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Influence of nicotinic acid and nicotinamide on diphosphopyridine nucleotide (DPN) synthesis in ascites tumor cells*

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IN THE biosynthesis of DPN from nicotinic acid (NA) and nicotinamide (NAM), desamido-DPN was identified as the common precursor in human erythrocytes and mouse liver tissue.[1, 2] Comparing the relative abilities of both types of cells to synthesize DPN from the respective vitamins, however, it is apparent that erythrocytes depend almost totally on NA and cannot utilize NAM to any appreciable extent, while liver tissue shows a preference for NAM as a DPN-precursor.

Roitt[3] demonstrated that alkylating agents of the ethyleneimine type inhibit glycolysis in tumor cells by interference with the synthesis of DPN. The decrease in the intra-cellular concentration of DPN associated with the action of the alkylating agent could be prevented when NAM was added to the incubation medium;[4] NA was ineffective in this respect. Concluding from these experiments that tumor cells may depend on NAM rather than NA for DPN-synthesis, it was desirable to investigate the specific utilization of these vitamins by a tumor and to compare the results with the biochemical transformations observed in a non-neoplastic tissue of the same animal.

From a group of 12 white Swiss–Webster mice (weight 50–60 g) with Ehrlich ascites tumor, 4 animals were injected intramuscularly with NA, 170 mg/kg, 4 received NAM in equal doses, and the remainder served as controls. Ten hours after the injections, concentrations of oxidized DPN in liver and tumor homogenates were estimated spectrophotometrically, using crystalline yeast alcohol dehydrogenase according to the method of Holzer.[5]

The results (see Table 1) indicate that the capacity of the tumor cells for DPN-synthesis from either NA or NAM is only about one-tenth that of liver tissue of the same animal. Comparing the specific utilization of the precursors for the synthesis of DPN, NAM was approximately four times as effective as NA in both liver and tumor, suggesting a similar biochemical mechanism for DPN-synthesis in these tissues. The limited response of the tumor cell is not related to insufficient transfer of the precursors into the ascitic fluid. No significant different in DPN levels was observed when NAM and NA were injected intraperitoneally instead of intramuscularly (unpublished data). The possibility that the tumor cell has a poor capacity to incorporate the precursors can be disregarded, since Holzer and Boltze[6] using ^{14}C-labeled NAM and NA showed that both vitamins penetrate freely into ascites tumor cells. Further investigations are needed to determine whether the same intermediates and enzymic sequences occur in this tumor.

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TABLE 1. DPN-CONCENTRATIONS IN ASCITES TUMOR CELLS AND LIVER TISSUE OF MICE 10 HR AFTER INTRAMUSCULAR ADMINISTRATION OF NICOTINAMIDE AND NICOTINIC ACID (170 MG/KG)

	No. of animal	Ascites tumor cells (μg of DPN/ml of packed cells)	Liver tissue (μg of DPN/g of wet weight)
A. Control animals	1	208	193
	2	194	263
	3	194	232
	4	197	215
	Mean	198	225
	S.D.	±7	±30
B. Nicotinamide	5	317	1451
	6	—	1351
	7	285	1260
	8	290	1520
	Mean	297 (+50%)	1395 (+521%)
	S.D.	±17	±114
C. Nicotinic acid	9	227	430
	10	230	530
	11	—	550
	12	235	655
	Mean	230 (+16%)	541 (+141%)
	S.D.	±4	±92

Two samples were lost by overalkalization. *t*-Test comparisons are significant at or beyond the 0·01 level of probability for B against A, C against A, and B against C, with respect to both ascites tumor cells and liver tissue. Figures in parentheses represent average increases over the control values.

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Separation of thymidine and its mono-, di- and tri-phosphate by paper chromatography

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THYMIDINE (TDR) and its various 5′-phosphorylated derivatives (thymidylic acid (TMP), thymidine diphosphate (TDP), and thymidine triphosphate (TTP)) have been separated readily from each other by column chromatography;[1, 2] however, it has been difficult to obtain clean separations using paper chromatography.[3] Although TDR and TMP were easily separable on paper, TDP and TTP, under the same conditions, appreciably overlapped, and the results could be interpreted only in a semi-quantitative manner.[3] Since paper chromatography often permits the separation of micro-quantities of materials, and is very convenient when dealing with a large number of samples, such a method for the efficient separation of TDR, TMP, TDP and TTP was sought. The present report describes a method, using Whatman cellulose paper AE30, which effectively separates the four compounds.