentration after laparotomy. The vertical bars represent twice the s.e.m. The control (zero-time) values were: $0.85 \pm 0.04 \mu\text{mol}$ of NAD/g wet wt. (five animals); $0.39 \pm 0.02 \mu\text{mol}$ of NADP/g wet wt. (five animals).

on the NADP concentrations, which fell by approx. 30% immediately after the operation and remained depressed for at least 48h. The lowest concentration of NADP occurred at 30h after partial hepatectomy and reflected a decrease of some 66%.

The fall in NAD concentration in the hepatectomized animals occurred in both the oxidized and reduced forms, with a slightly greater loss in the reduced form, as shown in Table 1. This caused an overall increase in the $[\text{NAD}^+]/[\text{NADH}]$ ratio in the partially hepatectomized animals as compared with the laparotomized animals. NADP^+ concentration fell at times to values beyond the sensitivity of this method of analysis, but as most of the hepatic NADP is in the reduced form it was primarily this component that exhibited the decreased concentration.

Course of regeneration. In Fig. 2 the changes in the total liver NAD and NADP contents expressed as $\mu\text{mol/whole liver per } 100\text{g body wt.}$ after partial hepatectomy are compared with the number of

Table 1. *Effect of partial hepatectomy and laparotomy on the redox state of hepatic NAD*

The treatment of animals and nicotinamide nucleotide assays were as described in Fig. 1 and the Methods section. The results are expressed as the means of five animals at each point.

Time after operation (h)	[NAD ⁺]/[NADH] ratio	
	After partial hepatectomy	After laparotomy
0	3.8	3.8
2	4.2	3.8
8	5.2	5.6
16	6.0	4.8
20	5.9	4.0
24	5.2	3.1
48	6.0	4.1

Fig. 2. Effect of partial hepatectomy on total hepatic NAD (●), NADP (▲) and numbers of nuclei (■). Operations were carried out on female albino rats at zero time and the whole liver contents of NAD, NADP and nuclei were determined as indicated in the Methods section at intervals after the operation. The results are expressed in μmol of NAD and NADP/whole liver per 100g body wt. and $10^{-6} \times \text{nuclei/whole liver per } 100\text{g body wt.}$ Each point is the average of five animals and the vertical bars represent twice the s.e.m. The control (zero-time) values were: $1.48 \pm 0.06 \mu\text{mol}$ of NAD/whole liver per 100g body wt. (five animals); $0.6 \pm 0.04 \mu\text{mol}$ of NADP/whole liver per 100g body wt. (five animals); 220×10^6 nuclei/whole liver per 100g body wt. (five animals).

nuclei in the liver. Since during the first 24h of regeneration the liver mass increased, expression of the NAD and NADP concentrations per g wet wt.

(see Fig. 1) might have given a misrepresentation of the true concentrations. In such circumstances any decrease observed could have been merely a reflection of increased liver size without a concomitant increase in total NAD and NADP. By expressing the results per whole liver this problem is circumvented, and it is clear that Fig. 1 does represent a genuine decrease in nucleotide concentration since the whole liver NAD and NADP contents also fell, by 25 and 33% respectively, during the first 24 h after partial hepatectomy. However, an 80% increase in the whole liver NAD content occurred between 24 and 30 h (rate of NAD increase = $0.13 \mu\text{mol/h}$ per total liver), coincident with the first mitosis. Further, the total liver NADP content also increased during the period 24–48 h, but at a rate ($0.006 \mu\text{mol/h}$ per total liver) that is approximately 10% of that of NAD synthesis over the same period. At 48 h the total liver NADP content had returned, within experimental limits, to the initial value.

Fig. 3. Effect of nicotinamide on the total liver content of NAD (●) and DNA (□) between 18 and 21 h after partial hepatectomy. Female albino rats were injected intraperitoneally with nicotinamide in 0.5 ml of 0.9% NaCl at the various doses shown 18 h after partial hepatectomy and killed 3 h later. The controls, also 18 h after partial hepatectomy (zero nicotinamide), were injected with 0.5 ml of 0.9% NaCl alone and killed 3 h later. The whole liver contents of NAD and DNA were determined as indicated in the Methods section. Each point represents the average of five animals and DNA determinations were carried out on pooled liver remnants. The vertical bars represent twice the s.e.m. The control (zero nicotinamide) values were: $1.3 \pm 0.08 \mu\text{mol}$ of NAD/whole liver per 100 g body wt. (five animals); 6.5 mg of DNA/whole liver per 100 g body wt. (five animals).

Dose-response to nicotinamide at 18 h after partial hepatectomy. The effect of injecting rats with various doses of nicotinamide 18 h after partial hepatectomy is shown in Fig. 3. The resultant total liver content of NAD/100 g body wt. 3 h after injection is compared with the total liver content of DNA per unit body weight at that time (i.e. 21 h after operation). Control animals received an injection of saline 3 h before being killed and the determination of DNA was carried out on pooled samples of liver remnants from at least five rats.

A dose of 500 mg of nicotinamide/kg body wt. produced the greatest rise in hepatic NAD content, a higher dose producing a slightly lower total content of NAD in the liver. A similar dose-response curve for nicotinamide-induced NAD synthesis has been reported for both normal and hypophysectomized rats where NAD content was assayed 5 h after nicotinamide injection (Greengard, Quinn & Reid, 1964). In this latter case also a slight decrease in NAD synthesis was observed at super-optimum doses (greater than 500 mg/kg body wt.) of nicotinamide. In this context it should be noted that the LD_{50} for nicotinamide injected into rats is 1.5 g/kg body wt. (Ellinger, Fraenkel & Kader, 1947). The highest concentration of NAD produced by nicotinamide injection in animals 18 h after partial hepatectomy was $2.2 \mu\text{mol/g}$ of liver and was some 20% lower than the NAD concentration produced 3 h after the injection of 500 mg of nicotinamide/kg body wt. into starved unoperated rats (Clark & Pinder, 1969). There was an inverse correlation between the NAD content of the liver and the total DNA content of the liver 3 h after the injection of nicotinamide, i.e. while the NAD increased the DNA decreased. It should be noted that the period 16–28 h after partial hepatectomy was one of very rapid DNA synthesis (see Fig. 5).

Nicotinamide injections at various times. In all the experiments reported in Fig. 4 nicotinamide (500 mg/kg body wt.) was injected into rats, at various times after partial hepatectomy, 3 h before they were killed and the resultant mean rate of NAD synthesis by the whole liver was calculated over that 3 h period. The results are shown in Fig. 4 and the rates of NAD synthesis are plotted as single points $1\frac{1}{2}$ h after the time of injection. It should be remembered, however, that these points actually represent the mean rate of NAD synthesis over the 3 h period immediately after the injection time. The rate of synthesis at zero time, calculated from the rate of synthesis of hepatic NAD by normal fed rats injected with the same amount of nicotinamide and the weight of liver remaining after partial hepatectomy, had a value of $0.93 \mu\text{mol}$ of NAD synthesized/h per 100 g body wt. During the first 10 h after partial hepatectomy the whole liver remnants showed a decreased ability to synthesize

Fig. 4. Nicotinamide-induced synthesis of hepatic NAD at intervals after partial hepatectomy. Female albino rats received intraperitoneal injections of 500mg of nicotinamide/kg body wt. in 0.5ml of 0.9% NaCl at intervals after partial hepatectomy and were killed 3h later. The NAD content of the liver was determined as indicated in the Methods section and the rate of synthesis of hepatic NAD was calculated by using data on the whole liver content of NAD at various times after partial hepatectomy derived from Fig. 2. The results are expressed as μmol of hepatic NAD synthesized/h per 100g body wt. Each point represents the mean rate of NAD synthesis over the 3h period after nicotinamide injection of at least five animals and is plotted as a point $1\frac{1}{2}$ h after the nicotinamide injection. The rate of synthesis of NAD at zero time was calculated from the portion of the liver remaining after partial hepatectomy and the rate of synthesis of hepatic NAD by fed rats similarly injected. The vertical bars represent twice the s.e.m. The control (zero time) value was $0.93 \pm 0.08 \mu\text{mol}$ of NAD synthesized/h per whole liver per 100g body wt. (five animals).

Fig. 5. Effect of partial hepatectomy on total hepatic NAD pyrophosphorylase activity (●), total hepatic NMN pyrophosphorylase activity (○) and total hepatic DNA content (□). Female albino rats were killed at intervals after partial hepatectomy and the enzyme activities and DNA content of the whole liver were measured as indicated in the Methods section. Enzyme activity of the whole liver is expressed as $\mu\text{mol/h}$ per 100g body wt. and DNA content of the whole liver as mg/100g body wt. The zero-time values are calculated from the weight of liver remaining after partial hepatectomy and values determined in normal fed female rats. Each point represents the average from at least four animals for enzyme activity determinations and at least six animals for DNA determinations. The vertical bars represent twice the s.e.m. The control (zero-time) values were: NAD pyrophosphorylase activity, $8.3 \pm 0.6 \mu\text{mol/h}$ per whole liver per 100g body wt. (four animals); NMN pyrophosphorylase activity, $0.33 \pm 0.03 \mu\text{mol/h}$ per whole liver per 100g body wt. (four animals); $4.4 \pm 0.25 \text{ mg}$ of DNA/whole liver per 100g body wt. (ten animals).

NAD from injected nicotinamide, falling to a rate of $0.65 \mu\text{mol/g}$ per 100g body wt. at 7h. This synthetic capacity then rose to a peak of $2.5 \mu\text{mol}$ of NAD synthesized/h per 100g body wt. 24–27h after the operation. This rate was almost 3 times the initial value. However, by 48h after operation the rate of synthesis had fallen to approx. $1.7 \mu\text{mol}$ of NAD/h per 100g body wt., i.e. 1.8 times the initial value.

Enzymic activities after partial hepatectomy. The activities of two enzymes of NAD synthesis, NAD pyrophosphorylase and NMN pyrophosphorylase, were assayed in tissue extracts of regenerating liver at various times after operation and these activities are recorded in Fig. 5. Also shown in Fig. 5 is the

total hepatic DNA expressed/unit body weight at various times after partial hepatectomy. During the first 12h of regeneration there was no significant change in the total hepatic enzyme activities or DNA content, although liver weight did increase slightly. From 12h onwards, however, there was a rapid increase in all three parameters, continuing until at least 48h after operation. Between 12 and 48h after operation the total hepatic DNA content increased by some 69% (to $8.5 \text{ mg}/100 \text{ g}$ body wt.), the total hepatic NAD pyrophosphorylase activity by approx. 88% (to $17.3 \mu\text{mol/h}$ per 100g body wt.) and the NMN pyrophosphorylase by approx. 83% (to $0.7 \mu\text{mol/h}$ per 100g body wt.). At no time was either of the enzyme activities lower than the initial

value and the activity of NAD pyrophosphorylase was always at least 20 times that of NMN pyrophosphorylase. Further, a comparison between the rate of NAD synthesis induced by nicotinamide shown in Fig. 4 and the activity of NAD pyrophosphorylase assayed *in vitro* reveals that the former is never greater than one-quarter of the latter. For instance, 24 h after partial hepatectomy the rate of total hepatic NAD synthesis induced by nicotinamide injection is $2.4 \mu\text{mol/h}$ per 100 g body wt., whereas the total hepatic NAD pyrophosphorylase activity is 4.7 times as great, at $11.4 \mu\text{mol/h}$ per 100 g body wt., and the total hepatic NMN pyrophosphorylase activity is only $0.55 \mu\text{mol/h}$ per 100 g body wt.

DISCUSSION

From the above results it is clear that regenerating rat liver, in common with other tissues undergoing rapid cell division, exhibits a decreased total content and concentration of the nicotinamide nucleotides. The effects of laparotomy and handling, in lowering the NADP concentration in a manner similar to partial hepatectomy, bear out the importance of adequate controls in regeneration studies, as previously emphasized by histochemical examination (Gahan, 1962). A similarity between the effects of partial hepatectomy and acute stress (induced by intraperitoneally administered Celite) on protein synthesis and ATP metabolism has also been reported (Majumdar, Tsukada & Lieberman, 1967; Ove, Takai, Umeda & Lieberman, 1967). Both these responses have been found to be adrenal-dependent in laparotomized animals only. Ishikawa (1969) has studied the nicotinamide nucleotide concentrations in rat liver during regeneration and reported, as the most significant feature, a dramatic increase in NAD⁺ concentration 8–12 h after operation. No such effect was found in the experiments reported here, but it is difficult to relate the two sets of experiments, since Ishikawa (1969) gave no data for control laparotomized animals and also expressed his data only per g wet wt. However, even as compared with the data reported here per g wet wt. it is apparent that the nucleotide concentrations reported by Ishikawa (1969) are generally lower than those reported in this paper. This may be due partly to differences in the assay techniques employed and partly to the inherent problems in considering changes in concentration of metabolites when the mass of the liver is also changing.

The first mitotic division occurred between 24 and 30 h after operation in these rats, in agreement with previous studies (Cater, Holmes & Mee, 1956; Harkness, 1957; Grisham, 1962). The timing of the decrease in liver NAD and NADP contents after partial hepatectomy is such that a minimum is reached just before the first mitosis.

Intraperitoneally injected nicotinamide seems to act primarily as a substrate for NAD synthesis (Clark & Pinder, 1969), and thus the extent of synthesis will reflect the maximum activities of the rate-limiting enzymes and the availability of other reactants, notably ATP and ribose 5-phosphate 1-pyrophosphate. The unique situation of NAD pyrophosphorylase, as the one enzyme of NAD synthesis located within the nucleus (Hogeboom & Schneider, 1952; Branster & Morton, 1956), has led to the suggestion that this will be the enzyme most important in the control of nicotinamide nucleotide synthesis and that a correlation exists between its activity and tissue concentrations of the nicotinamide nucleotides (Morton, 1961). From a consideration of the maximal activities of the enzymes synthesizing NAD, as assayed *in vitro*, it is evident that, under conditions of normal turnover and nicotinamide-induced NAD synthesis, the activity of NAD pyrophosphorylase is unlikely to be present in less than a tenfold excess (Myers, 1962; Greenbaum & Pinder, 1968). Thus, even in situations when changes in the activity of NAD pyrophosphorylase and tissue concentrations of the nicotinamide nucleotides are correlated, it is difficult to see why any functional relationship should be expected or deduced. Further, in the livers of rats subjected to various hormonal treatments (Greenbaum *et al.* 1965b) and carcinogens (Clark *et al.* 1966), rat mammary glands during the lactation cycle (Greenbaum & Pinder, 1968) and in the present work, no such correlation has been found. Indeed, in general, utmost caution should be exercised in any attempts to correlate changes in maximal enzyme activities *in vitro* and concomitant alterations in metabolic state.

NAD pyrophosphorylase activity did, however, show a positive correlation with DNA synthesis in the present study (Fig. 5), in rat mammary glands (Greenbaum & Pinder, 1968) and in various classes of rat liver nuclei (Haines *et al.* 1969). The total liver activity of a number of other enzymes has also been shown to increase during the period of rapid DNA synthesis in regenerating rat livers (Greenbaum, Greenwood & Harkness, 1954; Van Lancker & Sempoux, 1958).

The events after partial hepatectomy may be conveniently divided into three phases. During the first 12–15 h after operation there is a rapid increase in the rate of synthesis of RNA (Fujioka, Koga & Lieberman, 1963) and in the specific activity of RNA polymerase from hepatic nuclei and nucleoli (Tsukada & Lieberman, 1964), both changes beginning immediately after partial hepatectomy. The rate of destruction of ATP by 'briefly anoxic' livers is increased, as is the rate of incorporation of [³²P]P_i into ATP (Ove *et al.* 1967), both evidence of an increased ATP turnover. The total liver content

of all four nicotinamide nucleotides (NAD⁺, NADH, NADP⁺ and NADPH) fell. The decline in NAD appeared to be a function of partial hepatectomy, whereas the NADP decrease occurred in control laparotomized animals also and is probably the result of a non-specific stress reaction. At the same time the rate of NAD synthesis from injected nicotinamide fell transiently to 70% of the original, although the activities of NAD pyrophosphorylase and NMN pyrophosphorylase remained virtually unchanged. It is therefore reasonable to suggest that both in the normal regenerating liver and in liver under the influence of injected nicotinamide the rate of NAD synthesis is impaired by decreased availability of cofactors such as ribose 1-phosphate 5-pyrophosphate and ATP, which are also important for the synthesis of RNA. The normal rate of turnover of hepatic NADP has been estimated to be 4–5 h (Slater & Sawyer, 1966) and that of NAD to be 2 h (Greenbaum & Pinder, 1968). Thus the normal rate of breakdown would account for the falls in NAD and NADP concentrations, if synthesis were impaired.

The second phase of regeneration is from approx. 15 to 28 h after operation, during which time there is a rapid synthesis of DNA (Hecht & Potter, 1956, 1958; Bucher, Di Troia & Swaffield, 1961; Ove, Jenkins & Laszlo, 1969). The fall in hepatic NAD and NADP concentrations continued until a minimum was reached at 24 h after operation. The rate of NAD synthesis from nicotinamide was, however, increasing during this period from 1.3 to 2.5 μ mol of NAD synthesized/h per 100 g body wt. The injection of various doses of nicotinamide 18 h after partial hepatectomy induced an inverse relationship between NAD and DNA contents of the liver remnant. The activities of NAD pyrophosphorylase and NMN pyrophosphorylase also increased during this period, in agreement with Haines *et al.* (1969), who reported a correlation between DNA synthesis and NAD pyrophosphorylase activity in various classes of rat liver nuclei. The inverse correlation between NAD and DNA biosynthesis could be the result of competition for ATP, ribose 1-phosphate 5-pyrophosphate and adenine between the two synthetic pathways. It appears that during this phase of regeneration the increased capacity to synthesize hepatic NAD resulting from synthesis of the enzymes responsible was not normally realized and that DNA synthesis occurred at the expense of NAD synthesis. In this connexion Oide (1958) demonstrated that partially hepatectomized rats with an intrasplenic implantation of nicotinamide in a Carbowax pellet showed a decreased rate of mitosis. During the third phase of regeneration, studied from 28 to 48 h after operation, immediately after the first mitotic division, the total hepatic NAD content and

concentration increased rapidly and the total NADP content also increased, though at a lower rate. There was a decreased rate of synthesis of DNA and the capacity to synthesize NAD from nicotinamide was also decreased, despite a continued rise in the activities of NAD pyrophosphorylase and NMN pyrophosphorylase.

In conclusion, therefore, the present work emphasizes that substrates and cofactors may be more important in controlling the rate of synthesis of nicotinamide nucleotides than the maximum activities of the enzymes concerned. Further, it implies that the low concentrations of NAD and NADP recorded for various tissues undergoing rapid cell division are a result of the synthesis of nucleic acids tending to make less available the requisites of NAD biosynthesis.

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REFERENCES

- Branster, M. V. & Morton, R. K. (1956). *Biochem. J.* **63**, 640.
 Briggs, M. H. (1960). *Nature, Lond.*, **187**, 249.
 Bruce, H. M. & Parkes, A. S. (1949). *J. Hyg., Camb.*, **47**, 202.
 Bucher, N. L. R. (1963). *Int. Rev. Cytol.* **15**, 245.
 Bucher, N. L. R., Di Troia, J. F. & Swaffield, M. N. (1961). *Fedn Proc. Fedn Am. Soc. exp. Biol.* **20**, 286.
 Burch, H. B. & Von Dippel, P. (1964). *J. biol. Chem.* **239**, 1898.
 Burton, K. (1955). *Biochem. J.* **61**, 473.
 Caiger, P., Morton, R. K., Filsell, O. H. & Jarrett, I. G. (1962). *Biochem. J.* **85**, 351.
 Cater, D. B., Holmes, B. E. & Mee, L. K. (1956). *Acta radiol.* **46**, 655.
 Clark, J. B., Greenbaum, A. L. & McLean, P. (1966). *Biochem. J.* **98**, 546.
 Clark, J. B. & Pinder, S. (1969). *Biochem. J.* **114**, 321.
 Dawkins, M. J. R. (1959). *Proc. R. Soc. B*, **150**, 284.
 de Burgh, P. M. (1957). *Aust. J. Sci.* **20**, 86.
 Dietrich, L. S., Fuller, L., Yero, I. L. & Martinez, L. (1966). *J. biol. Chem.* **241**, 188.
 Ellinger, P., Fraenkel, G. & Kader, M. M. A. (1947). *Biochem. J.* **41**, 554.
 Fujioka, M., Koga, M. & Lieberman, I. (1963). *J. biol. Chem.* **238**, 3402.
 Gahan, P. B. (1962). *Biochem. J.* **84**, 118P.
 Greenbaum, A. L., Clark, J. B. & McLean, P. (1965a). *Biochem. J.* **95**, 161.
 Greenbaum, A. L., Clark, J. B. & McLean, P. (1965b). *Biochem. J.* **96**, 507.
 Greenbaum, A. L., Greenwood, F. C. & Harkness, R. D. (1954). *J. Physiol., Lond.*, **125**, 251.
 Greenbaum, A. L. & Pinder, S. (1968). *Biochem. J.* **107**, 64.
 Greengard, P., Quinn, G. P. & Reid, M. B. (1964). *J. biol. Chem.* **239**, 1887.

- Grisham, J. W. (1962). *Cancer Res.* **22**, 842.
- Haines, M. E., Johnson, I. R., Mathias, A. P. & Ridge, D. (1969). *Biochem. J.* **115**, 881.
- Harkness, R. D. (1957). *Br. med. Bull.* **13**, 87.
- Hecht, L. I. & Potter, V. R. (1956). *Cancer Res.* **16**, 988.
- Hecht, L. I. & Potter, V. R. (1958). *Cancer Res.* **18**, 186.
- Higgins, G. M. & Anderson, R. M. (1931). *Archs Path.* **12**, 186.
- Hogeboom, G. H. & Schneider, W. C. (1952). *J. biol. Chem.* **197**, 611.
- Ishikawa, H. (1969). *J. Fac. Sci. Tokyo Univ.* section 4, **11**, 589.
- Jedeikin, L. A. & Weinhouse, S. J. (1955). *J. biol. Chem.* **213**, 271.
- Kornberg, A. (1950). *J. biol. Chem.* **182**, 779.
- Majumdar, C., Tsukada, K. & Lieberman, I. (1967). *J. biol. Chem.* **242**, 700.
- Morton, R. K. (1958). *Nature, Lond.*, **181**, 540.
- Morton, R. K. (1961). *Aust. J. Sci.* **24**, 260.
- Myers, D. K. (1962). *Can. J. Biochem. Physiol.* **40**, 619.
- Nemeth, A. M. & Dickerman, H. (1960). *J. biol. Chem.* **235**, 1761.
- Oide, H. (1958). *Gann*, **49**, 49.
- Ove, P., Jenkins, M. D. & Laszlo, J. (1969). *Biochim. biophys. Acta*, **174**, 629.
- Ove, P., Takai, S., Umeda, T. & Lieberman, I. (1967). *J. biol. Chem.* **242**, 4963.
- Slater, T. F. & Sawyer, B. C. (1966). *Biochem. Pharmac.* **15**, 1267.
- Stirpe, F. & Della Corte, E. (1968). *Biochem. J.* **108**, 89.
- Tsukada, K. & Lieberman, I. (1964). *J. biol. Chem.* **239**, 2952.
- Van Lancker, J. L. & Sempoux, D. G. (1958). *Archs Biochem. Biophys.* **77**, 129.