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CONTENT OF NICOTINAMIDE COENZYMES IN NORMAL AND REGENERATING RAT LIVER

F. Pasos and V. I. Telepneva

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The liver regenerating after partial hepatectomy can be regarded as a model for use in studying the metabolic processes and regulatory mechanisms that lie at the basis of cell differentiation, growth, and division. Radical changes in metabolism take place in the regenerating liver within a few hours after the operation [11]. A characteristic feature of this period of regeneration is activation of enzymes related to cell proliferation, whereas activity of enzymes maintaining the specific functions of the liver may be sharply depressed [6].

A reduction of 30% in the total content of nicotinamide coenzymes (NAD^+ + NADH and $NADP^+$ + NADPH) has been observed 24 h after partial hepatectomy [8], but these four dinucleotides have not been determined separately. Yet it may be expected that during regeneration both the total content and the ratio between oxidized and reduced nicotinamide coenzymes, reflecting the functional state of the tissue, will differ from the corresponding values in the intact liver.

The aim of this investigation was to study the time course of changes in the content of nicotinamide coenzymes in rat liver after partial hepatectomy in the initial period of regeneration.

EXPERIMENTAL METHOD

Noninbred male rats weighing 150-200 g were used. Partial hepatectomy was performed under ether anesthesia by the method of Higgins and Anderson, and the middle and left lateral lobes were removed. The content of nicotinamide coenzymes in the regenerating liver was studied 4, 18, 24, and 32 h after the operation. Lobes of the liver removed during partial hepatectomy served as the control. The content of reduced and oxidized nicotinamide coenzymes (NAD^+, NADH, $NADP^+$, and NADPH) was determined after preliminary freezing of the liver tissue with liquid nitrogen, followed by extraction by the method described previously [3], with certain modifications as follows. First, to extract reduced nicotinamide coenzymes an aqueous, and not an alcoholic, solution of 0.1 M Na_2CO_3 was used. Second, reduced nucleotides, extracted under the conditions specified previously [3], ensuring destruction of oxidized forms of the coenzymes, were determined after their spontaneous oxidation, which took place while alkaline extracts were kept in a refrigerator at 4°C. The criterion of completeness of oxidation of NADH and NADPH was absence of changes in optical density at 340 nm after addition of 0.1 ml of a solution of phenazine methosulfate (1 mg/ml) to a cuvette containing Tris-HCl buffer and 0.5 ml of the alkaline extract. Because of the low NADH content in the liver, its level was determined after spontaneous oxidation, by the method in [12]. Activity of NAD-kinase, catalyzing NADP synthesis from NAD and ATP, was determined in extracts obtained after homogenization of a weighed sample of liver with five volumes of 0.02 M solution of $KHCO_3$, pH 7.4, containing 1 mM EDTA, followed by centrifugation by the method described previously [1]. The numerical results were subjected to statistical analysis.

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TABLE 1. Content of Nicotinamide Coenzymes (in μg/g wet weight of tissue) in Liver of Intact Rats (M ± m)

Time of year	NAD^+	NADH	$NADP^+$	NADPH
February–March (23)	235,0±6,4	66,6±4,3	73,3±2,5	148,0±4,6
October–November (21)	261,2±5,5	43,7±2,6	77,4±2,5	136,6±5,3

Legend. Here and in Table 2, number of animals given in parentheses.

EXPERIMENTAL RESULTS

The total NAD(H) content was found to be 1.5 times higher (Table 1) than the corresponding value for NADP(H). The oxidized form of NAD was more abundant than the reduced form, whereas most NADP was in the reduced form. The results on the ratio between oxidized and reduced forms of the dinucleotides are in good agreement with information in the literature, but they differ somewhat from them quantitatively. We obtained higher values for $NADP^+$ and lower values for NADH and NADPH.

Considerable individual fluctuations were observed in the levels of nicotinamide coenzymes in the liver of the different animals, although in all cases the investigation was conducted on rats of the same sex and age (the animals were equal in weight). Significant seasonal changes were found in the NAD^+ ($P < 0.01$) and NADH ($P < 0.001$) contents in the rat liver studied at different times of the year (Table 1). The $NADP^+$ and NADPH levels showed smaller fluctuations: Differences between the corresponding values are not statistically significant.

These observations on considerable individual fluctuations in nicotinamide coenzyme levels in the liver are confirmed by data in the literature [4, 5, 7, 10, 12].

The content of nicotinamide coenzymes in the regenerating liver was determined 4, 18, 24, and 32 h after partial hepatectomy. Usually when metabolic disturbances in the regenerating liver are studied, the liver of animals undergoing mock operations is used as the control. Considering the possibility of considerable individual fluctuations in the dinucleotide content in the liver, we used as the control lobes of the liver removed during the operation, and we compared results obtained in the experiment with the control at the corresponding time.

No differences were found in the content of nicotinamide coenzymes in the control and regenerating lobes of the liver 4 h after the operation. The results (Table 2) show that no significant differences were present likewise 18 h after the operation. Significant differences in the content of all four nicotinamide coenzymes in the control and regenerating lobes of the liver did not appear until 24 h after hepatectomy. Meanwhile the ratio between the oxidized and reduced forms of NAD also changed. Whereas the NAD^+ level in the regenerating liver rose to 125.6% compared with the control, the NADH content fell sharply to only 42.2% (in both cases $P < 0.001$). The total NAD^+ + NADH content in the regenerating liver under these circumstances did not change significantly. By 32 h after the operation the NAD^+ content in the regenerating liver was close to normal again ($P > 0.05$), whereas the NADH level remained low ($P < 0.05$).

The reason for the changes discovered in the ratio of NAD^+ and NADH in the regenerating liver is not yet clear. Our data on the NAD^+ and NADH levels in the regenerating liver differ from those in the literature [8]. The authors cited in [8] observed the greatest fall (by 33%) in the total NAD^+ and NADH content 24 h after the operation, whereas at this time we found an increase in the content of oxidized and a decrease in the content of reduced NAD.

Our results of determination of the $NADP^+$ and NADPH levels 24 h after hepatectomy revealed a definite decrease in the content of both forms of the dinucleotide ($P < 0.01$) by contrast with the corresponding values for NAD^+ and NADH (Table 2). Ferris and Clark also observed the maximal decrease in the total content of $NADP^+$ + NADPH at this time [8]. The level of $NADP^+$ and NADPH in the regenerating liver remained lower than the control 32 h after the operation also ($P < 0.05$).

TABLE 2. Content of Nicotinamide Coenzymes (in μg/g wet weight of tissue) in Regenerating Rat Liver (M ± m)

Time after operation, h		NAD^+	NADH	$NADP^+$	NADPH	NAD^+ + NADH	$NADP^+$ + NADPH
18	Control (6)	222,3±19,6	46,1±4,6	70,6±4,5	153,4±18,7	268,4	224,0
	Experiment (6)	193,3±24,1	31,8±6,2	52,6±6,3	116,6±15,2	225,1	169,2
24	Control (16)	205,7±9,3	31,0±2,4	74,6±3,1	161,3±8,5	236,7	235,9
	Experiment (16)	258,5±6,9	13,2±0,4	51,5±2,2	116,4±11,9	271,7	167,9
32	Control (8)	236,9±13,7	46,0±3,9	70,7±3,4	142,1±6,7	282,9	212,8
	Experiment (8)	205,6±10,9	32,9±2,3	57,6±3,0	106,5±12,3	238,5	163,6

The fall in the NADP(H) level in the regenerating liver could be due to changes in activity of NAD-kinase, which is responsible for NAD synthesis, or of enzymes which catalyze the dinucleotide. Investigation of NAD-kinase activity in the liver of the hepatectomized rats 24 h after the operation revealed an increase in enzyme activity from 11.4 ± 0.9 to 16.4 ± 1.3 nanomoles/mg protein/h ($P < 0.01$). The results were confirmed by an investigation [2] which revealed considerable increase in NAD-kinase activity in the regenerating liver 48 h after the operation.

The absence of any direct relationship between the potential activity of NAD-kinase, measured *in vitro* 24 h after the operation, and the NADP(H) level in the liver, which was maximally depressed at this time, is noteworthy. It is evidently not the activity of this enzyme, but the availability of substrates, especially ATP, that is of decisive importance for NADP synthesis in liver cells *in vivo*. This suggestion is confirmed by the results of a study of the effect of ethionine on the nicotinamide coenzyme levels in rat liver [9]. Determination of the (NAD^+ + NADH) and ($NADP^+$ + NADPH) levels 5 h after administration of ethionine, lowering the ATP concentration in the liver tissue by 80%, revealed a very small decrease in the total NAD(H) content, whereas the NADP(H) level was depressed by 30-40%. NAD-kinase activity, determined in liver extracts in this case, was actually raised a little. The authors cited explained differences in the liver coenzyme levels after administration of ethionine on the grounds that K_m^{ATP} for NAD-pyrophosphorylase, synthesizing NAD from nicotinamide mononucleotide and ATP, is two orders of magnitude lower than K_m for NAD-kinase. The reason for the lowered $NADP^+$ + NADPH level in the regenerating liver may perhaps be the lesser accessibility of ADP for NADP synthesis, despite the increased NAD-kinase activity observed 24 h after the operation. This explanation is supported by our results obtained when injecting nicotinamide into hepatectomized rats. Intraperitoneal injection of nicotinamide (500 μg/kg) 18 h after the operation led to an increase in the NAD(H) content without any effect on the NADP(H) level.

As regards enzymes catalyzing NAD and NADP in the regenerating liver, preliminary results show no change in their activity, or even a small decrease, at the times of investigation.

Contrary to our expectations, during the first hours (4 and 18 h) after hepatectomy, no changes were thus found in the content of any of the four nicotinamide coenzymes; this can evidently be explained by the absence of any significant changes in the state of the enzyme systems responsible for synthesis and breakdown of these binucleotides in the regenerating rat liver at the above times.

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STRUCTURAL CHANGES IN ERYTHROCYTE MEMBRANES IN NEPHROPATHY

O. M. Panasenko, R. I. Shalina, and O. A. Azizova

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One of the main problems in modern scientific and practical obstetrics is the study of mechanisms of the pathogenesis of late toxemia of pregnancy (LTP). It is now known that nephropathy is accompanied as a rule by disturbance of the circulation [3]. This is manifested in particular by changes in the properties of the blood cells [3, 5, 6, 8]. One of the most important links in the chain of pathogenesis of this polyetiologic disease is disturbance of the respiratory function of the erythrocytes, specifically, depression of their ability to give up oxygen to the tissues [6, 8], with consequent hypoxia [3, 8]. With the development of LTP the lipid composition of the erythrocyte plasma membranes is also known to change: The content of phospholipids (PL) and cholesterol (Ch) increases, while at the same time the content of free fatty acids and of triglycerides decreases [13]. In nephropathy the aggregating power of the erythrocytes has been shown to be increased, whereas their electrophoretic mobility is reduced [5]. The causes of disturbance of erythrocyte function have not been established. It can be tentatively suggested that these disturbances are connected with structural modifications of erythrocyte plasma membranes.

The aim of this investigation was to study structural changes in the erythrocyte membrane of patients with nephropathy of different degrees of severity. The spin probe method, capable of determining precise changes in the structure of biomembranes [9], was used for this purpose.

EXPERIMENTAL METHOD

Erythrocytes from the blood of 15 healthy pregnant women and also of 39 women with a mild (20) and a severe (19) degree of nephropathy (according to the Wittlinger scale) were studied. Blood from nine healthy nonpregnant women was used as the control. Erythrocytes were isolated by triple centrifugation in NaCl solution (150 mM) containing 10 mM Tris-HCl, pH 7.3, for 10 min at 1000g. EPR spectra were recorded on an E-4 radiospectrometer (Varian, USA) at 37°C. The spin probes used were 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl (probe I, from Syva, USA), 2,2,6,6-tetramethyl-4-dodecyl-Δ^3-dihydropiperidine-1-oxyl (probe II, synthesized at the Research Institute for Biological Testing of Chemical Compounds), and 2,2,6,6-tetramethyl-4-(4-phenylbutinyl)-piperidine-1-oxyl (probe III, synthesized at the Institute of Chemical Physics, Academy of Sciences of the USSR). EPR spectra and structural formulas of the probes are given in Fig. 1. The probes were added to the erythrocyte suspension in the form of a solution in ethanol. The concentration of the probe in the specimen was 5×10^{-5} M, of ethanol 2%, and of erythrocytes 10^{10} cells/ml. To characterize behavior of the probes in the membrane the following parameters were used: the degree of order S, describing the mobility of the fatty-acid chains of PL, and the isotropic constant of hyperfine interaction a, from the values of which the parameter of hydrophobicity h was determined, giving an estimate of the degree of polarity of the environment of the nitroxyl fragment of the probe [9].

$$h = (a_w - a)/(a_w - a_o),$$

where a_w and a_o were determined for the probe in an aqueous solution of NaCl (150 mM) con-

Laboratory of Biophysical Principles of Membrane Pathology, Research Institute of Physicochemical Medicine, N. I. Pirogov Second Moscow Medical Institute. Department of Obstetrics and Gynecology, Pediatric Faculty, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 4, pp. 434-437, April, 1985. Original article submitted April 21, 1984.

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