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Differences in pentobarbital disappearance rate in rats bearing two lines of Walker carcinosarcoma 256

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That modifications of drug metabolism may be induced in the host by the presence of an experimental tumour is widely documented [1–3]. The metabolism of pentobarbital is markedly prolonged in Walker carcinosarcoma 256 bearing rats, as observed *in vivo*, in the microsomal fraction and in isolated perfused liver [1, 4, 5].

Our previous data pointed out that both liver metabolic capacity and "factors" in blood from tumour bearing animals were responsible for the differences observed [1].

In an attempt to find a possible explanation we continued this study but we encountered an unexpected variable in the same experimental tumour deriving from two different lines. This paper compares the kinetics of pentobarbital disappearance in perfused liver and *in vivo* in two different lines of Walker carcinosarcoma 256.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 200 g (CD-COBS, Charles River, Italy) were maintained in standardized conditions (22°, 60 per cent of relative humidity); Altromin-MT diet (Rieper, Italy) and water were supplied *ad libitum*.

Two lines of Walker carcinosarcoma 256 (received from Dr. I. Wodinsky, A. D. Little, Cambridge, U.S.A., and from Dr. D. Gericke, Hoechst Labs., Frankfurt, Germany) were transplanted subcutaneously 12 days before the host animals were used for perfusion experiments or for evaluation of sleeping time.

The liver was isolated under anaesthesia (chloralose 60 mg/kg body wt, sodium phenobarbital 50 mg/kg body wt) by the usual surgical technique, and the portal vein and biliary duct were cannulated [6].

The perfusion medium contained 1/3 defibrinated heparinized blood, 1/3 homologous serum and 1/3 Krebs Ringer bicarbonate buffer pH = 7.4. Sodium pentobarbital 25 μg/ml was added to this medium; the total volume of medium was proportional to the liver wt, i.e. 6 ml/min/g of liver (other details of the perfusion technique and the apparatus used have been reported elsewhere [7].

Pentobarbital was assayed by a spectrofluorimetric method by which the drug can be detected at a concentration of 0.5 μg/ml with an average recovery of 94 ± 1 per cent (8).

Microsomal activity. The pellet of liver microsomes was prepared by calcium precipitation and suspended either in 154 mM KCl buffered by 50 mM phosphate (pH = 7.4) or in sera of both normal and tumour bearing rats [9].

The sleeping time induced by pentobarbital (25 mg/kg i.p.) was determined by following the duration of loss of righting reflex.

RESULTS AND DISCUSSION

Average body wt of control rats was 219 ± 2 g as opposed to 211 ± 3 g (line A) and 193 ± 11 g (line B) in tumour bearing animals. The average liver wt of normal rats was 10.8 ± 0.4 g and that of Walker 256 bearing animals 11.7 ± 0.4 g (line A) and 10.3 ± 0.7 g (line B). In the growth of both lines of tumours there were also no differences: the average wt of the Walker carcinosarcoma 256 was 17.5 ± 2.5 in line A and 17.2 ± 3.0 g in line B. However, we observed different kinetics of pentobarbital disappearance from the perfusion medium in the two lines of Walker carcinosarcoma (Table 1).

Table 1. First order rate constants, half-life of pentobarbital and correlation coefficient in perfused liver

Experimental condition*	First order rate constant ($K_1 \times 10^{-3}$) min^{-1}	Half-life of pentobarbital ($t_{\frac{1}{2}}$) min	Correlation coefficient (r)
Normal liver*			
normal blood (mean ± S.E.)	24.1 ± 1.6	29.0 ± 1.9	0.996
Tumour liver A*			
tumour blood A (mean ± S.E.)	9.0 ± 0.8	77.8 ± 7.2‡	0.993
Tumour liver B*			
tumour blood B (mean ± S.E.)	4.2 ± 0.4	157.6 ± 12.9§	0.987
Normal liver*			
tumour blood A (mean ± S.E.)	20.5 ± 1.2	32.6 ± 1.7	0.983
Normal liver*			
tumour blood B (mean ± S.E.)	11.2 ± 2.4	66.8 ± 12.0†	0.990
Tumour liver A*			
normal blood (mean ± S.E.)	11.5 ± 0.4	60.7 ± 2.0§	0.996
Tumour liver B +			
normal blood (mean ± S.E.)	8.8 ± 1.6	80.8 ± 8.4‡	0.995

* Mean of three experiments.
Initial blood level of pentobarbital was 23.2 ± 0.2.
† $P < 0.05$
‡ $P < 0.01$ } vs controls.
§ $P < 0.001$

Table 2. Comparison of sleeping time and levels of serum pentobarbital at the beginning and end of narcosis in normal and tumour bearing rats

	Control	Walker A	Walker B
Onset of narcosis (min ± S.E.)*	9.1 ± 0.5	7.5 ± 0.4	4.3 ± 0.2§
Serum pentobarbital (μg/ml ± S.E.) at the beginning of narcosis†	22.0 ± 0.4	22.9 ± 0.7	27.7 ± 1.3‡
Sleeping time (min ± S.E.)*	32.4 ± 1.6	45.5 ± 4.0‡	82.7 ± 5.5§
Serum pentobarbital (μg/ml ± S.E.) at the end of narcosis†	12.7 ± 0.6	13.5 ± 0.9	13.9 ± 0.4

* Mean of fifteen determinations.
† Mean of eight determinations.
Pentobarbital was given intraperitoneally at the dose of 25 mg/kg.
‡ $P < 0.01$ compared to controls.
§ $P < 0.001$ compared to controls.

Pentobarbital concentration in the medium decreased by a first order kinetics between 10 and 120 min after the start of liver perfusion. The half-life ($t_{\frac{1}{2}}$) of the drug was 29.0 min in normal rat liver, 77.8 min (line A) and 157.6 min (line B) in the liver of tumour bearing rats (Table 1).

In the previous work the half-life of pentobarbital was 32.2 min in the normal controls and 323 min in Walker carcinosarcoma bearing rats (1). These data indicate that the inhibitory capacity of the experimental tumour kept in our laboratory is modified. This strong inhibition was observed both with liver and blood from tumour bearing rats [1]. Walker 256 carcinosarcoma has been reported as being associated with inhibition of the activities of drug metabolizing enzymes in liver microsomes [5, 10]. Previously Toporek found that whole blood from Walker 256 tumour bearing rats carried an "inhibitory signal" which decreased serum protein synthesis of perfused normal rat livers [11]. This "signal factors" activity is located in the albumin fraction of "tumour blood" [12].

Our cross perfusions (normal liver—tumour blood and vice versa) demonstrate that while the metabolic activity of liver is impaired in both lines of Walker tumour bearing rats, only blood from animals bearing line B significantly decreases the rate of pentobarbital disappearance during the perfusion of normal liver (Table 1). An attempt to measure directly the effect of the serum on pentobarbital transformation by liver microsomes did not show any statistically significant change in normal serum or serum from animals bearing A and B lines. Serum from normal and tumour bearing rats (both lines) in fact decreases liver microsomal activity to a similar extent. We also investigated whether pentobarbital distribution was different in normal and tumoral liver. Using short single-pass perfusion without recirculation of the medium, we found no difference in the amount of pentobarbital extracted by the isolated liver.

A study *in vivo* of the pentobarbital-induced sleeping time in animals bearing the two lines of Walker 256 carcinosarcoma confirmed the differences observed in liver perfusion. Table 2 shows the time elapsed between the injection of pentobarbital and onset of narcosis. The differences in these findings might be related to the distribution of pentobarbital being altered because of diminished adipose tissue in tumour bearing animals [13]; pentobarbital levels in blood at the beginning of narcosis support this assumption. Sleeping time is prolonged in both groups of tumour bearing animals, like the half-life of pentobarbital in perfused liver. Almost identical blood levels of the drug are found at the moment of awakening [14].

These findings are consistent with the hypothesis that the tumour influences liver metabolism rather than sensitivity to pentobarbital as previously suggested by Rosso and Beck [4, 14].

In conclusion it should be stressed that many of the contradictory results found in the literature, concerning drug sensitivity and drug metabolism in tumour bearing animals, may be explained by changes in the characteristics of the experimental tumours arising from their repeated transplantation in animals.

Repeated transplantations of experimental tumours provoke modified metabolic activities in the host.

The rats bearing two lines of Walker carcinosarcoma 256 show different kinetics of pentobarbital metabolism in liver perfusions which are related to variation in sleeping time *in vivo*.

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