Synthesis of Diphosphopyridine Nucleotide by the Livers of Mice Bearing Transplantable Tumors.* (22618)

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It was shown(2) that a single injection of acetylpodophyllotoxin-ω-pyridinium chloride into mice bearing Sarcoma 37 induced marked damage in tumors, and that the capacity of the damaged tumors to synthesize diphosphopyridine nucleotide (DPN) from nicotinamide mononucleotide (NMN) was markedly reduced. The livers from these animals, however, exhibited an increased ability to synthesize DPN. When the tumor tissue had only 40% of the activity of control tumors, the livers from the treated mice had an activity of about 130% of the values of the livers of untreated tumor-bearing animals. It should be noted, however, that this capacity to synthesize DPN is still less than that of livers from non-tumor-bearing, untreated animals; in untreated mice the presence of week-old Sarcoma 37 lowered ability to synthesize DPN by about 50%. The synthetic activity with respect to DPN in livers from tumor-bearing mice decreased(2) rapidly to about the fourth day after tumor transplantation; by the sixth day about half of the activity had disappeared. These studies have now been extended to other tumors and tissues.

The present report deals with the DPN-synthetic capacity of livers from animals bearing a variety of transplanted tumors and the extent to which removal of tumor restores the DPN-synthetic function.

Methods and materials. In the first experiment a group of CAF_1 mice [(BALB/c x A)F_1] was inoculated in the tail with implants of Sarcoma 37. The tumor grew slowly; 10 days after implantation, it was approximately 0.6 cm in diameter. Sarcoma 37, implanted in the skin of CAF_1 mice, grew to a diameter of approximately 0.6 cm in 6 days. Lymphoma 1 and Lymphoma 2 also were implanted subcutaneously in CAF_1 mice. Leukemia 1210 was implanted subcutaneously in CDBA hybrids [(BALB/c x DBA/2)F_1] while [(C_3H x $C_{57}BL/6$)F_1] mice were employed for subcutaneous transplantation of Hepatoma 129. The ascitic form of Leukemia L-4946 was implanted intraperitoneally in (C_3H x AKR)F_1 hybrid mice.§ The rate of synthesis of DPN by liver and tumor homogenates was followed as described previously(2). Blood, drawn directly from the heart and homogenized with distilled water for enzyme determinations, from both tumor-bearing and control animals, was examined in the same way.

Results. Since the livers from mice bearing Sarcoma 37 showed(2) a decreased ability to synthesize DPN from NMN, tumors in the tail were removed, surgically, by cut-

TABLE I. Synthesis of DPN by Livers from Mice Bearing 10-Day-Old Sarcoma 37 in the Tail.

	DPN formed/mg liver N/hr		
	Control mice (no tumor), μmoles	Mice bearing tumor, μmoles	Relative activity, %
♂	.46	.25	54
♀	.46	.31	67
	48 hr after removal of tumor by caudectomy on 10th day		
♂	.49	.46	94
♀	.46	.46	100

Tumor approximately 0.6 cm wide.
Each value is the mean of at least 4 separate livers.

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§ This tumor arose in Dr. Law's laboratory on 11/27/51 in an AKR female mouse from transplanted thymic tissue. It has been carried as a solid tumor for several generations by Dr. Law, and also has been carried in the ascites form for 90 generations.

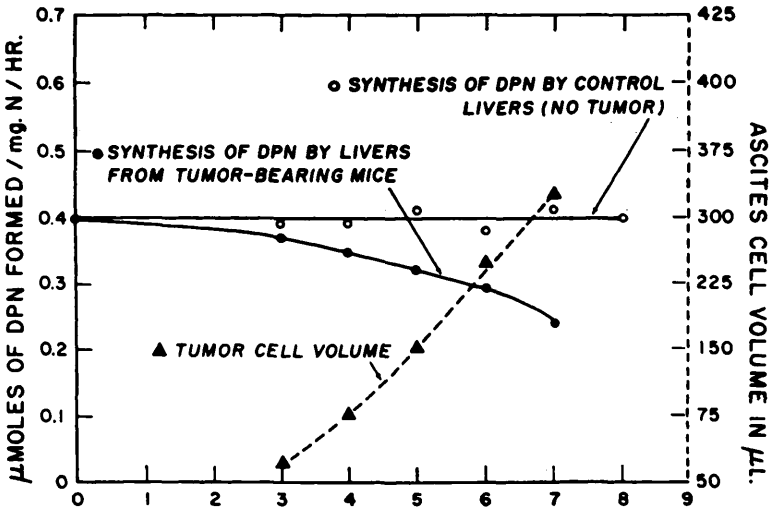

FIG. 1. Synthesis of DPN by livers from mice bearing lymphocytic leukemia L-4946 in the ascites form.

TABLE II. Synthesis of DPN by Livers from Mice Bearing 6-Day-Old Sarcoma 37 in the Skin

	DPN formed/mg liver N/hr		
	Control mice (no tumor), μmoles	Mice bearing tumor, μmoles	Relative activity, %
♂	.48	.31	65
♀	.49	.32	65
	2 days after sham operation	2 days after removal of tumor	
♂	.49	.48	98
♀	.50	.49	98

Tumor approximately 0.6 cm wide.
Each value is the mean of at least 3 separate livers.

ting off the tails to determine whether there would be restoration of the capacity of the livers to synthesize DPN. The findings are summarized in the first 2 tables.

Table I shows that the synthesis of DPN by livers from mice bearing 10-day-old sarcoma 37 in tail was about 60% of the value given by livers from control mice. In all these experiments, at least 4 animals were employed in each group; the results obtained were almost identical within each group and hence only the average values are given. About 48 hours after removal of the tumor on 10th day, the enzyme activity of the livers returned to normal.

The results with livers from mice bearing intradermal tumors are listed in Table II. Sarcoma 37 was implanted in the skin in such a way that, after 6 days, it was banana-shaped, and approximately 0.6 cm wide. Here, too, only 65% of the activity of control livers was obtained. From another group, tumor tissue was removed surgically‖ on the 6th day as completely as possible (nembutal anesthesia, 80 μg/g), the skin sewed, and 600 units of penicillin administered to minimize infection. Two days later the animals were sacrificed for determination of DPN-synthesizing capacity of their livers. It was found that, after removal of tumor, the enzyme activity of the livers returned to normal. Sham operation in control animals had no effect on the synthesis of DPN by their livers. These experiments were repeated at least 3 times each, with excellent reproducibility; hence only the mean values are given in Table II.

Shacter reported(3) that the glutathione level in livers from mice bearing lymphocytic leukemia L-4946 decreased to about 70% of the levels in control livers. Accordingly, biosynthesis of DPN by the livers from mice bearing this tumor in ascites form was next investigated. Fig. 1 shows the synthesis of DPN by the livers of mice bearing lymphocytic leukemia L-4946 ascites with relation to the volume of tumor cells. As the total volume of the tumor cells‖ increased, the synthe-

‖ By Betty Achinstein of this laboratory.

TABLE III. Synthesis of DPN by Homogenates of Tumor and of Liver.

Tissues*	Control mice (no tumor) Synthesis of DPN,† μmoles	Tumor-bearing mice Synthesis of DPN,† μmoles	Tumor-bearing mice Age of tumors, days
Sarcoma 37	—	.18	6
Liver	.48	.25	
Lymphoma 1	—	.19	15
Liver	.48	.27	
Lymphoma 2	—	.24	15
Liver	.49	.34	
Hepatoma 129	—	.25	15
Liver	.42	.28	
Leukemia 1210	—	.30	6
Liver	.49	.35	
Leukemia L 4946 ascites	—	.16	7
Liver	.41	.24	

* CAF_1 [(BALB/c × A) F_1] mice were employed in groups for control and bearing Sarcoma 37, Lymphoma 1, and Lymphoma 2. CDBA hybrids [(BALB/c × $DBA/_2$)F_1] were used for Leukemia 1210; [C_3H × $C_{57}BL/6)F_1$] for Hepatoma 129; and C_3H × AKR F_1 hybrids for Leukemia L 4946.
† Expressed as μmoles of DPN formed/mg N/hr.

sis of DPN by the livers of the animals decreased; 7 days after transplant, the volume of packed tumor cells had increased to 340 μl while the capacity of the livers from these mice to synthesize DPN had decreased from 0.40 to 0.24 μmoles DPN formed, *i.e.*, a drop of 40%.

Table III summarizes the results on synthesis of DPN by livers and tumors from mice bearing Lymphoma 1, Lymphoma 2, Hepatoma 129 and Leukemia 1210. In all these cases, the livers from tumor-bearing mice showed a 30 to 50% lowered capacity to synthesize DPN than livers from control mice. The values on the synthesis of DPN by the tumors such as Leukemia L 4946, Sarcoma 37, Lymphoma 1, Lymphoma 2, Hepatoma 129 and Leukemia 1210 were from 0.16 to 0.30 μmoles DPN formed. In most of the tumors the DPN synthesizing activity was 70% of that of the livers from mice bearing these tumors. The tumors from mice bearing Leukemia 1210 or Hepatoma 129 showed an activity of 86 and 89% of the activity of livers from these animals.

Fig. 2 summarizes capacity of the blood from mice bearing intramuscular Sarcoma 37 to synthesize DPN. Within 24 hours after tumor transplantation, enzyme activity of whole blood from mice receiving the implant dropped to about 50% of the activity of blood from control mice, and remained at this level.

Discussion. The data presented here extend our previous work(2) on synthesis of DPN by the livers from mice bearing Sarcoma 37. It was found that the livers from mice bearing various transplantable tumors, such as a Lymphoma, Lymphocytic leukemia or a Hepatoma, also showed this lowered capacity to synthesize DPN. With ascites tumor also, as the tumor cell volume increased the capacity of the liver to synthesize DPN decreased. Surgical removal of the tumor was followed by return of DPN synthetic capacity of the liver to normal. DPN synthesis by blood dropped sharply within 1 day after inoculation of tumor into mice. Preliminary experiments on injection of normal tissues, such as liver, kidney, spleen or heart, into

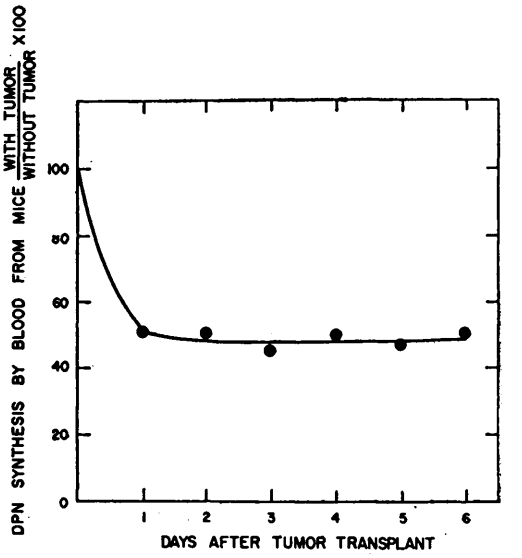

FIG. 2. Synthesis of DPN by blood from mice bearing Sarcoma 37. Range of control values: .123-.129 μmoles of DPN formed per mg N per hr. The data are given in terms of percentages of the control values, *i.e.*,

$$\frac{\text{DPN synthesis by blood from tumor-bearers}}{\text{DPN synthesis by blood from control mice}} \times 100.$$

‖ We are indebted to Dr. Shacter for furnishing the homogenates of livers from mice bearing L-4946 in the ascites form and also for providing information on the cell volume of the tumor.

mice failed to produce inhibitory effect on DPN-synthesizing enzyme of the blood of these animals. Information is also being gathered on the effect of transplanted tumors on DPN synthesis by tissues other than liver or blood. Growing tumors may exert their influence on DPN synthesis by systemic metabolic competition with other tissues. On the other hand, the thesis that the tumor contains an inhibitor which is transmitted through the blood and which blocks its synthetic capacity to form DPN is also being explored. Search is being made for a humoral factor either in tumor tissue or in tissues from tumor-bearing hosts which may inhibit the synthesis of DPN from NMN.

Summary. 1. Homogenates of liver from mice bearing transplanted tumors (Sarcoma 37, Lymphoma 1, Lymphoma 2, Leukemia L 1210, Hepatoma 129 and Leukemia L-4946) exhibited a drop of 30 to 50% in capacity to synthesize DPN. 2. Surgical removal of Sarcoma 37 restored the DPN-synthesizing capacity of the livers to the level of livers from control mice. 3. Within one day after inoculation of tumor into mice, ability of the blood to synthesize DPN dropped about 50%.

1. Waravdekar, V. S., Powers, O., and Leiter, J., *Proc. Am. Assn. Cancer Res.*, 1956, v2, 155.
2. ———, *J. Nat. Cancer Inst.*, 1956, v17, 145.
3. Shacter, B., *Proc. Am. Cancer Res.*, 1956, v2, 146.

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Proteolytic Enzymes. III. Circulating Esterase Activities after Parenteral Trypsin and Chymotrypsin in Rabbits.* (22619)

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The hydrolysis of *p*-toluenesulfonyl-L-arginine methyl ester (TAMe) by trypsin, and of acetyl-L-tyrosine ethyl ester (ATEE) by chymotrypsin was first reported in 1948(1,2). The method used, based on titration of acid liberated by hydrolysis of the ester, was rapid, relatively accurate, and required low enzyme concentrations. We were interested in measuring circulating enzyme activity after parenteral administration of trypsin and chymotrypsin and chose the esterase method because of these advantages.

Materials and methods. Crystalline preparations of trypsin (Armour D-531-157) and chymotrypsin (Armour D-530-165) were employed. The respective hemoglobin activities (3) of these lyophilized preparations were 1.47 and 0.78 μg tyrosine μg/minute. Rabbit plasma was prepared by mixing 9 parts of blood with 1 part of 3.2% sodium citrate, followed by centrifugation. Imidazole buffer was prepared by adding 1.75 g imidazole to 90 ml of 0.1 *N* HCl. The pH was adjusted to 7.4 and the volume brought to 100 ml with distilled water. This solution was diluted 1:10 with saline for use. TAMe, the substrate for trypsin, was present in the final reaction mixture at a concentration of 0.02 M. For chymotrypsin, ATEE was used in a concentration of 0.006 M.

Esterase determinations were carried out separately at 37°C in a small temperature-controlled water bath. Reaction volumes were constant at 4.0 ml, and mixing was continuous with a magnetic stirrer. All determinations were run at pH 7.4. One ml imidazole buffer was added to the reaction mixture except when plasma was present. Saline was used as the diluent for all reagents. Rate of substrate hydrolysis was measured by determining time required at pH 7.4 for liberation of an amount of acid neutralized by 0.01 ml of 0.1 *N* NaOH.

Results. In vitro studies. In the absence of plasma, reaction velocity was linearly re-

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