Evidence That Inhibition of Hepatic Drug Oxidation by Tumors is Mediated by a Circulating Humor[1] (36361)

BADI M. BOULOS,[2] MARGARET MACDOUGALL, DON W. SHOEMAN, AND DANIEL L. AZARNOFF

Clinical Pharmacology Toxicology Center, Departments of Medicine and Pharmacology, University of Kansas Medical Center, Kansas City, Kansas 66103

Tumor-bearing animals show a decrease in hepatic catalase and microsomal drug oxidizing activity even in the absence of liver metastases (1–6). A polypeptide, toxohormone, has been isolated from the tumor tissue and demonstrated to inhibit microsomal drug metabolism *in vivo* and *in vitro* (7–9).

The effect of tumors on hepatic drug metabolism was studied using hexobarbital which gives a type I spectral shift when added to microsomes, and aniline, which gives a type II shift (10), as substrates. Using parabiotic rats, a method commonly employed to study tumor–hormonal relationships, we present evidence that elaboration of a circulating substance is the mechanism by which tumors decrease hepatic microsomal drug metabolizing activity.

Materials and Methods. Male, littermate, F344, Simonsen rats were used for this study. The animals were fed Purina rat chow and water *ad libitum*. Between four and six weeks of age, the animals were anesthetized with ethyl carbamate (1 g/kg intraperitoneally) and the skin, scapula, and ilium but no muscle of each of a pair of littermates sutured together. Rat pairs were allowed to recover for two weeks before tissue was implanted. The tumor studied was an actinomycin-induced fibrosarcoma carried through numerous transplantations in rats. In one group of parabiotic rats, a 1 mm^3 piece of tumor was transplanted into the flank of one of each pair. Groups of tumor-bearing and non-tumor-bearing individuals, nonparabiotic rats were similarly prepared. Another group of non-tumor-bearing parabiotic rats served as controls. Parabiotic animals were housed one pair per cage and the nonparabiotic animals individually. The rooms were maintained at 72 ± 2°F and had constant 12 hr light and dark cycles.

When the tumor size reached 3–4 cm^3, hepatic microsomal enzyme activity was estimated *in vitro*. Animals were killed at 9:00 AM by decapitation, the livers removed and chilled in ice-cold 1.15% KCl. Each liver was weighed, homogenized in 2 vol cold 1.15% KCl using a close-fitting motor driven teflon pestle, and centrifuged at 0° for 20 min at 9,000*g*. The supernatant was filtered through a layer of gauze to remove fat particles. Duplicate aliquots of each liver was assayed for their capacity to oxidize aniline (2.32 m*M*) and hexobarbital (0.58 m*M*) (11).

At the end of the prescribed incubation period, the flasks containing aniline were assayed for the formation of *p*-aminophenol (12) and those containing hexobarbital were assayed for the amount of unchanged hexobarbital (13). The protein content of each flask was determined by the method of Lowry *et al.* (14).

A factor analysis was used to detect significant differences between the means.

Results. The presence of tumor tissue in one of a parabiotic pair of rats depresses the metabolism of the two substrates studied in both animals equally (Table I). The hepatic drug metabolism of individual tumor-bearing rats is also depressed compared to nontumor-bearing controls. Parabiosis *per se* does not influence the microsomal drug oxidation of either animal of the pair.

[1] Supported by grant GM 15956 from the U.S. Public Health Service.

[2] Trainee in Clinical Pharmacology (GM 01342).

TABLE I. Drug Metabolism in Tumor and Non-tumor-bearing Parabiotic and Individual Rats.[a, b]

	n[a]	Hexobarbital (μmoles metabolized/mg protein/30 min)	Aniline (μmoles metabolized/mg protein/15 min)
Group I			
Nonparabiotic tumor-bearing animals	20	2.2 ± 0.4	15 ± 1
Parabiotic animals			
tumor-bearing member	8	2.0 ± 0.3	11 ± 1
non-tumor-bearing member	7	2.5 ± 0.2	14 ± 0.4
Group II			
Nonparabiotic non-tumor-bearing animals	31	4.4 ± 0.3	21 ± 1
Parabiotic animals			
one non-tumor-bearing member	11	4.2 ± 0.7	25 ± 2
other non-tumor-bearing member	11	4.2 ± 0.7	29 ± 3

All values in Group I are different from the corresponding value in Group II. $p = <.05$.
[a] n = number of animals. Value for each animal is mean of duplicate determination.
[b] Mean ± SEM.

Discussion. The presence of certain tumors (Walker 256 Carcinosarcoma, Flexner Jobling Sarcoma, Sarcoma 45, T8 uterine epithelioma of Güeren) has been reported (4, 5, 15) in animals to be associated with inhibition of hepatic drug oxidation. The clinical observation that cancer patients are more sensitive to barbiturates than are normal individuals suggests a similar effect in humans (16). The response is not universal, however, since no inhibition was noted when the transplanted adenocarcinoma R-3230AC was studied (17). Correlation between the degree of inhibition of microsomal drug metabolism and size of the tumor is good, the maximum effect being observed when the tumor is greater than 10% of the body weight (1). It has also been shown that removal of the tumors restores the metabolic rate to normal (3).

Toxohormone, isolated and partially purified from tumor tissue, inhibits microsomal durg oxidation when injected into non-tumor-bearing animals (7, 18). This observation does not constitute proof that this substance circulates in the blood and is responsible for the inhibition of drug metabolism documented *in vivo*. Parabiotic animals have a common circulation so that substances produced in one of the pair will reach the other member only if the substance circulates in the blood. The transplantable fibrosarcoma used in this study does not metastasize. Therefore, the inhibition of hepatic drug metabolism in the non-tumor-bearing member of the parabiotic pair constitutes evidence that the tumor does elaborate a substance which circulates in the blood.

Recently, Kato *et al.* (19) have demonstrated that toxohormone reduces the binding of hexobarbital, aminopyrine, and aniline to cytochrome P_{450}. The activity of the microsomal NADPH-lined electron transport system and content of cytochrome P_{450} are also decreased in the livers of tumor-bearing rats (4).

Summary. Transplantation of a nonmetastasizing fibrosarcoma into one member of a parabiotic pair of rats produced inhibition of hepatic microsomal drug oxidation in both members. This observation is considered evidence that this tumor secretes a circulating substance which inhibits hepatic drug metabolism.

1. Kampschmidt, R. F., Adams, M. E., and

McCoy, T. A., Cancer Res. **19**, 236 (1959).

2. Wilson, J. T., Proc. Soc. Exp. Biol. Med. **128**, 445 (1968).

3. Wilson, J. T., J. Pharmacol. Exp. Ther. **160**, 179 (1968).

4. Kato, R., Takanaka, A., Takahashi, A., and Onoda, K., Jap. J. Pharmacol. **18**, 224 (1968).

5. Kato, R., Takanaka, A., and Oshima, T., Jap. J. Pharmacol. **18**, 245 (1968).

6. Foussard-Blanpin, O., C. R. Acad. Sci. (Paris) **161**, 896 (1967).

7. Nakahara, W., and Fukouka, F., Gann **52**, 197 (1961).

8. Holmberg, B., Eur. J. Cancer **4**, 271 (1968).

9. Garvie, W. H. H., and Grant, R. M., Brit. J. Cancer **25**, 166 (1971).

10. Shenkman, J. B., and Sato, R., Mol Pharmacol. **4**, 613 (1968).

11. Sladek, N. E., and Mannering, G., Mol. Pharmacol. **5**, 174 (1969).

12. Kato, R., and Gillette, J., J. Pharmacol. Exp. Ther. **150**, 279 (1965).

13. Brodie, B. B., and Burns, J. J., J. Pharmacol. Exp. Ther. **109**, 26 (1953).

14. Lowry, O. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

15. Franchi, G., and Rosso, R., Biochem. Pharmacol. **18**, 236 (1969).

16. Mark, L. C., J. Clin. Pharmacol. Ther. **4**, 504 (1963).

17. Wilson, J. T., Biochem. Pharmacol. **17**, 1449 (1968).

18. Hozume, M., Ohashi, M., and Ono, T., Gann **56**, 313 (1965).

19. Kato, R., Takansha, A., and Takahashi, A., Gann **61**, 359 (1970).

Received Nov. 8, 1971. P.S.E.B.M., 1972, Vol. 139.