Biochemical Pharmacology, Vol. 17, pp. 1485–1488. Pergamon Press. 1968. Printed in Great Britain

ACTION OF PODOPHYLLIC ACID ON MALIGNANT TUMORS—I.
DISTRIBUTION OF TRITIATED PODOPHYLLIC ACID ETHYL HYDRAZIDE IN SUBCELLULAR FRACTIONS OF MOUSE MAMMARY TUMORS

JOHN G. GEORGATSOS, T. KAREMFYLLIS and A. SYMEONIDIS

Laboratory of Biochemistry, Theagenion Cancer Institute, Thessaloniki, Greece

(*Received* 26 *January* 1968; *accepted* 13 *March* 1968)

Abstract—The intracellular distribution of radioactivity in mammary tumors has been studied following administration of tritiated podophyllic acid ethyl hydrazide to tumor-bearing R III mice. Over 90 per cent of the label is found in the 100,000 *g* supernatant, two thirds of which is associated with two protein fractions clearly separable from one another by means of gel filtration through Sephadex G-100.

THE METAPHASE arresting compound podophyllotoxin has been shown by a number of investigators to inhibit tumor growth in cell cultures as well as to cause tumor regression when administered to human patients with tumors. (See recent review[1]). The fact remains that very little is known about the molecular basis of either the metaphase arresting capabilities of this drug, or its tumor inhibiting effect. Furthermore it has not been established whether there is a correlation between this substance's antitumor action and its effect on cell multiplication. No effects of podophyllotoxin on various enzyme systems could be established[2, 3] while concentrations of two podophyllotoxin derivatives that completely inhibited cell multiplication did not affect DNA synthesis as measured by tritiated thymidine incorporation into P-815 mastocytoma cells.[4] Studies on the distribution of podophyllic acid ethyl hydrazide in a number of organs of experimental animals and men have been reported.[5] In a human brain tumor radioactivity was localized mainly in the periphery of the tumor, following injection of tritiated podophyllic acid ethyl hydrazide into the patient 1 hr prior to tumor excision.[5]

In this communication the subcellular distribution of radioactivity in mouse mammary tumors and livers is reported, following intraperitoneal administration of tritiated podophyllic acid ethyl hydrazide to tumor-bearing mice.

METHODS

RIII/HeSy mice bearing spontaneous mammary tumors were used throughout this work. An ethanolic solution containing 60 μC of podophyllic acid ethyl hydrazide labeled with tritium at the 4'-methoxy group (specific radioactivity 175 μC/mg)*, was

* The tritiated podophyllic acid ethyl hydrazide was a generous gift of SANDOZ Ltd., Basle Switzerland.

administered i.p. to each animal in groups of 5 mice. Twenty four hr later an additional dose of 60 μC was similarly given to each mouse and 4 hr later the animals were sacrificed with ether. The tumors and livers were immediately excised and placed in chilled 0·25 M sucrose solution. The subcellular distribution of the administered radioactive podophyllotoxin derivative was studied by fractionating the pooled tissues in sucrose solutions according to the method of Hogeboom.[6] The nuclear and mitochondrial fractions were further purified by three washings with 0·25 M sucrose. Tumor DNA was prepared according to the method of Kay *et al.*[7] The final step of dissolving the DNA in salt solution and precipitating it by means of ethanol was repeated several times. Perchloric acid hydrolysis of DNA and paper chromatography of the bases were performed as described by Wyatt.[8] The supernatant solution following the initial homogenization for obtaining the nucleoproteins was not discarded but was further centrifuged at 100,000 *g* for 60 min. The 100,000 *g* supernatant was filtered through a 3 × 60 cm column of Sephadex G-100 in 0·15 M NaCl. Radioactivity was determined in a SELO liquid scintillation counter after dissolving the samples in hyamin. Specific radioactivity of DNA is defined as cpm/mg of radioactive substance.

RESULTS

Table 1 shows the distribution of radioactivity in the subcellular fractions of mouse mammary tumors. Since the nuclear fraction possesses radioactivity, the possibility was investigated whether a portion of it resides in the DNA. Indeed following isolation of the tumor DNA it was found that the preparation was radioactive. However, upon repeated dissolutions of the DNA in salt solution and reprecipitations by means of ethanol the specific radioactivity of the preparation falls to very low levels (Table 2). Following perchloric acid hydrolysis of the DNA, the bases isolated were totally unlabeled. Fig. 1 shows the distribution of radioactivity in the 100,000 *g* supernatant of the tumors. The major portion of the radioactivity is specifically bound unto two proteins or groups of proteins that are clearly separable from one another, as well as from the major protein peak of the supernatant. A third non-protein bound radioactivity peak is also present.

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN THE SUBCELLULAR FRACTIONS OF MOUSE MAMMARY TUMORS FOLLOWING ADMINISTRATION OF TRITIATED PODOPHYLLIC ACID ETHYL HYDRAZIDE INTO TUMOR-BEARING RIII MICE

Subcellular fraction	cpm/g of tissue		% Radioactivity	
	tumor	liver	tumor	liver
Nuclear	48,800	20,180	7·3	1·6
Mitochondrial	4773	11,770	0·7	0·9
Microsomal	8368	42,580	1·3	3·4
Supernatant	603,730	1,173,330	90·7	94·1

DISCUSSION

The major portion of the radioactivity of tritiated podophyllic acid ethyl hydrazide resides in the soluble fraction of the mouse mammary tumor. As expected, there is no base labeling of nuclear DNA, even though there is radioactivity associated with the

TABLE 2. SPECIFIC RADIOACTIVITY OF MOUSE MAMMARY TUMOR DNA FOLLOWING ADMINISTRATION OF TRITIATED PODOPHYLLIC ACID ETHYL HYDRAZIDE TO TUMOR-BEARING RIII MICE

No. of precipitations of DNA from solution	Specific radioactivity
1	2068
2	3099
3	993
4	331
5	298
6	77

DNA molecule that by chemical standards contains no more than 0·3% protein as impurity. Removal of the DNA-associated radioactivity can be accomplished only by repeated precipitations of the DNA preparation by means of ethanol and acetone. Whether this weak association of the DNA with the antimitotic compound is of any physiological significance, remains to be seen.

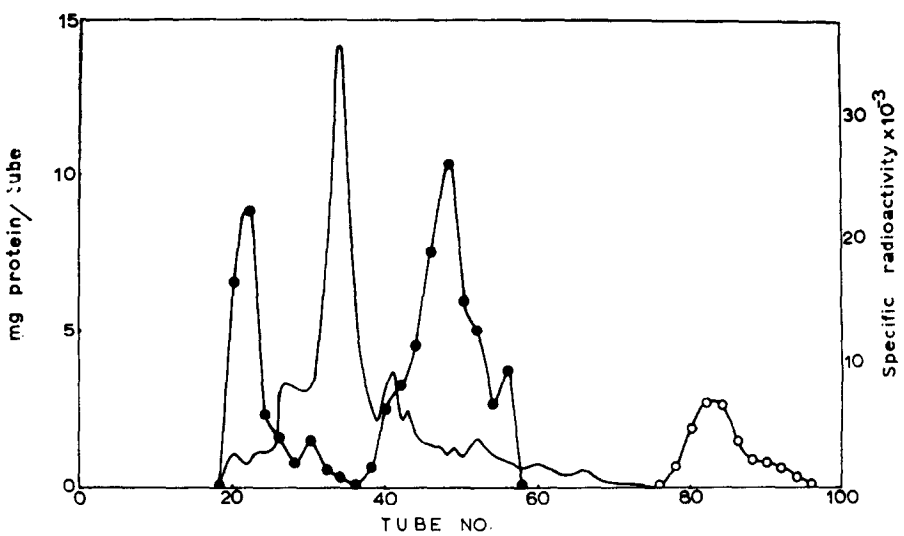

FIG. 1. Gel filtration of 100,000 *g* supernatant of mammary tumor following administration of tritiated podophyllic acid ethyl hydrazide to tumor-bearing RIII mice. Filtration medium 0·14 M NaCl Fraction size 10 ml———protein concentration, ●———● specific radioactivity, ○———○ radioactivity per tube.

Approximately two thirds of the radioactivity in the soluble portion of the tumor homogenate is associated with proteins (Fig. 1). It is apparent that the radioactivity is not randomly distributed among all the proteins in the 100,000 *g* supernatant, since there are two distinct classes of proteins with high specific radioactivity, clearly separable from one another. It can not be said at the moment whether the unchanged podophyllic acid moiety is attached to the protein fractions, or else some metabolic product is associated with one, or both of these fractions. It would be interesting to

know whether these fractions show metaphase arresting and/or tumor inhibiting properties, particularly since protein-bound drugs are rarely biologically active, unless they are released from their protein complexes.

REFERENCES

1. H. SAVEL, in *Progress in Experimental Tumor Research* (Ed. F. HOMBURER), vol. 18, p. 189. S. Kager, Basel (1966).
2. M. SOLDATI and A. FIORETTI, *Tumori* **50**, 101 (1964).
3. E. KAISER and PANTLITSCHKO, *Klin. Med., Wien* **20**, 368 (1955).
4. K. BATZ, F. KALBERER and H. STAHELIN, *Experientia* **20**, 524 (1964).
5. H. STAHELIN and A. CERLETTI, *Schw. Wschr* **42**, 1490 (1964).
6. G. HOGEBOOM, in *Methods in Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN), vol. 1, p. 16. Academic Press, New York (1955).
7. E. R. M. KAY, N. S. SIMONS and A. DOUNCE, *J. Am. Chem. Soc.* **74**, 1724 (1952).
8. G. R. WYATT, in *The Nucleic Acids* (Eds. E. CHARGAFF and J. N. DAVIDSON), vol. 1, p. 243. Academic Press, New York (1955).