Effect of an Induced Synthesis of Pyridine Nucleotides in Vivo on the Metabolism of Ribonucleic Acid

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SUMMARY

The effect of a nicotinamide-induced stimulation of DPN synthesis on the metabolism of ribonucleic acid (RNA) in the normal and hypertrophying rat kidney was studied. The behaviors of the nuclear and cytoplasmic RNA were compared.

The turnover of RNA, as well as the neosynthesis of RNA and proteins during renal hypertrophy, were reduced to an extent proportional to the increase in DPN synthesis. The inhibition of RNA metabolism began in the nucleus, and the labile RNA was probably involved. A hypothesis is discussed which relates the inhibition of RNA synthesis by nicotinamide to a competition resulting from the utilization of ATP both for DPN synthesis and for the formation of nucleoside triphosphates and of RNA in the cell nucleus. Thus, DPN biosynthesis and nucleo-cytoplasmic relationships appear to be important factors in growth regulation.

Materials and Methods

In total, 100 adult male rats, originated from repeated crosses between brothers and sisters of an already homogeneous Wistar strain, were used.

Action of nicotinamide on a compensatory renal hypertrophy of 26 hours and on the resting kidney.

—in the first three lots of eight rats each, two rats were used as controls, two received nicotinamide (two injections of 50 mg/100 gm body weight with a 12-hour interval [13]); four others underwent nephrectomy, and, of these, two were given two injections each of nicotinamide under the conditions indicated above. Two hours after the operation all the animals were given an intramuscular injection of P³¹ (300 μc/100 gm body weight) and were sacrificed after a further 24 hours.

Action of nicotinamide on hypertrophy of longer

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duration, either 4 or 5 days.—In five other groups of four rats each, all the animals underwent nephrectomy, but only two of each group received, twice a day until sacrifice, either 50 mg. (Exp. 1, 2, 3), or 65 mg. (Exp. 4, 5) of nicotinamide per 100 gm. body weight by intraperitoneal injection. The hypertrophied kidneys were compared with the kidneys removed during nephrectomy. For Experiment 1 the conditions were: age of the rat, 5.5 months, 4 days' hypertrophy; Experiment 2: age, 5.5 months, 5 days' hypertrophy; Experiment 3: age, 4 months, 5 days' hypertrophy; and Experiments 4 and 5: age, 6 months, 5 days' hypertrophy.

Comparative action of nicotinamide on the nuclear and cytoplasmic fractions of renal tissue after 18 hours.—In each of the remaining eight lots of ten rats, five animals received intraperitoneal injections of nicotinamide (50 mg/100 gm body weight) 18 hours before sacrifice (18), and five were used as controls. Injections of P32 (300 μc/100 gm body weight) were given either 90 minutes (two experiments), 7 hours (three experiments), or 18 hours (three experiments) before sacrifice. Preparation of nuclei was carried out according to Chauveau (8) by centrifugation of the fresh homogenized tissue in 2.2 M sucrose containing 0.002 M CaCl2 for 1 hour at 40,000 X g.

Nucleic acids and proteins were estimated according to the following procedure. On treatment of the homogenized tissue with cold 0.6 N perchloric acid, the acid-insoluble fraction was precipitated and then washed with cold water and ethanol. Lipides were extracted by boiling ethanol and ether, and the protein residue was dried. The dry residue was hydrolyzed with N/2 KOH at 37° C. (38). Deoxyribonucleic acid and proteins were precipitated from the hydrolysate with concentrated perchloric acid; the oligodeoxyribonucleotides were extracted with 5 per cent trichloroacetic acid at 90° C., and the deoxyribose was estimated according to Dische (7, 10). The acid-soluble fraction of the alkaline hydrolysate was neutralized with cold KOH and passed down a column of charcoal. This treatment separates non-nucleotidic phosphorus compounds from the ribonucleotides which remain adsorbed on the charcoal. The nucleotides are eluted with a mixture of ethanol, water, and ammonia (50/40/10) (v/v/v). Radioactivity measurements were carried out with solutions of infinite thickness in a Geiger-Müller counter (mica window 1.8 mg/sq cm). Phosphorus was determined by the method of Briggs (5) and ribose according to Mejbaum (25). When tissues of different rats are compared, we always refer to the relative specific activity of RNA—i.e., the ratio of the specific activity of the ribonucleic phosphorus to the specific activity of the acid-soluble PO4– (20).

DPN was assayed by the fluorometric method of Lowry (17), in which the DPN is converted to a fluorescent compound by treatment with strong alkali. By this procedure triphosphopyridine nucleotide (TPN) cannot be separated from DPN. Hence, the results reported in this paper include both DPN and TPN and are given as DPN. Reduced DPN and TPN were destroyed by acid and were not estimated under our conditions. In fact TPN represents about 10 per cent of the pyridine nucleotides which appear after nicotinamide injection, while the reduced forms of DPN and TPN are found in very small amounts (18). The UV-absorption at 260 μ of the acid-soluble fraction (E260) was also studied, according to Kaplan (13). In agreement with this author, we observed a rigorous proportionality between the increase of E260 and the amount of DPN synthesized (Chart 1).

RESULTS

Action of nicotinamide on the turnover of RNA in resting and hypertrophying kidney (Chart 2).—The incorporation of P32 into the RNA of kidney homogenates 24 hours after the first nicotinamide injection was decreased by about 25 per cent. Moreover, although in the hypertrophying kidney of a normal animal the turnover of ribonucleic...
phosphorus increased during the first 24 hours following nephrectomy, when the animal received nicotinamide the increase no longer appeared. However, in these animals nephrectomy also stimulated the RNA turnover, as can be seen if one compares in Chart 2 the relative specific activities of RNA in the hypertrophying kidney and in the normal kidney of the nicotinamide-injected rat (respectively, \( h_n \) and \( n \)). No significant variation in the amount of RNA was observed within these 24 hours of the experiment.

**Action of nicotinamide on the neosynthesis of RNA during renal hypertrophy.**—The increase of the absolute quantity of RNA of the hypertrophied kidney, 5 days after nephrectomy, in animals with and without nicotinamide injections in comparison to the kidney removed during the operation is presented in Chart 5. The increase in weight as well as the increase in protein and RNA contents observed in the hypertrophied kidney (column \( H \)) was inhibited nearly 80 per cent by nicotinamide (column \( HN \)). The injection of the DPN precursor caused a twofold increase of the acid-soluble nucleotides and a sevenfold increase of DPN content. It is important to note that in the hypertrophied kidneys of the normal animals \( (H) \) there was also an increase in DPN which was the result of the general enlargement of the renal cytoplasmic mass during the hypertrophy. This augmentation no longer appeared if results were expressed per gram of tissue. It should also be noted that, during the first weeks of the compensatory hypertrophy, the DNA did not vary, and the number of cells per unit weight remained constant (18).

By varying the experimental conditions as described in the methods, we were able to modify the level of the inhibition of RNA formation by nicotinamide. Results of five experiments are shown in Chart 4. When young rats were used and 500 mg nicotinamide/kg was injected, the ratio of RNA formation to that of DPN was 1:2.5 (Exp. 3). When the hypertrophy was reduced to 4 days or when 650 mg nicotinamide/kg was injected, this ratio was close to 1:80 (Exps. 1, 4, 5). The linear relationship obtained shows that the inhibition of RNA synthesis was proportional to the extent of DPN synthesis after nicotinamide injection.

Nicotinamide administration for 5 days failed to reduce the RNA content of the resting renal tissue.

**Action of nicotinamide on the nuclear RNA fraction.**—In Table 1 is shown the incorporation of
Our results show clearly that an increased synthesis of DPN, after 24 hours, considerably inhibited the incorporation of P32 into the RNA of the resting kidney. Simultaneous renal hypertrophy and nicotinamide injection showed a summation of their respective effects on RNA turnover. This would point to an equilibrium between RNA and DPN formation. Furthermore, nicotinamide not only modified the incorporation of P32 into RNA but actually inhibited the RNA and the protein neosynthesis which normally occur after a few days of renal hypertrophy. The extent of this inhibition by the DPN precursor was dependent upon the experimental conditions (age of the rats, duration of hypertrophy, dose of nicotinamide). Why in this last experiment, when the total homogenate was examined, a difference of —17 per cent between nicotinamide-injected and normal rats was observed; an important part of the radioactivity at this time belonged to the nuclear RNA, and the large decrease of nuclear radioactivity was seen also to a lesser extent in the homogenate.

**DISCUSSION**

The use of compensatory renal hypertrophy (18, 23) allows the comparison of a nonproliferating adult organ with the same tissue resuming its growth under the action of a known physiological phenomenon.

It must be recalled that the increase in the cellular DPN, observed after parenteral administration of nicotinamide in the rat, represents a net synthesis of DPN. An inhibition by nicotinamide of the DPNase activity of the cell can in no respect account for this phenomenon, according to Kaplan et al. (13).
tinamide injected), but we always observed a strict inverse proportionality between the rate of RNA synthesis and the amount of DPN formed. Thus, the greater the RNA production, the smaller the induced DPN synthesis and vice versa. According to our data, which agree with those of De Burgh (6), RNA metabolism and DPN metabolism seem to compete in both resting and growing tissues.

In an attempt to localize this relationship in the cell, we found that 13 hours after nicotinamide injection the specific activity of nuclear RNA was already diminished about 20 per cent (when the P^32 was given several hours before death), whereas under the same condition there was no variation in the turnover of the whole cellular RNA. Since a considerable inhibition of the renewal of the cellular RNA by nicotinamide occurred after 24 hours, the question of the influence of nuclear RNA on the synthesis of cytoplasmic RNA is raised. Numerous biochemical approaches to this problem have been attempted, particularly with radioactive substances. Although a more rapid incorporation of isotope into the nuclear RNA was observed (39), much information refuting the concept of a precursor role of this RNA toward cytoplasmic RNA is gathered (1, 9, 37). In our experiments the successive inhibitions of RNA turnover first in the nucleus and afterward in the cytoplasm show clearly that we are dealing with a biochemical process acting on the metabolism of nuclear RNA, which, shortly afterwards, also affects the cytoplasmic RNA. Many recent data point toward an actual influence of some species of RNA of the nucleus on the cytoplasm (27, 29); it appears that our experiments in vivo support this hypothesis. In this manner a part of the nuclear RNA can be assimilated to a messenger RNA (4). Actually, when the P^32 was injected a short time (90 minutes) before the animal was killed, the inhibition of the turnover of the nuclear RNA was 2.5 times greater (Table 1). Therefore, it seems probable that the labile RNA (4) is directly involved in the DPN competition.

The localization in the nucleus of the RNA-DPN correlation which was observed indicates that the mechanism involved affects the biosynthesis of these compounds, since the nucleus is the site of pyridine nucleotides synthesis (12). There may exist an equilibrium based on the competitive utilization of the adenosine compounds for the formation of both RNA and DPN. Shuster et al. (35) showed that the administration of nicotinamide provokes an increase in the incorporation of formate-C^14 in the adenine compounds of the acid-soluble nucleotides, in DPN, and in RNA, and causes an increased quantity of AMP to appear in the medium. These facts confirm the existence of a common precursor for DPN and RNA. It must be emphasized that these data do not contradict ours: they concern a stimulation in the synthesis of the adenylc precursors of DPN, a reflection of which is also found in RNA, but they do not involve a net synthesis of RNA. In fact, it is now established that the requisite adenylc precursor for both RNA and DPN is adenosine triphosphate (16, 22). Therefore, the equilibrium which has been observed would rest on a competition for ATP between the nicotinamide mononucleotide and a nuclear RNA. Since, in the nucleus, the pool of ATP is relatively small (36) and the metabolism of RNA active, our interpretation might explain the facts.

According to our hypothesis, the RNA-DPN relationship will depend chiefly on the metabolic pathway producing ATP from AMP, and a feedback mechanism may occur, ATP being necessary for the synthesis and turnover of DPN and the presence of DPN ensuring the oxidative phenomena (28). The study of the relations between the energy metabolism of the cell and the RNA-DPN equilibrium may furnish a direct demonstration. Nevertheless, an indirect support for this opinion is already given by the fact that nicotinamide administration for 5 days does not reduce the quantity of renal RNA, despite the inhibition of turnover of RNA produced during the first 24 hours. The newly formed DPN would enhance the biosynthesis of ATP, and the cell could then re-establish the normal RNA metabolism. However, in a proliferating tissue—e.g., a hypertrophying kidney, in which the amount of ATP utilized for RNA synthesis is greater—this feedback mechanism does not take place.

It must be recalled that, in connection with cancer problems, mechanisms of growth regulation based on DPN metabolism (26) as well as on other coenzymes such as flavine adenine dinucleotide (28, 32) have been previously proposed. As for us, we have described (31) the effect of nicotinamide injections on the RNA metabolism of two types of hepatoma. According to our views, the important decrease in DPN metabolism observed in rapidly proliferating tissues would account for the enhanced production of RNA, since in these tissues ATP would no longer be used for the synthesis which normally ensure cellular differentiation (30). These mechanisms may therefore explain some differences between normal and pathological growth. Attempts to attribute the decrease of DPN synthesis in tumors to an increase of the
ATPase activity (14) contradict the in vivo observations that the ATP level is increased in the cancerous tissue (11, 34).  

Finally, it should be emphasized that an important growth factor such as thyroxine influences the RNA-DPN equilibrium. The thyroid hormone on the one hand inhibits the DPN synthesis which occurs after nicotinamide injection (2) and on the other hand intensifies the turnover and the net synthesis of RNA and causes an actual renal hypertrophy (21).

Thus, in conclusion, DPN biosynthesis appears as an important factor distinguishing adult differentiated tissues from rapidly proliferating tissues.

REFERENCES


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