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PROLONGED METABOLISM OF PENTOBARBITAL IN ISOLATED PERFUSED LIVER OF TUMOR BEARING RATS

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Abstract—The rate of pentobarbital disappearance from the medium was followed during the perfusions of livers isolated from normal and Walker 256 tumor-bearing rats. The half life of pentobarbital found when tumor liver was perfused with tumor blood was ten times higher (323 min) than that found in normal conditions. The cross experiments—normal liver perfused with tumor blood and *vice versa*—demonstrated that blood of tumor-bearing animals decreased the rate of pentobarbital metabolism by liver tissue.

PREVIOUS studies[1,2] demonstrated that a single dose of pentobarbital (25 mg/kg body wt. i.v.) markedly increases the sleeping time in Sprague–Dawley male rats as compared to normal animals. It was also shown, that pentobarbital levels are higher in plasma and brain of tumor bearing animals than in control rats. These data were confirmed both in Wistar rats bearing Walker 256 carcinosarcoma[3] and in Swiss–Webster tumor bearing mice.[4]

As it is known, the pharmacological effect of pentobarbital *in vivo* is usually proportional to its amount available at the receptor site and therefore greatly influenced by many factors, among which the metabolic process represents a very important step. In order to analyze the rate of pentobarbital hepatic metabolism, the technique of perfusion of isolated liver has been used, so that the difficulties connected with both *in vitro* systems, structure damage from tissue homogenization and *in vivo* systems, distribution and binding of the drug, could be overcome.

METHODS AND MATERIALS

Male Sprague–Dawley rats, both bearing Walker 256 carcinosarcoma tumor and control animals, were used as donors of liver and blood for the perfusion experiments. The animals were maintained on the laboratory diet ALAL 56 and water *ad lib.*

The animals were anesthetized with a mixture of chloralose (60 mg/kg body wt. i.v.) and sodium phenobarbital (50 mg/kg body wt. i.v.). It was found in preliminary experiments that the narcosis does not interfere with the assay of pentobarbital.

The liver was isolated by the usual surgical technique; portal vein and biliary duct were cannulated.[5] 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 the perfusion medium. The total volume of the medium was proportional to the liver weight, i.e. 5 ml of medium/1 g of liver. The organ was perfused by recirculation; the flow of the medium was 1 ml/min/g of liver. The portal pressure was 70–100 mm of water. The perfusion apparatus consisted

of a soft container for the isolated organ, medium sampler, rotating tubular oxygenator, thermostatized blood containers, roller pump and electronic manometer. A dialyzing unit could be included in the perfusion circuit.*

Pentobarbital was assayed by a spectrofluorimetric method which allows to detect the drug in concentration of 0·1 μg/ml with an average recovery of 90 ± 6 per cent.[6]

RESULTS AND DISCUSSION

The average body weight of both normal and tumor bearing animals was 220 ± 10 g. The average liver weight of normal rats was 10·26 ± 0·26 g and that of tumor bearing animals was 13·22 ± 1·23 g.

The average weight of the Walker 256 carcinosarcoma tumor was 25·07 ± 4·47 g, 12 days after transplantation.

Pentobarbital concentration in the medium decreased by a first order reaction rate from 10 to 180 min after the start of liver perfusion, both in normal and tumor bearing rats (see Tables 1 and 2). The rate constant of pentobarbital disappearance (K_1) was $21{\cdot}4 \times 10^{-3}$ min^{-1} in perfusion of normal rat liver and $2{\cdot}14 \times 10^{-3}$ min^{-1} in perfusion of liver of tumor bearing animal.

TABLE 1. PENTOBARBITAL CONCENTRATION IN THE PERFUSION MEDIUM μg/ml OF MEDIUM

Perfusion (min)	Normal liver + normal blood mean ±S.E.*	Tumor liver + tumor blood mean ±S.E.†
0	25·00	25·00
10	15·28 ± 0·78	17·90 ± 0·69
30	11·37 ± 0·94	15·65 ± 1·10
60	7·94 ± 0·61	15·15 ± 1·03
120	1·91 ± 0·33	13·70 ± 0·71
180	0·72 ± 0·40	11·92 ± 0·89

* Mean of seven experiments.
† Mean of four experiments.
Linearity (correlation coefficient):
r = 0·9926 r = 0·9779
First order rate constants:
$K_1 = 21{\cdot}4 \times 10^{-3}$ min^{-1} $K_1 = 2{\cdot}14 \times 10^{-3}$ min^{-1}
Half life of pentobarbital:
$t_{1/2}$ = 32·2 min $t_{1/2}$ = 323 min

The respective half life (t ½) of the drug was 32·2 min in normal rat liver and 323 min in the liver of tumor bearing rats (Table 1).

The half life of pentobarbital in the perfused liver of normal animals is in good agreement with data recently published.[7]

The respective sleeping time observed *in vivo* in normal control rats was 31 ± 4 min and 65 ± 8 min in Walker tumor bearing animals[1] (107 per cent increase). The difference between the rate constants of pentobarbital decrease in the liver perfusion is higher with respect to the difference between the sleeping times. Indeed, it was shown[7] that the difference between the distribution volumes of pentobarbital *in vivo* and in

* I. Bartošek, A. Guaitani and S. Garattini, in preparation.

TABLE 2. PENTOBARBITAL CONCENTRATION IN THE PERFUSION MEDIUM μg/ml OF MEDIUM

Perfusion (min)	Normal liver + tumor blood mean ±S.E.*	Tumor liver + normal blood mean ±S.E.†
0	25·00	25·00
10	14·16 ± 0·59	16·64 ± 0·96
30	13·38 ± 0·64	14·63 ± 0·68
60	10·16 ± 0·66	10·60 ± 0·70
120	5·82 ± 0·95	8·68 ± 0·78
180	3·92 ± 0·90	5·78 ± 0·81

* Mean of five experiments.
† Mean of seven experiments.
Linearity (correlation coefficient):
r = 0·9953 r = 0·9890
First order rate constant:
$K_1 = 7{\cdot}8 \times 10^{-3}$ min^{-1} $K_1 = 6{\cdot}0 \times 10^{-3}$ min^{-1}
Half life of Pentobarbital:
$t_{1/2}$ = 88 min $t_{1/2}$ = 116·5 min

liver perfusion influences the values of the drug half life in blood. This may partly explain the differences between the disappearance rate of the drug *in vivo*[1,2] and in the perfused liver.

The rapid initial decrease of pentobarbital concentration in medium between 0 and 10 min of liver perfusion corresponds to the saturation of the organ with the drug. This saturation proceeds also by a first order rate as it was observed during single pass perfusion of the liver (data not reported in detail). It is known[8] that most of the drug remains in circulation; only about 15 per cent of the substance is bound to liver tissue and 4 per cent is excreted in bile. Pentobarbital present in blood is bound to 45 per cent to plasma proteins and the remaining substance is free.[9]

As it is known,[10] blood of Walker 256 tumor bearing rats carries an "inhibitory signal" which decreases protein synthesis in the perfused liver. The role of "tumor" blood was evident in perfusions of both "normal" and "tumor" liver when the results are compared with the respective experiments performed with "normal" blood. The "inhibitory signal" is present in the albumin fraction of "tumor" blood.[11]

It was, therefore, speculated that a similar mechanism could explain the impairment of the metabolism of pentobarbital in tumor bearing animals.

Cross experiments—perfusion of "tumor" liver with "normal" blood and perfusion of "normal" liver with "tumor" blood–confirmed that "tumor" blood carried an "inhibitory signal" decreasing pentobarbital metabolism. The first order rate constant characterizing pentobarbital disappearance decreased 2·7 times when "normal" liver was perfused with "tumor" blood. The same decrease—2·8 times—was observed when "tumor" liver was perfused with "tumor" blood (Table 2).

It is tempting to assume that the "inhibitory signal" is related to the presence of a growth process. In this respect, it is interesting to recall that in partially hepatectomized rats the weight of the regenerated liver has recovered after 4–6 days while the detoxication of pentobarbital remains low during the first 7 days.[12] It seems to be of interest

to establish which step of pentobarbital metabolism is mostly influenced by these "inhibitory signals."

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