BIPHASIC EFFECT OF DOXAPRAM ON HYPNOTIC ACTIVITY OF PENTOBARBITAL IN MICE

KEN-ICHI SASAKI, SHINOBU FURUSAWA, AND GIICHI TAKAYANAGI

Cancer Research Institute, Tohoku College of Pharmaceutical Sciences, 4-4-1, Komatsushima, Sendai, 983, Japan

(Received July 27, 1980)

The effect of doxapram, a respiratory stimulant, on the pentobarbital sleeping time was investigated in mice. The sleeping time induced by the intraperitoneal injection of pentobarbital was prolonged 0–120 min after the administration of doxapram (25–100 mg/kg, *i.p.*). The pretreatment with doxapram 60 min before had no effect on the anesthetic time induced by ether and on the sleeping time induced by the intracerebroventricular injection of pentobarbital, while increased the lethality of pentobarbital only slightly and the levels of pentobarbital in the plasma and brain significantly. The activities of pentobarbital oxidase and aminopyrine N-demethylase in the $9000 \times g$ supernatant fraction of the liver were inhibited by the pretreatment with doxapram 60 min before the test. On the other hand, 12–24 hr after the injection of doxapram the pentobarbital sleeping time was markedly shortened. Thus, the biphasic effect of doxapram, prolongation at first and shortening later, on the pentobarbital sleeping time was observed. It is possible that doxapram inhibits the hepatic microsomal drug-metabolizing enzymes without an increase in the sensitivity of the central nervous system at first and stimulates these enzymes during the second phase.

Keywords—doxapram; pentobarbital; sleeping time; biphasic effect; hepatic microsomal drug-metabolizing enzymes; mice

INTRODUCTION

Doxapram, a pyrrolidinone derivative, is a nonspecific analeptics capable of producing widespread stimulation of the central nervous system.¹⁾ Apparently doxapram is a relatively specific respiratory stimulant^{2,3)} and has a wider margin of safety than other analeptic drugs such as nikethamide and dimorpholamine.⁴⁾ Cohn⁵⁾ found that nikethamide had no effect on the amylobarbitol sleeping time and doxapram potentiated the action of amylobarbitol in rats. Furthermore, Pleuvry *et al.*⁶⁾ reported that doxapram potentiated the pentobarbital sleeping time in mice.

The present *in vivo* and *in vitro* studies were designed to investigate the mechanisms of interaction between doxapram and pentobarbital in mice.

MATERIALS AND METHODS

Animals—Male ddY mice weighing 20–22 g were used. One dose group consisted of 10–18 mice except for measurement of enzyme activity and pentobarbital level. The mice were fed with a commercial diet (Nippon Clea Co., CE-2) and allowed access to water *ad libitum*.

Drugs—The following drugs were used in this study. Doxapram (1-ethyl-4-(2-morpholino-ethyl)-3,3-diphenyl-2-pyrrolidinone hydrochloride hydrate, Dopram[®], Kissei Yakuhin Kogyo Co.), bemegride (Megibal[®], Takeda Yakuhin Kogyo Co.), dimorpholamine (Theraptique[®], Eisai Co.), resibufogenin (Respigon[®], Taisho Seiyaku Co.), pentobarbital (sodium salt, Tokyo Kasei Kogyo Co.), trichloroethanol (Tokyo Kasei Kogyo Co.), ether (Wako Junyaku Co.), chlorpromazine hydrochloride (Shionogi Seiyaku Co.), SKF-525A (2'-(diethylamino)ethyl-2,2-diphenylvalerate hydrochloride, Smith-Klein and French Laboratories). Drugs for injection were

dissolved in physiological saline. All drugs were administered intraperitoneally except in the case of intracerebroventricular injection of pentobarbital.

Measurement of Sleeping Time — Pentobarbital or trichloroethanol sleeping time was defined as the time between the loss and the recovery of righting reflex. The intracerebroventricular injection of pentobarbital (80 μg /mouse) was carried out according to the method of Haley-McCormick.⁷⁾ All experiments were carried out at 20–25°.

Ether-Anesthesia — A mouse was placed in a beaker (1 liter) containing 0.7 ml of ether. The beaker was sealed with cellophane paper. After mice were anesthetized, they were removed from the beaker 1 min after the loss of righting reflex. Anesthetic time was expressed as the time between the loss and the recovery of righting reflex.

Measurement of Body Temperature — Rectal temperature of mouse was measured by a thermometer with thermister element (Shibaura Electronics, MGA-3). Mice having 37–38° of body temperature were employed in this experiment after selection by measuring twice at 15 min intervals.

Acute Toxicity — Lethality of mice was observed continuously for 72 hr.

Enzyme Assays — The activities of aminopyrine *N*-demethylase and aniline hydroxylase were assayed by determining 4-aminoantipyrine formation⁸⁾ and *p*-aminophenol formation,⁹⁾ respectively. The activity of pentobarbital oxidase was assayed by the method of Brodie *et al.*¹⁰⁾

The pooled livers of 3–5 mice were homogenized in 4 volumes of 0.154 M KCl in a Potter-Elvehjem type homogenizer. The homogenate was centrifuged at 9000 $\times g$ for 20 min to make sediment consisting nuclei and mitochondria.

The supernatant fraction (1.5 ml) was mixed with 10 μmol glucose-6-phosphate, 0.5 μmol NADP, 50 μmol nicotinamide, 25 μmol MgCl_2 , 0.8 ml of 0.1 M phosphate buffer (pH 7.4), 0.2 ml of the substrates (3 μmol aminopyrine, 3 μmol aniline, or 0.6 μmol pentobarbital) and water to a final volume of 3 ml.

Doxapram was given in volume of 0.2 ml

instead of 0.2 ml of water. The mixtures were incubated for 30 min at 37° for aminopyrine *N*-demethylation and aniline hydroxylation and for 60 min at 37° for pentobarbital oxidation.

The activity of glucuronyl transferase in the liver was determined by the modified method of Hollman and Touster.¹¹⁾ To assay the enzyme activity, 0.3 ml of 9000 $\times g$ supernatant fraction (0.154 M KCl) of the liver was mixed with 0.06 ml of 0.0017 M *p*-nitrophenol in 0.5 M phosphate buffer (pH 7.3) and 0.1 μmol of UDP-glucuronic acid in 0.1 ml of water to a total volume of 0.6 ml. The reaction was stopped after the incubation for 30 min at 37° by the addition of 2.4 ml of 0.1 N trichloroacetic acid. Tubes were centrifuged, and the supernatant was poured into 0.06 ml of 10 N KOH. The absorbancy was measured at 400 nm. Protein content was determined by the method of Lowry *et al.*¹²⁾

Determination of Pentobarbital Concentrations in Plasma and Brain — The mice were decapitated at 15, 30, 60, 90, 120 and 240 min after the injection of pentobarbital. Blood and brain were collected for the assay of pentobarbital by the method of Brodie *et al.*¹⁰⁾ Brains were homogenized in 4 volumes of 1.15% KCl. The homogenate or plasma was further transported in 1 M acetate buffer (pH 5.0). Pentobarbital was extracted with petroleum ether containing 1.5% isoamyl alcohol, and petroleum ether phase was shaken with 0.4 M phosphate buffer (pH 11.0). Then the optical density of aqueous phase was measured at 240 nm with an ultraviolet spectrophotometer (Hitachi 124 type).

Statistical Evaluation — Student's *t* test was used to determine the statistical significance.

RESULTS

1. Effect of Doxapram Treatment on Pentobarbital Sleeping Time

As shown in Table I and Fig. 1, the pentobarbital sleeping time in mice was markedly changed by the intraperitoneal administration of doxapram. The mean sleeping time $\pm$ S.E. of intraperitoneal administration of pentobarbital (50 mg/kg) was 51.6 ± 4.0 min.

When doxapram was given with pentobarbital

TABLE I. *Effect of Doxapram on the Hypnotic Activity of Pentobarbital*

Drugs	Pentobarbital sleeping time		
	Intraperitoneal injection (min) ^{a)}	Intracerebroventricular injection (sec) ^{b)}	Pentobarbital-pretreated mice (min) ^{c)}
Control (Saline)	51.6 ± 4.0 (10)	252.9 ± 24.7 (15)	18.6 ± 3.5 (10)
Doxapram 10 mg/kg	62.1 ± 5.8 (10)	—	47.3 ± 3.7 (10)***
Doxapram 25 mg/kg	93.8 ± 8.8 (10)***	250.0 ± 21.2 (18)	76.5 ± 6.3 (10)***
Doxapram 50 mg/kg	178.6 ± 18.1 (10)***	288.3 ± 27.1 (15)	106.2 ± 5.7 (10)***
Doxapram 100 mg/kg	413.3 ± 29.9 (10)***	316.7 ± 33.2 (15)	282.1 ± 16.4 (10)***

Results are expressed as the mean ± S.E. The number of animals are shown in parentheses. a) Doxapram was injected intraperitoneally with pentobarbital (50 mg/kg, *i.p.*) simultaneously. b) Doxapram was injected intraperitoneally 60 min before the intracerebroventricular injection of pentobarbital (80 µg/animal). c) Mice were rendered tolerant to pentobarbital by the intraperitoneal injection (50 mg/kg) 4 times every 12 hr. Doxapram was injected 12 hr after the final injection of pentobarbital. Sixty min after doxapram dosing pentobarbital (50 mg/kg, *i.p.*) was injected and the sleeping time was measured. *** indicates significant from control at the level of $p < 0.001$.

simultaneously, the pentobarbital sleeping time was markedly prolonged. Pentobarbital sleeping time of the groups with 50 mg/kg, *i.p.*, or 100 mg/kg, *i.p.*, of doxapram was prolonged 3.5-fold or 8.0-fold as compared with that of control group. Thus, doxapram caused a dose-dependent

FIG. 1. *Relationship between Pretreatment Time with Doxapram (25 mg/kg, *i.p.*) and Pentobarbital Sleeping Time*

Groups of 10 mice were used. The dotted line indicates the sleeping time induced by 50 mg/kg, *i.p.*, of pentobarbital alone as control (51.6 ± 4.0 min). Doxapram was injected intraperitoneally at the represented time before the intraperitoneal injection of pentobarbital (50 mg/kg). *, ** and *** indicate significant difference from control at the level of $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

prolongation in the pentobarbital sleeping time in the case of simultaneous injection.

By measurement at various time intervals after the injection of doxapram, a biphasic variation in the pentobarbital sleeping time was observed (Fig. 1). Pentobarbital sleeping time was markedly prolonged 60 min after the administration of doxapram (25 mg/kg, *i.p.*). However, the duration of hypnotic action returned to the normal range after 4 hr, and was markedly shortened 24 hr after the administration of doxapram.

In a group of mice receiving 50 mg/kg/day, *i.p.*, of doxapram for 2 days, the pentobarbital sleeping time decreased significantly 24 hr after the final injection of doxapram (13.5 ± 1.9 min). Similarly, in case of consecutive administration of 50 mg/kg/day of doxapram for 5 or 7 days, no significant difference was observed in the pentobarbital sleeping time 24 hr after the final injection of doxapram as compared with that of the treatment with doxapram for 2 days. In contrast to daily injected group, doxapram at 50, or 100 mg/kg, *i.p.*, 4 times every 12 hr markedly depressed the action of pentobarbital, namely, the pentobarbital sleeping time 12 hr after the final injection of doxapram was 3.7 ± 0.5 min or 0 min, respectively.

TABLE II. *Effect of Doxapram on the Lethality of Pentobarbital*

Pretreatment (i.p.)		Lethality of Pentobarbital (72 hr) (mg/kg)	
		100	130
Control		0/10	6/10
Doxapram	10 mg/kg	—	7/10
Doxapram	25 mg/kg	0/10	8/10
Doxapram	50 mg/kg	0/10	9/10
Doxapram	100 mg/kg	2/10	10/10
SKF-525A	20 mg/kg	1/10	9/10
SKF-525A	40 mg/kg	4/10	10/10

Pentobarbital was injected intraperitoneally 60 min after the injection of doxapram or 30 min after the injection of SKF-525A.

The mean sleeping time $\pm$ S.E. after the intracerebroventricular administration of pentobarbital (80 μ g/animal) was 252.9 ± 24.7 sec. By the pretreatment with each dose of doxapram 60 min before the administration of pentobarbital, no significant change was observed in the sleeping time induced by intracerebroventricular injection of pentobarbital.

On the other hand, mice were rendered tolerant to pentobarbital by *i.p.* injection of pentobarbital (50 mg/kg) 4 times every 12 hr. The mean sleeping time $\pm$ S.E. induced by pentobarbital (50 mg/kg) after *i.p.* administration of pentobarbital (50 mg/kg) 4 times every 12 hr was 18.6 ± 3.5 min. The degree of tolerance is determined by a decrease in hypnotic response to pentobarbital as estimation of sleeping time. A dose-dependent potentiation on the action of pentobarbital by doxapram was also seen in pentobarbital-tolerant mice (Table I).

2. Effect of Doxapram on the Lethality and the Hypothermic Action of Pentobarbital

The effect of doxapram on the lethality of pentobarbital was examined and the result was shown in Table II. By the pretreatment with doxapram (50 or 100 mg/kg) or SKF-525A (20 or 40 mg/kg), the lethality of pentobarbital at a dose of 100 mg/kg or 130 mg/kg slightly increased. Figure 2 shows the effects of pentobarbital and doxapram on the body temperature. A single dose

of 25 mg/kg of doxapram had little effect on the body temperature. However, doxapram potentiated the hypothermic action of pentobarbital.

3. Effect of Doxapram on Plasma and Brain Pentobarbital Concentrations in Pentobarbital-Treated Mice

Figure 3 shows the pentobarbital concentra-

FIG. 2. *Effect of Doxapram on the Hypothermic Action of Pentobarbital*

Doxapram (25 mg/kg, *i.p.*) was injected 60 min before the injection of pentobarbital (50 mg/kg, *i.p.*). * and ** indicate significant difference from pentobarbital (50 mg/kg)-group at the level of $p < 0.05$ and $p < 0.01$, respectively.

Symbols; — Doxapram (25 mg/kg) alone, ● — ● Pentobarbital (50 mg/kg) alone, ○ — ○ Doxapram (25 mg/kg) + Pentobarbital (50 mg/kg).

tions in the plasma and brain in mice after the administration of 50 mg/kg of pentobarbital, and both concentrations were elevated by the injection of 25 mg/kg of doxapram. The rate of decay of pentobarbital in both plasma and brain may be less in doxapram-pretreated group than that of control groups.

4. Biphasic Change in Hepatic Microsomal Drug-Metabolizing Enzyme Activity by Doxapram

Effect of doxapram on the activity of hepatic microsomal drug-metabolizing enzyme was examined by a single or consecutive administration of drug. The activity of enzyme was significantly changed after the administration of doxapram. As shown in Fig. 4, the activities of aminopyrine *N*-demethylase and pentobarbital oxidase were markedly inhibited 60 min after the administration of a single dose of doxapram (100

mg/kg), and the activities of UDP-glucuronyl transferase and aniline hydroxylase were slightly inhibited by the injection of a single dose of doxapram. After the consecutive injection of doxapram (100 mg/kg/12 hr $\times$ 4), activities of aminopyrine *N*-demethylase and pentobarbital oxidase were significantly higher than that of control group. But no effect on the activities of UDP-glucuronyl transferase and aniline hydroxylase was observed.

Activities of the four enzymes tested were examined *in vitro*. As shown in Table III, doxapram added to an incubation mixture showed

FIG. 3. Time Course of Pentobarbital Level in Plasma and Brain after Injection of Pentobarbital. Doxapram (25 mg/kg, i.p.) was injected 60 min before the injection of pentobarbital (50 mg/kg, i.p.). Each point represents the mean of the two numbers obtained from pooled mixture of the plasma or brain of 7 mice.

Symbols; ● — ● pentobarbital (50 mg/kg) alone, ○ — ○ Doxapram (25 mg/kg) + Pentobarbital (50 mg/kg).

FIG. 4. Effect of Doxapram on Drug-metabolizing Enzyme Activities in Hepatic 9000 $\times$ g Supernatant Fraction of Mice in Vivo

Results are expressed as the mean of at least four experiments. The animals were sacrificed by decapitation, their livers removed immediately, and enzyme activities determined. The activities of UDP-glucuronyl transferase, aniline hydroxylase and aminopyrine *N*-demethylase in supernatant fraction (9000 $\times$ g) of homogenate of the liver were determined after the incubation with each substrate for 30 min at 37°. The reaction mixture for assay of pentobarbital oxidase activity was incubated for 60 min at 37°. Hepatic enzyme activities of control mice were as follows. — a) 0.40 ± 0.02 (p-nitrophenol nmol/30 min/mg protein), b) 2.06 ± 0.17 (p-aminophenol nmol/30 min/mg protein), c) 1.23 ± 0.21 (4-aminoantipyrine nmol/30 min/mg protein), d) 116.94 ± 14.34 (pentobarbital nmol/60 min/mg protein).

Symbols; □ 60 min after injection of doxapram (100 mg/kg, i.p.), ■ 12 hr after the final injection of doxapram (100 mg/kg/12 hr $\times$ 4, i.p.).

TABLE III. *Metabolic Inhibition by Doxapram in Vitro*

Enzymes	% inhibition of enzyme activity by doxapram (M)		
	3.3×10^{-5}	3.3×10^{-4}	1.7×10^{-3}
UDP-glucuronyl transferase	0	0	19
Aniline hydroxylase	0	28	36
Aminopyrine <i>N</i> -demethylase	13	30	—
Pentobarbital oxidase	23	53	100

Each value represents the mean of two experiments obtained from pooled mixture of the livers of 5 mice.

some inhibitory actions on pentobarbital oxidase but showed a weak inhibitory action on UDP-glucuronyl transferase.

DISCUSSION

Doxapram has been shown to be a potent respiratory stimulant. It has also been reported that doxapram can produce marked arousal effects in respiratory depression due to overdosage of barbiturates or other anesthetics in animals¹³⁾ and in man.¹⁴⁾

On the other hand, Cohn⁵⁾ found that doxapram potentiated the sleep induced by amylobarbitol in rats but analeptics such as nikethamide had no effect on amylobarbitol sleeping time. Further, Pleuvry *et al.*⁶⁾ recently demonstrated that doxapram prolonged pentobarbital sleeping time and increased the duration of the respiratory depression caused by pentobarbital in mice through a mechanism involving increased blood concentration by pentobarbital.

The present investigation revealed a biphasic effect of doxapram on the pentobarbital sleeping time and on hepatic microsomal enzyme activity in mice. It is apparent that the effect of doxapram on the hypnotic action of pentobarbital is dependent on the time intervals of pretreatment with doxapram as shown in Fig. 1. Pentobarbital sleeping time was significantly prolonged within 2 hr after and shortened 12 to 24 hr after the administration of doxapram. In pentobarbital (50 mg/kg/12 hr $\times$ 4)-pretreated mice the administration of doxapram 60 min before the injection of

pentobarbital produced a significant increment of pentobarbital sleeping activity.

However, no effect was observed on the ether-anesthetic time in mice by the pretreatment with doxapram 60 min before. Similarly, no effect of doxapram on the trichloroethanol-induced sleeping time in mice was also observed. Since it is known that trichloroethanol is inactivated by glucuronic acid conjugation process in the liver, it seems likely that doxapram has no effect on metabolism as to glucuronic acid conjugation.

Furthermore, other respiratory stimulants such as dimorpholamine (20 mg/kg) and resibufogenin (4 mg/kg) prolonged the pentobarbital sleeping time about 1.6–2.0-fold as compared with control mice when these drugs were administered 30 min prior to the injection of pentobarbital. But the effect of bemegride (10 mg/kg) on pentobarbital sleeping action was not seen in this experiment. Trichloroethanol-induced sleeping time was slightly prolonged by the pretreatment with dimorpholamine or resibufogenin 30 min before the injection.

On the toxicity of doxapram, it was reported that morphine^{15,16)} and pentobarbital⁶⁾ enhanced the toxicity of doxapram in mice. In present experiment, the effect of doxapram on the lethality of pentobarbital was examined and it was shown that the lethality of pentobarbital was slightly increased in doxapram-treated mice.

The hypothermic action of pentobarbital was potentiated by the pretreatment with doxapram. The duration of hypothermic action of pentobarbital after the injection of doxapram agreed with

initial effect of doxapram on pentobarbital sleeping time in mice.

The intracerebroventricular administration of pentobarbital to animal induces direct action to the central nervous system except for some metabolisms of pentobarbital in the liver. The treatment with doxapram 60 min before the intracerebroventricular administration of pentobarbital was not effective on the sleeping activity induced by pentobarbital. With respect to this finding, it is unlikely that doxapram enhances the sensitivity of central nervous system to pentobarbital.

Several investigators have clearly shown that barbiturate levels in the brain is important in determining the pharmacological activity.¹⁷⁻¹⁹⁾ Figure 3 also shows the time course of pentobarbital levels in the plasma and brain of mice treated with doxapram 60 min prior to the administration of pentobarbital. In doxapram-treated mice higher concentrations of pentobarbital in the plasma and brain were observed than those in mice treated with pentobarbital alone. From the above results, it is apparent that doxapram increased the levels of pentobarbital in the plasma and brain and prolonged the sleeping time induced by pentobarbital. It seems likely that doxapram potentiates the sleep induced by pentobarbital by inhibiting the metabolism of pentobarbital.

Therefore, the effect of doxapram on the activity of drug-metabolizing enzymes in the mouse liver was examined *in vivo* and *in vitro*.

In vivo, the effect of doxapram on the activity of enzyme was dependent on the number of administration of doxapram as shown in Fig. 4. The activities of aminopyrine *N*-demethylase and pentobarbital oxidase were markedly inhibited 60 min after the single administration of doxapram (100 mg/kg). However, these enzyme activities were markedly enhanced 12 hr after the consecutive administration of doxapram (100 mg/kg/12 hr $\times$ 4). No change in aniline hydroxylase activity was seen 60 min after the single administration or 12 hr after the consecutive administration of doxapram. Activities of UDP-glucuronyl transferase

decreased significantly 60 min after the single administration of doxapram but did not vary 12 hr after the consecutive administration of doxapram.

In vitro, doxapram inhibited strongly the activity of pentobarbital oxidase and inhibited slightly those of aminopyrine *N*-demethylase, aniline hydroxylase and UDP-glucuronyl transferase in the 9000 $\times$ *g* supernatant in the liver homogenate.

These data suggest that the variation of pentobarbital sleeping time by doxapram may be the result of the variation of the activities of hepatic drug-metabolizing enzymes and a subsequent change of pentobarbital levels in the plasma and brain without causing changes in the sensitivity of central nervous system to pentobarbital.

REFERENCES

- 1) L.S.Goodman and A.Gilman: "The Pharmacological Basis of Therapeutics," 5th ed., Macmillan, New York, 1975, pp. 363-364.
- 2) W.H.Funderburk, K.L.Oliver and J.W.Ward: Electrophysiologic analysis of the site of action of doxapram hydrochloride, *J.Pharmacol.Exp.Ther.*, **151**, 360-368 (1966).
- 3) T.Kuga and J.Naito: Effect of doxapram on the central nervous system, *Nippon Yakurigaku Zasshi*, **69**, 701-710 (1973).
- 4) W.H.Funderburk and R.S.Alphin: Electrical changes in the CNS produced by a new respiratory stimulant, AHR-619, *Fed. Proc.*, **21**, 324 (1962).
- 5) M.L.Cohn: Doxapram hydrochloride, *Brit. J. Anaesth.*, **46**, 169-170 (1974).
- 6) B.J.Pleuvry, S.E.Maddison and B-B.Shahal: Interaction between doxapram and pentobarbital in the mouse, *J. Pharm. Pharmacol.*, **30**, 522-523 (1978).
- 7) T.J.Haley and W.G.McCormick: Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse, *Brit. J. Pharmacol.*, **12**, 12-15 (1957).
- 8) B.B.Brodie and J.Axelrod: The fate of aminopyrine (pyramidon) in man and methods for the estimation of aminopyrine and its metabolites in biological material, *J. Pharmacol. Exp. Ther.*, **99**, 171-184 (1950).
- 9) H.Kitagawa, S.Yoshida and T.Kamataki: Changes of sleeping time, liver microsomal drug metabolizing enzyme activity and accumulation to various organs by long term administration (1 month) of pentobarbital in cats, *Yakugaku Zasshi*, **88**, 954-957 (1968).
- 10) B.B.Brodie, J.J.Burns, L.C.Mark, P.A.Lief, E.Bernstein and E.M.Papper: The fate of pentobarbital in man and

- dog and a method for its estimation in biological material, *J. Pharmacol. Exp. Ther.*, **109**, 26–34 (1953).
- 11) S.Hollman and O.Touster: Alterations in tissue levels of uridine diphosphate glucose dehydrogenase, uridine diphosphate glucuronic acid pyrophosphatase and glucuronyl transferase induced by substances influencing the production of ascorbic acid, *Biochim. Biophys. Acta*, **26**, 338–352 (1962).
- 12) O.H.Lowry, N.J.Rosebrough, A.L.Farr and R.J.Randall: Protein measurement with the Folin phenol reagent, *J. Biol. Chem.*, **193**, 265–275 (1951).
- 13) J.W.Ward and B.V.Franko: A new centrally acting agent (AHR-619) with marked respiratory stimulating pressor, and “awakening” effects, *Fed. Proc.*, **21**, 325 (1962).
- 14) A.A.Sanchez-Salazar, W.E.Pembleton and C.M. Banerjee: Doxapram hydrochloride as a respiratory stimulant in man, *Anesthesiology*, **24**, 808–812 (1963).
- 15) S.M.Gregoretti and B.J.Plevry: Interactions between morphine and doxapram in the rabbit and mouse, *Brit. J. Anaesth.*, **49**, 323–329 (1977).
- 16) B.J.Plevry: A study of the enhanced toxicity of doxapram in rodents treated with narcotic analgesics, *Brit. J. Anaesth.*, **50**, 451–458 (1978).
- 17) A.Jori, A.Bianchetti and P.E.Prestini: Relations between barbiturate brain levels and sleeping time in various experimental conditions, *Biochem. Pharmacol.*, **19**, 2687–2694 (1970).
- 18) M.E.Greig and T.C.Mayberry: The relationship between cholinesterase activity and brain permeability, *J. Pharmacol. Exp. Ther.*, **102**, 1–4 (1951).
- 19) J.Noordhoek: Pharmacokinetics and dose-sleeping time lines of hexobarbital in mice, *Eur. J. Pharmacol.*, **3**, 242–250 (1968).