Biochemical Pharmacology, Vol. 22 pp. 2429–2431. Pergamon Press, 1973. Printed in Great Britain.

ACCELERATED CATABOLISM OF HYDROCORTISONE IN ISOLATED PERFUSED LIVER OF TUMOR-BEARING RATS

IVAN BARTOŠEK, VIVIANA MARC, AMALIA GUAITANI and SILVIO GARATTINI
Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea, 62, 20157 Milano, Italy

(*Received* 20 *February* 1973; *accepted* 27 *March* 1973)

Abstract—The rate of hydrocortisone disappearance from the medium was followed during the perfusion of livers isolated from normal and Walker 256 tumor-bearing rats. The half-life of hydrocortisone found during the perfusions of tumor livers with tumor blood was only about half (12·8 min) of that found during perfusions of normal livers with normal blood (22·4 min). The cross experiments, normal livers perfused with tumor blood and vice versa, demonstrated that blood does not influence the rate of hydrocortisone disappearance, which is determined exclusively by the state of liver tissue.

THE METABOLISM of drugs in the liver *in vivo* and *in vitro* is altered in the presence of tumors.[1–5] The technique of perfusion of isolated liver overcomes some of the difficulties in the interpretation of results from *in vitro* systems, such as structure damage due to tissue homogenization, and from *in vivo* systems, such as distribution and binding of the drug. Previous studies[6,7] have demonstrated that the rate of pentobarbital disappearance from the medium during perfusion of livers isolated from tumor-bearing animals is slower than in similar experiments with normal livers. Several experiments[8] with hydrocortisone, both *in vivo* and *in vitro*, which showed a faster disappearance rate of hydrocortisone and a higher formation of 6-β-hydroxyhydrocortisone in tumor-bearing rats prompted us to re-examine the influence of tumors on hepatic metabolism, using as substrate a drug which is closely related to naturally occurring hormone found in rats, i.e. corticosterone.

MATERIALS AND METHODS

Male Sprague–Dawley rats (CD–COBS from Charles River, Italy) bearing Walker 256 carcinosarcoma tumor and/or normal animals were used as donors of livers and blood for the perfusion experiments. The animals were maintained on a laboratory diet (Charles River 4 RF 26) and water *ad lib.* The tumor-bearing rats were used 12 days after subcutaneous transplantation of Walker carcinosarcoma 256. The tumor development and general condition of the animals were controlled accurately before the experiment. The animals used for preparation of blood were sacrificed at 9 a.m., avoiding any stress. The liver was isolated under anesthesia (chloralose 60 mg/kg body wt, sodium phenobarbital 50 mg/kg body wt) by the usual surgical technique: the portal vein and biliary duct were cannulated.[9] The perfusion medium contained one-third defibrinated heparinized blood, one-third homologous serum and one-third Krebs–Ringer bicarbonate buffer, pH 7·4. Free hydrocortisone was dissolved in a

minimal quantity of ethanol (0·1 ml) and added to the perfusion medium in order to achieve the final concentration of 20 μg/ml; the total volume of medium was proportional to liver weight, i.e. 5 ml/g of liver tissue. The organ was perfused by recirculation; the flow of the medium was 1 ml/min/g of liver (other details of the perfusion technique and the apparatus used have previously been reported[10]).

Samples of medium were taken after 5, 10, 15, 20 and 30 min of liver perfusion. Hydrocortisone levels in the perfusion medium were assayed by a modified spectrophotofluorimetric method,[11,12] which allows detection of the hormone in concentrations of 0·2 μg/ml with an average recovery of 98 ± 3 per cent.

RESULTS AND DISCUSSION

Both normal and tumor-bearing rats of the same age had an average total body wt of 235 ± 10 g; the average liver weight of normal rats was 10·3 ± 0·4 g and that of tumor-bearing animals was 12·0 ± 0·4 g. The average weight of the Walker 256 carcinosarcoma tumors was 32·9 ± 1·6 g, 12 days after transplantation.

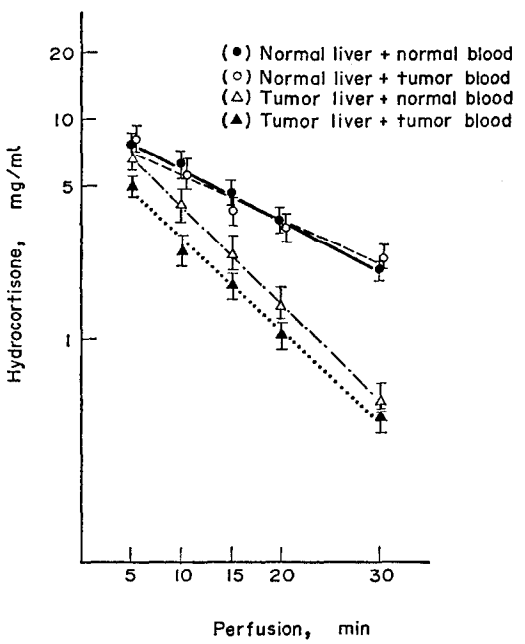

FIG. 1. Hydrocortisone disappearance from the medium during the perfusion of isolated rat liver. The initial concentration of hydrocortisone was 20 μg/ml of perfusion medium. (●), Normal liver + normal blood; (○), normal liver + "tumor" blood; (△), "tumor" liver + normal blood; (▲), "tumor" liver + "tumor" blood.

Hydrocortisone levels in the medium decreased by a first-order reaction rate during liver perfusion, both in normal and tumor-bearing rats (see Fig. 1). The rate constants K_1 and respective half lives $t\frac{1}{2}$ are reported in Table 1. The value of $t\frac{1}{2}$ indicates that the presence of tumors in rats substantially changes the disappearance rate of hydrocortisone from the perfusion medium.

Cross experiments, i.e. perfusion of tumor liver with normal blood and vice

TABLE 1. HYDROCORTISONE DISAPPEARANCE FROM THE PERFUSION MEDIUM

Liver	Blood	$K_1 \times 10^{-3}$ min^{-1} (mean ± S.E.)	Half life $t\frac{1}{2}$ min (mean ± S.E.)	Exp. No.
Normal	Normal	30·9 ± 1·7	22·4 ± 0·4	7
Tumor	Tumor	53·9 ± 5·7*	12·8 ± 1·2*	5
Normal	Tumor	29·1 ± 0·5	23·8 ± 0·1	6
Tumor	Normal	51·2 ± 3·8*	13·5 ± 1·8*	6

* $P < 0{\cdot}01$ with respect to controls.

versa, exclude the possibility that blood or serum from tumor-bearing animals modifies the performance of liver tissue, as is evident from the data summarized in Table 1.

In order to explain the increased rate of disappearance of hydrocortisone, it may be recalled that the presence of a tumor may be regarded as a continuous stress which increases the production of corticosteroids. This is indicated by unpublished results[8] showing an elevated plasma corticosterone levels in tumor-bearing rats. Enhanced levels of blood corticosteroids increase the capacity of the liver to metabolize these hormones,[13] and other drugs such as barbiturates.[14,15]

However, in another investigation using similar experimental conditions, pentobarbital disappeared 10 times more slowly from the medium during the perfusion of liver obtained from tumor-bearing rats than from control.[6] Another difference in the disappearance of pentobarbital or hydrocortisone is seen in the cross experiments: the blood of tumor-bearing animals influences the disappearance rate of pentobarbital[6] but not of hydrocortisone.

The observation of an increased rate of hydrocortisone disappearance in the presence of tumor may be of significance when steroid hormones are used for therapy in cancer patients.

Acknowledgements—This work was supported by a Contract from Consiglio Nazionale delle Ricerche, No. 71.00821.04. We wish to thank Misses I. Simonazzi and A. Guindani for their skilful technical assistance and Miss T. Colombo for tumor transplantations.

REFERENCES

1. R. ROSSO, E. DOLFINI and M. G. DONELLI, *Eur. J. Cancer* **4**, 133 (1968).
2. R. ROSSO, M. G. DONELLI, G. FRANCHI and S. GARATTINI, *Eur. J. Cancer* **7**, 565 (1971).
3. G. A. J. GOODLAD and C. M. CLARK, *Biochem. J.* **80**, 49P (1961).
4. R. KATO, A. TAKANAKA and T. OSHIMA, *Jap. J. Pharmac.* **18**, 245 (1968).
5. J. T. WILSON, *Biochem. Pharmac.* **17**, 1449 (1968).
6. I. BARTOŠEK, A. GUAITANI and M. G. DONELLI, *Biochem. Pharmac.* **21**, 2359 (1972).
7. G. C. SHARMA, S. GARB and B. DE BOER, *Fedn Proc.* **29**, 757 (1970).
8. V. MARC, in preparation.
9. J. KVĚTINA and A. GUAITANI, *Pharmacology* **2**, 65 (1969).
10. I. BARTOŠEK, A. GUAITANI and S. GARATTINI, *Pharmacology* **8**, 244 (1972).
11. R. GUILLEMIN, *J. Lab. clin. Med.* **53**, 830 (1959).
12. M. A. STOCKHAM, *J. Endocrinol.* **26**, IV (1963).
13. T. UETE and A. J. BLAIR JR., *Metabolism* **14**, 904 (1965).
14. C. W. DRIEVER and F. W. BOUSQUET, *Life Sci.* **4**, 1449 (1965).
15. H. REMMER, *Naunyn-Schmiedeberg's Archs exp. Path. Pharmak.* **233**, 184 (1958).