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Studies on Drug Metabolism. I. Effect of Respiratory Oxygen on the Duration of Pentobarbital induced Sleep

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The relationship between the duration of pentobarbital induced sleep and respiratory oxygen tension was investigated in mice and cats. Pentobarbital, when administered to cats in a high dose (70 mg/kg), resulted in irregular respiration, and prolonged biologic half life. And found that the characteristic curve of disappearance of pentobarbital obtained from a high dose administration of pentobarbital were caused by low activity of liver microsomal oxidative enzyme and not by the tissue accumulation.

The low activity was assumed to be caused by insufficiency of oxygen required by oxidative enzymes.

Pharmacological action of a drug can be determined by studying its absorption from intestine, sensitivity of site of action, excretion by kidney and the rate of metabolism in liver microsomal enzyme system.

Recent studies have shown that the activity of the drug metabolizing enzymes play an important role on the duration of drug action.

Also indications are that in various oxidative reactions, aromatic and aliphatic hydroxylation, N– and O–dealkylation, deamination, sulfoxidation and N–oxidation, hydroxylation occur as a common step.[2)]

According to the classification of Mason,[3,4)] the microsomal enzymes are mixed function oxidases.

Oxidative enzymes show activities only under aerobic condition, but nitro reductase loses most of its activity under this condition, while azo reductase retaines most of its activity under aerobic condition *in vitro*. Requirement of oxygen for oxidative metabolism suggests that the duration of sleep induced by pentobarbital may be altered by controlling the respiratory oxygen tension.

When pentobarbital, a depressant of central nervous system, administered in high doses intraperitoneally to the cat, the plasma biologic half life obtained was abnormal type compared with the case of low dose administration.

The cause why the characteristic curve is obtained will be discussed in this paper.

Materials and Methods

Animals——Male cats weighing 2—4 kg and male mice weighing 15—20 g were used for the drug localization studies and for measurement of the duration of the drug induced sleep.

Materials——Sodium pentobarbital was injected into all aminals intraperitoneally. Sodium nitrite was used to induce methemoglobinemia and after pretreatment by this agent its effect on the duration of pentobarbital induced sleep was determined.

Analytical Method of Pentobarbital——A 2 ml quantity of tissue homogenate or plasma was pipeted into a 60 ml glass stoppered bottle, followed by the addition of 2 ml of pH 5.5 citrate buffer and 25 ml of heptane containing 1.5 percent of isoamyl alcohol. After shaking for 45 minutes, the mixture was cen-

1) Location: *1–33 Yayoi–cho, Chiba.*
2) B.B. Brodie, J.R. Gillette and B.N. La Du, *Ann. Rev. Biochem.*, **27**, 427 (1958).
3) H.S. Mason, *Advan. Enzymol.*, **19**, 79 (1957).
4) H.S. Mason, *Science*, **125**, 1185 (1957).

trifuged for 5 minutes at 3000 rpm. To another glass stoppered bottle containing 4 ml of pH 11.0 phosphate buffer, 20 ml of the solvent phase was transfered, shaken for 5 minutes and centrifuged for 5 minutes at 3000 rpm. The heptane phase was removed by a fine-tipped pipet. The pH 11.0 phosphate buffer phase was transfered to a quarz cuvette, and the optical density was read at 241 mμ and 260 mμ by HITACHI spectrophotometer. From the calibration curve obtained by pure sodium pentobarbital, the tissue pentobarbital concentration was determined.

Measurement of Duration of Sleep Induced by Pentobarbital——Duration of sleep of mice was measured by righting reflex and that of cats by the eye lid reflex.

Tissue Localization of Pentobarbital in Cats——Pentobarbital 35 mg/kg or 70 mg/kg was injected into the cats, and after 2 or 6 hr, the cats were killed by exanguination. Various tissues, that is, lung, heart, skeletal muscle, liver, kidney, brain medulla and brain cortex were homogenized with 4 volumes of ice cold 1.15 percent KCl solution by Waring blender for 2 minutes, and the pentobarbital concentration was measured by the analytical method described above.

Measurement of Duration of Pentobarbital Induced Sleep at Various Oxygen Tensions in Mice——Mice were placed in two cages, the oxygen tensions of which were maintained at 20 and 10 percent respectively. After 1 hr, they were injected intraperitoneally with 40 mg/kg of pentobarbital and immediately homogenized after cutting of the tails with 4 volumes of ice cold KCl solution. Complete homogenization of the body was obtained in 10 minutes using the Waring blender. The homogenates were used for determination of pentobarbital by the analytical method described above.

Results and Discussion

When a high dose of pentobarbital (70 mg/kg) was injected intraperitoneally into cats, they fell into a deep sleep and their respiration became feeble, but animals administered a low dose of pentobarbital (35 mg/kg) did not show the interrupted respiration. The plasma biologic half life of pentobarbital administered at a low dose was 6.75 hr, and the disappearance from the blood was found to fall lineally. But when a high dose of this drug was administered, the plasma biologic half life looked unusual, that is, for several hours from the time of injection, the biologic half life was very long (30 hr) and after this, the biologic half life shortened to 9.25 hr (Fig. 1).

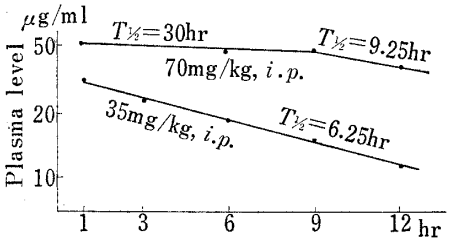

Fig. 1. Plasma Level of Pentobarbital in Cats

awake plasma level: 42 μg/ml in the case of 70 mg/kg inj.
28.5 μg/ml in the case of 35 mg/kg inj.

One of the cause which the biologic half life of pentobarbital was prolonged is assumed to be the high accumulation to various tissues. And if tissue distribution has an important role to the prolonged biologic half life, pentobarbital must be accumulated very much in the case

Table I. Distribution of Pentobarbital in Various Tissues of Cats at 2 and 6 Hours after Intraperitoneal Injection

Dose (mg/kg)	35			70		
Hours after inj. / Tissues	2 hr μg/g	6 hr μg/g	%[a]	2 hr μg/g	6 hr μg/g	%[a]
Lung	15.9	5.4	33.9	37.7	25.6	67.9
Heart	29.8	4.6	15.4	71.5	16.7	23.3
Skeletal muscle	15.9	4.4	27.6	37.7	23.6	62.5
Liver	29.8	4.9	16.4	48.4	24.3	50.2
Kidney	19.9	0.7	3.5	97.3	45.1	46.3
Fat	25.8	15.4	59.6	33.8	43.2	127.8
Brain medulla	59.6	7.4	12.4	—	28.6	—
Brain cortex	63.6	5.0	7.8	47.7	29.2	61.2
Plasma	27.6[b]	11.8[b]	42.7	47.0[b]	41.4[b]	88.0

a) 6 hr distribution volume (μg/g)/2 hr distribution volume (μg/g) × 100
b) μg/ml

of 70 mg/kg administration than in the case of 35 mg/kg administration. And that accumulated pentobarbital must be released from the tissue into the blood rapidly. Distribution of low and high dose of pentobarbital in various tissues of cats at 2 hr and 6 hr after intraperitoneal injection is shown in Table I.

In the case of 35 mg/kg of pentobarbital administration, the plasma level at 2 hr was 27.6 μg/ml and 11.8 μg/ml at 6 hr after administration. On the other hand, in the case of 70 mg/kg administration of pentobarbital, the plasma level at 2 hr was 47.0 μg/ml, 41.4 μg/ml at 6 hr, it is evident that the decrease of pentobarbital in the plasma level in the case of 35 mg/kg administration was more rapid than that in the case of 70 mg/kg of pentobarbital administration. And also in all tissues, more pentobarbital remained in 70 mg/kg administered group than in 35 mg/kg group in absolute amount and in percent (6 hr/2 hr).

This evidence suggests that in the high dose administration, the low degree of pentobarbital disappearance from plasma does not depend on the sufficient supply of pentobarbital by the tissues. Because if it depends only on the release of the drug from the tissues, pentobarbital concentrations of the tissues at 6 hr might have to be much lower. On the other hand, if liver microsomal pentobarbital metabolizing enzyme is depressed by any factors, only in the case of high dose administration, it may be acceptable that disappearance of pentobarbital in either plasma or tissues is slow. Because of these reasons, the prolonged biologic half life in the plasma can be attributed to the low activity of the enzyme and not by recycling of the drug from tissues to plasma.

Pentobarbital is a respiratory depressant as described above, the experiments for the effect of the oxygen in the mice were tested as follows. When mice are injected pentobarbital intraperitoneally, they metabolized it rapidly. But when the mice were placed in a cage with a low oxygen tension, the metabolic rate of pentobarbital was lowered (Table II).

Table II. The Effect of Oxygen Tension on the Metabolism of Pentobarbital by Mice (Whole Body Homogenate) during a Short Period

Oxygen (%)	Percent of pentobarbital metabolized during 1 to 2 minutes after injection
20 (air)	80.4±7.8[a] (5)
10 (air— N_2=1:1)	48.7±6.2[a] (5)

a) standard deviation
Figure in brackets are number of mice used.

The mice put in a cage with 20 percent oxygen (air) for 1 hour metabolized in a short time about 80 percent of the drug injected, while the mice with 10 percent oxygen metabolized only about 49 percent of the injected amount. This suggests that the mice kept for 1 hour in an environment of low oxygen tension do not contain in the blood enough oxygen for enzyme system to act to its full capacity.

Table III. Duration of Pentobarbital induced Sleep of Mice under Various Oxygen Tension

Oxygen (%)	Duration of sleep (min)
95	32.6± 8.5[a] (20)
60	48.9±14.2 (20)
20	66.2±16.0 (20)
10	75.9±16.3 (20)

a) standard deviation
Figure in brackets are number of mice used.

Further, the effect of the oxygen tension on the duration of pentobarbital induced sleep was investigated in Table III.

Mice were injected with 60 mg/kg of pentobarbital intraperitoneally and placed in a cage with varying oxygen tension slept for different durations. For example, the mice in 95 percent of oxygen tension slept only for about 33 minutes while the mice in 10 percent of the tension slept for 75.9 minutes. This finding agreed with those from the *in vitro* study. The mice in the high oxygen tension can incorporate the oxygen into the blood, and the oxygen essential for liver microsomal oxidative enzyme system to act to its full capacity can be supplied. Therefore, full activity of the enzyme induced by sufficient supply of oxygen might have shortened the duration of pentobarbital induced sleep. Table IV shows the effect of sodium nitrite pretreatment on the duration of sleep by pentobarbital.

Table IV. The Effect of Sodium Nitrite Pretreatment on the Duration of Pentobarbital induced Sleep

$NaNO_2$ (mg/kg, *s.c.*)	Pentobarbital (mg/kg, *i.p.*)	Interval between $NaNO_2$ and pentobarbital injection (min)	Duration of sleep (min)
100	—	—	0
—	60	—	20.8
100	60	30	50.4
200	60	30	308.0
200	60	5	127.8

Although sodium nitrite has no anesthetic action, the injection of the chemical (200 mg/kg) into mice might result in more complete methemoglobinemia in 30 minutes. After intervals of 5 and 30 minutes from the injection of 200 mg/kg of sodium nitrite, 60 mg/kg of pentobarbital were administered to mice and the duration of sleep was measured by righting reflex. Mice pretreated with sodium nitrite 5 minutes before the pentobarbital injection slept 6 times as long as the controls, whereas mice pretreated with sodium nitrite 30 minutes prior to the drug injection slept for 15 times as long as the controls. It is reasonable that sodium nitrite made methemoglobinemia and then inhibited oxygen incorporation into hemoglobin, and the oxygen could not be transported enough from lung to liver drug oxidative enzyme system. The prevention of sufficient oxygen transport may have caused decreased activity of the oxidative enzyme, and thus probably resulted in the prolongation of the duration of sleep.