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ACTION OF PODOPHYLLIC ACID ON MALIGNANT TUMORS—II.

EFFECTS OF PODOPHYLLIC ACID ETHYL HYDRAZIDE ON THE INCORPORATION OF PRECURSORS INTO THE NUCLEIC ACIDS OF MOUSE MAMMARY TUMORS AND LIVERS *IN VIVO*

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Abstract—Administration of podophyllic acid ethyl hydrazide to mice bearing spontaneous mammary tumors, suppresses the incorporation of radioactive precursors into the nucleic acids of the neoplastic tissue. This suppression was also observed in the case of liver nucleic acids but with exceptions in a number of the precursors employed.

MOST of the cytostatic agents employed in cancer chemotherapy have either been proved to affect some phase of nucleic acid metabolism in the malignant tissues, or else there is circumstantial evidence pointing to that effect.[1, 2] The well known mitotic inhibitor colchicine has also been shown to suppress DNA synthesis.[3] The ethyl hydrazide of podophyllic acid, a metaphase-arresting agent that has found use in the chemotherapy of solid tumors, was shown to inhibit protein synthesis both in transplanted tumors as well as in liver of rats. Altaner *et al*, who performed this work, presume that this effect on protein synthesis is a secondary reflection of a primary disorder in RNA synthesis.[4]

In this paper evidence is presented that podophyllic acid ethyl hydrazide inhibits the incorporation of radioactive precursors into the nucleic acids of mammary tumors and, to a lesser extent, livers of mice.

METHODS

RIII/HeSy mice with spontaneous mammary tumors were used throughout this work. Groups of 3 animals were given three times at 24-hr intervals i.p injections of 100γ/g body wt. podophyllic acid ethyl hydrazide (SPI Sandoz), a generous gift of Sandoz, A. G., Basel, Switzerland, diluted by means of isotonic NaCl solution. Four hr after the last administration, the treated animals as well as 3 controls received i.p. 10 μc of the ^{14}C-labeled compounds or 30 μC ^{32}P-labeled phosphate, or 100 μC ^{3}H-labeled guanosine. The animals were sacrificed by ether 2 hr later and the tumours as well as the livers were excised and placed in chilled isotonic NaCl solution. Each precursor was studied with a single batch of pooled tissue.

The nucleic acids were isolated as follows: The tissues were homogenized in a Virtis '23' Homogenizer (Arthur H. Thomas Co. Philadelphia Pa. U.S.A.) for 3 min in 0·9% NaCl solution. The homogenate was centrifuged at 5000 *g* for 30 min. DNA was extracted from the sediment according to the procedure of Kay *et al.*[5] RNA

was prepared by extracting the 5000 *g* supernatant solution with phenol and precipitating the aqueous phase with ethanol. The RNA preparation was further treated with pancreatic DNase I (purchased from Worthington, U.S.A.). Acid hydrolysis of the nucleic acids with perchloric acid and separation of the bases by means of paper chromatography in isopropanol-HCl, were carried out as described by Wyatt.[6]

All radioactive precursors were purchased from Amersham, Bucks, England.

Radioactivity of ^{14}C and ^{32}P-labeled preparations was measured in a SELO low background flow counter, while of the ^{3}H-labeled substances the measurements were made in a SELO liquid scintillation counter.

RESULTS

The incorporation of radioactive precursors into the nucleic acids of tumor-bearing RIII mice is illustrated in Figs. 1 and 2. Table 1 shows the labeling of bases when the respective nucleosides were administered to the animals. In all instances of nucleic acids isolated from the malignant tissue there is a clear inhibition of the incorporation of precursors in the SPI-treated animals. This inhibition is not so apparent with the liver nucleic acids.

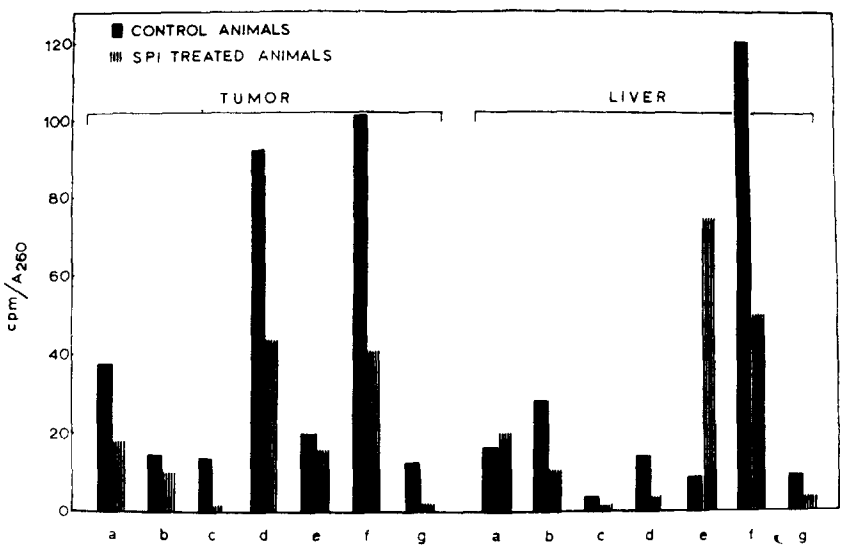

FIG. 1. Incorporation of nucleic acid precursors into the DNA of mammary tumors and livers of RIII mice treated with podophyllic acid ethyl hydrazide. The specific radioactivity (cpm/A260) of the DNA preparations was determined after administration of the following precursors; a, adenine-8-^{14}C; b, adenosine-8-^{14}C; c, uridine-6-^{14}C; d, thymidine-6-^{14}C; e, guanosine-^{3}H; f, cytidine-8-^{14}C; g, ^{32}P orthophosphate. The value for thymidine should be multiplied by a factor of 10.

DISCUSSION

The results presented in this communication show that incorporation of precursors into the nucleic acids of mouse mammary tumors is inhibited in animals receiving podophyllic acid ethyl hydrazide. All seven precursors administered were incorporated to a lesser extent into the SPI-treated animals than to controls. No replicates were performed for each precursor separately. However, this lack of replicates is safely

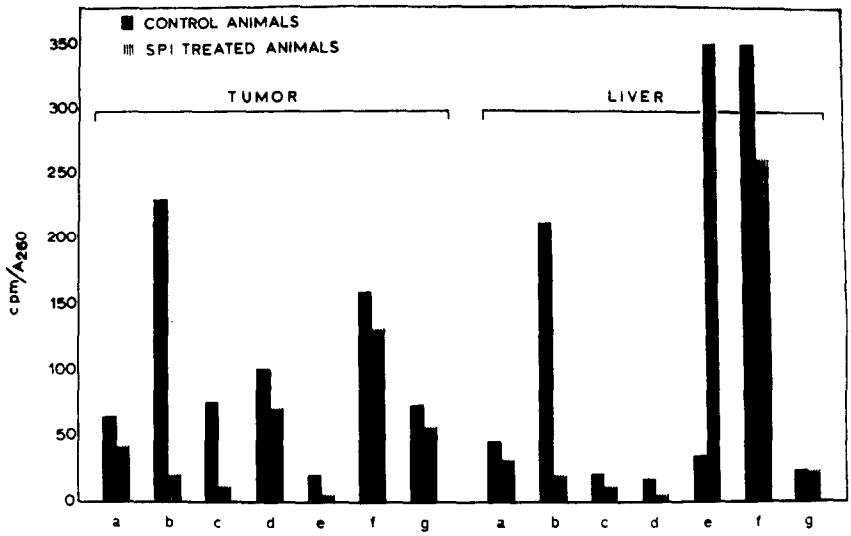

FIG. 2. Incorporation of nucleic acid precursors into the RNA of mammary tumors and livers of RIII mice treated with podophyllic acid ethyl hydrazide. The specific radioactivity (cpm/A 260) of the RNA preparations was determined after administration of the following precursors: a, adenine-8-^{14}C; b, adenosine-8-^{14}C; c, uridine-6-^{14}C; d, thymidine-6-^{14}C; e, guanosine-^{3}H; f, cytidine-8-^{14}C; g, ^{32}P orthophosphate.

TABLE 1. INCORPORATION OF RADIOACTIVITY INTO THE BASES OF NUCLEIC ACIDS OF MOUSE MAMMARY TUMORS AND LIVERS WHEN THE RESPECTIVE ^{14}C-LABELED NUCLEOSIDES WERE ADMINISTERED TO CONTROL MICE AS WELL AS MICE TREATED WITH PODOPHYLLIC ACID ETHYL HYDRAZIDE

Incorporation was calculated as cpm/mmole of base. The numbers denote the per cent labeling of the bases in SPI-treated animals as compared to the respective controls.

	Adenine	Thymine	Guanine	Cytosine	Uracil
Tumor DNA	31	21	8	46	11*
Liver DNA	31	118	105	31	68*
Tumor RNA	34	74†	18	63	6
Liver RNA	25	110†	250	56	37

* Measured as thymine.
† Measured as uracil.

offset by the use of various precursors. The results of Figs. 1 and 2 and of Table 1 do not completely coincide. The differences are mainly due to the interconversion of bases prior to the incorporation of the triphosphates into the nucleic acids, so that the label is isolated not only in the base administered, but also in other bases. The proportion of interconversion varies of course from base to base. This fact has been studied in several instances in the course of this work, but is not reported herein since it is a well known and often reported phenomenon. Still, no matter which set of results one examines the antimitotic agent suppresses the incorporation of precursors into the nucleic acids of the malignant tumors. This suppression is generally less pronounced in the case of liver nucleic acids.

The inhibitory effect of podophyllic acid ethyl hydrazide on nucleic acid synthesis is similar to the effect reported for colchicine, by Ilan and Quastel (1966), who suggest that colchicine may inhibit DNA and, to a lesser extent, RNA synthesis by combining with the DNA template. They base this claim mainly on two lines of evidence: (1) The incorporation of adenine-8-^{14}C into the DNA of Ehrlich ascites carcinoma cells is fully protected from the inhibitory effect of colchicine when thymus DNA is incubated with the culture, and (2) the optical rotation of a mixture of DNA and colchicine is significantly different than the sum of the rotations of the two substances taken separately.

In the preceding paper we reported the isolation of radioactive DNA when tritium-labeled podophyllic acid ethyl hydrazide was administered to the animals. However, it was pointed out that the specific radioactivity of the DNA declined with the frequency that the preparation was precipitated by means of ethanol and washed with acetone, indicating that the interaction of DNA with the podophyllotoxin derivative could not have been very strong. Still, in view of the present results even this weak binding may be of biological significance.

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