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FURTHER STUDIES ON THE INHIBITION AND STIMULATION OF MICROSOMAL DRUG-METABOLIZING ENZYMES OF RAT LIVER BY VARIOUS COMPOUNDS

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Abstract—A variety of drugs produce an inhibition of the *in vitro* and *in vivo* metabolism of pentobarbital, hexobarbital, meprobamate, carisoprodol and strychnine and most of these drugs produce after 36–48 hr a stimulation of the *in vitro* and the *in vivo* metabolisms of pentobarbital *et al.* On the other hand, a number of drugs, known as the stimulator of the microsomal drug-metabolizing enzymes, produce an inhibition of these enzymes, if they are added directly to the incubation mixture or given to the rats shortly before the administration of pentobarbital *et al.* It would therefore appear that many drugs cause the biphasic effects on the microsomal drug-metabolizing enzymes, chlorcyclizine, phenaglycodol, thiopental, hydroxyzine, benadryl, zoxazolamine, phenylbutazone, SKF 525A, DPEA, MG 3062.

There is no direct relationship between these effects and glutethimide, phenaglycodol and thiopental stimulate the drug metabolism more markedly than SKF 525A, DPEA and MG 3062, and the latter compounds inhibit the drug metabolisms more markedly than the former. Phenobarbital, meprobamate and urethan have strong stimulatory action, but they have no or only very weak inhibitory action, while JB 516, ipronizaide, imipramine and azacyclonol have strong inhibitory action, but they have no stimulating action.

The results of the present studies are of practical importance in the evaluation of the action of two compounds administered simultaneously or after short time intervals.

THE ADMINISTRATION of a number of compounds to rats causes biphasic effects on the metabolism of barbituarates. For example, Serrone and Fujimoto[1] found hexobarbital metabolism is inhibited when MPDC (N-methyl-3-piperidyl-(N′,N′)-diphenyl carbamate) is given 1–6 hr before the barbiturate, but is accelerated when MPDC is administered 24–48 hr before hexobarbital. Similarly, SKF 525A(β-diethylaminoethyl-diphenylpropylacetate HCl),[2–4] Lilly 18947 (2,4-dichloro-6-phenylphenoxyethyl-N-N-diethylamine HBr),[5, 6] DPEA (2,4-dichloro-6-phenylphenoxyethylamine)[6] and MG 3062 (phenyl-(4-chlorophenyl)-4-piridylmethanol)[7] are known to prolong the action of a variety of drugs, such as hexobarbital, pentobarbital, meprobamate and strychnine

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by blocking liver microsomal enzyme systems, but we recently reported that these inhibitors can stimulate barbiturate metabolism when they are given to rats 1–2 days before the drugs.[8] Moreover, pretreatment of rats with chlorcyclizine,[9] glutethimide,[3, 4, 10–14] or phenaglycodol enhance the metabolism of barbiturates and wide variety of other drugs, but when these compounds are added to incubation mixtures containing rat liver microsomes, they strongly inhibit the metabolism of pentobarbital and meprobamate.[15]

These findings suggested the possibility that biphasic effects on the drug metabolizing enzymes in liver microsomes might be caused by all of the compounds which accelerate drug metabolism in rats. Evidence presented in this paper shows that a wide variety of compounds in rats produce biphasic alterations in the metabolism of pentobarbital, meprobamate and carisoprodol. However, there is no correlation between the effectiveness of a compound in inhibiting drug metabolism 1–6 hr after its administration and its effectiveness in accelerating drug metabolism 24–48 hr after its injection. Some compounds, such as SKF525 A and DPEA, strongly inhibit drug metabolism during the first phase, and accelerate drug metabolism only slightly during the second phase. In contrast, other compounds such as glutethimide and phenaglycodol inhibit drug metabolism only slightly during the first phase and markedly stimulate the drug metabolism during the second phase. The evidence further shows that many compounds added to incubation mixtures are potent inhibitors of drug metabolism, but that some of these compounds in rat alters the activity of the drug metabolizing enzymes only slightly, whereas others markedly enhance the activity of these enzymes.

EXPERIMENTAL

Determination of the metabolism of pentobarbital, meprobamate and carisoprodol in living rats

Female rats (Sprague-Dawley strain, 110 g) were treated with SKF 525A, DPEA, MG 3062, chlorcyclizine, glutethimide or phenaglycodol. At various times thereafter pentobarbital (20 mg/kg), meprobamate (150 mg/kg) and carisoprodol (150 mg/kg) were administered (i.p.). The animals were then killed 1 or 3 hr later and their serum and brain level of pentobarbital, meprobamate and carisoprodol were determined.

Treatment of animals for in vitro *experiments*

Male rats (Sprague-Dawley strain, 80 g) received various drugs intraperitoneally. The animals were killed 48 hr later by decapitation and their livers removed immediately.

Enzyme assays

The livers of 3–5 rats were pooled and homogenized with 3 volumes of 1·5% KCl using a Potter-Elvehjem type homogenizer. The homogenate was centrifuged at 8,500 *g* for 15 min to sediment nuclei and mitochondria. 3 ml of the supernatant fraction were mixed with 20 μmole glucose-6-phosphate, 0·4 μmole TPN, 50 μmole nicotinamide and 75 μmole $MgCl_2$ and 100 μmole KCl, 1·3 ml of either 0·1 M phosphate buffer (pH 7·4) or 0·1 M tris-buffer (pH 8·2) (for strychnine), 0·2 ml of the substrates and water to a final volume of 5 ml. The final concentrations of hexobarbital, pentobarbital, meprobamate, carisoprodol and strychnine were 4×10^{-4}, 2×10^{-4},

3×10^{-4}, 3×10^{-4}, and 2×10^{-4}, respectively. The inhibitors were given in volume of 0·1 ml instead of 0·1 ml of the buffers. Some inhibitors, which could not be dissolved in distilled water, were dissolved in propylenglycol and diluted with distilled water. The maximum finale concentration of propylenglycol was 0·3%, such concentration itself did not produce significant inhibition and addition of propylenglycol up to the final concentration of 0·3% did not potentiate the action of the inhibitory drugs which is easily dissolved in water. The mixtures were incubated for 1 hr in an atmosphere of air at 37°. 2 ml aliquots of the reaction mixtures were then assayed for the amount of unchanged substrates.

In some experiments the liver was sliced with a microtome and the liver slices (500 mg) were suspended in a Warburg-flask which contained 6 ml of Krebs phosphate buffered Ringer (pH 7·4 or pH 8·2 for strychnine) and 0·2 ml of the substrates and incubated in a Dubnoff shaking incubator in an atmosphere of oxygen at 37° for 1 hr. The reaction mixtures were then homogenized and 2 ml aliquots were assayed for estimating the disappearance of the substrates. The microsomes were separated with the centrifuge (10,500 *g*, 60 min) by using isotonic sucrose solution (0·25 M) and the activity of microsomal TPNH oxidase was determined according to the methods of Gillette *et al.*[16]

Chemical procedure. The determination of the concentration of hexobarbital, pentobarbital, meprobamate, carisoprodol and strychnine in the incubation mixture and in the serum and brain were carried out according to the methods of Cooper and Brodie,[17] Brodie *et al.*,[18] Hoffmann and Ludwig,[19] Kato *et al.*,[20] and Kato *et al.*[21] respectively. Microsomal RNA and protein were determined according to the methods of Schneider[22] and Lowry *et al.*[23] respectively.

RESULTS

Inhibition and stimulation of the in vivo *metabolism of hexobarbital, pentobarbital, meprobamate and carisoprodol by various compounds*

As shown in Fig. 1, the rate of metabolism of pentobarbital in rats was markedly inhibited 30 min after the administration of SKF 525A, DPEA, chlorcyclizine, glutethimide and phenaglycodol (the first phase, the inhibitory phase). However, the rate of metabolism of the barbiturate returned to the normal range 12 hr later, and was markedly enhanced 48 hr after administration of these compounds (the second phase, the stimulatory phase).

It is of interest to note that SKF 525A and DPEA more strongly inhibited the pentobarbital metabolism than glutethimide and phenaglycodol; in contrast glutethimide and phenaglycodol more markedly increased the pentobarbital metabolism than SKF 525A and DPEA.

A similar biphasic variation in meprobamate metabolism was also observed (Fig. 2). Again, the metabolism of meprobamate was more markedly inhibited by DPEA and MG 3062 than by glutethimide and phenaglycodol; whereas it was more markedly increased by glutethimide and phenaglycodol than by DPEA and MG 3062. Figure 3 shows the typical contrast results in the decreasing rate of the serum concentration of pentobarbital, between the rats pretreated 30 min before with DPEA and chlorcyclizine and the rats pretreated 48 hr before. The decreasing rate of the serum pentobarbital concentration was very slow in the rats pretreated 30 min before with

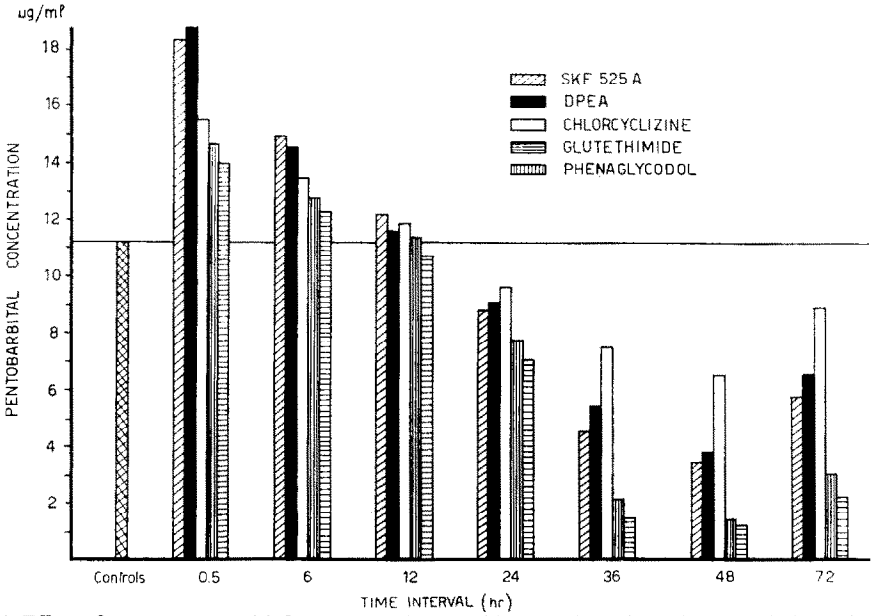

FIG. 1. Effect of pretreatment with SKF 525 A, DPEA, chlorcyclizine, glutethimide and phenaglycodol on the *in vivo* metabolism of pentobarbital.

SKF 525 A (50 mg/kg), DPEA (50 mg/kg), chlorcyclizine (25 mg/kg), glutethimide (40 mg/kg) and phenaglycodol (60 mg/kg) were injected intraperitoneally. Pentobarbital (20 mg/kg) were intraperitoneally administered at different time intervals after the pretreatments and the serum pentobarbital concentrations were determined 1 hr later. Female rats, weighing about 110 g were used. The values given represent averages obtained from at least six animals.

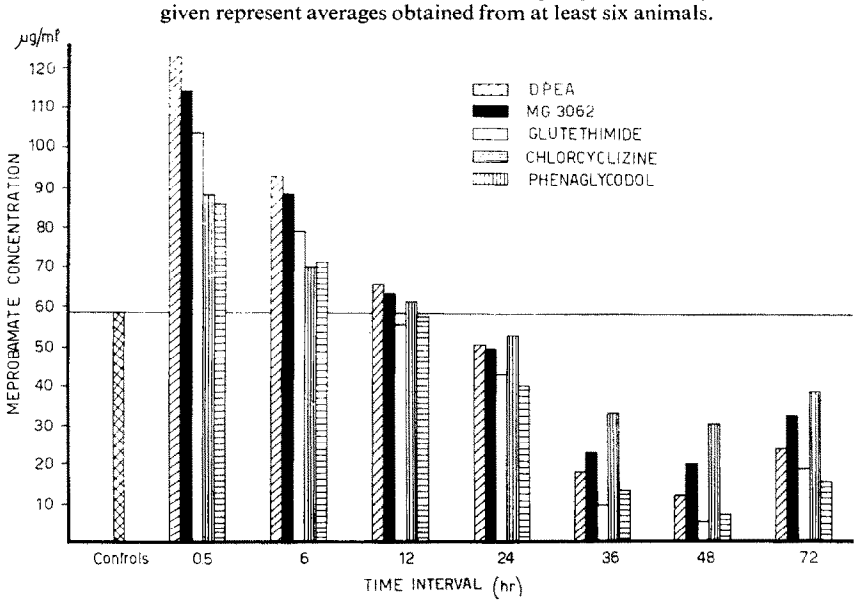

FIG. 2. Effect of pretreatment with DPEA, MG 3062, glutethimide, chlorcyclizine and phenaglycodol on the *in vivo* metabolism of meprobamate.

DPEA (50 mg/kg), MG 3062 (50 mg/kg), glutethimide (40 mg/kg), chlorcyclizine (25 mg/kg) and phenaglycodol (60 mg/kg) were intraperitoneally injected. Meprobamate (150 mg/kg) were intraperitoneally administered at different time intervals after the pretreatments and the serum meprobamate concentrations were determined 3 hr later. Female rats, weighing about 110 g were used. The values given represent averages obtained from at least six animals.

DPEA and chlorcyclizine (showing with I after the drugs) and, on the contrary, in the rats pretreated 48 hr before (II) the very fast decreasing rate was observed.

Figures 4 and 5 show the similar results concerning the decreasing rate of the serum concentration of meprobamate and carisoprodol in the rats pretreated with SKF

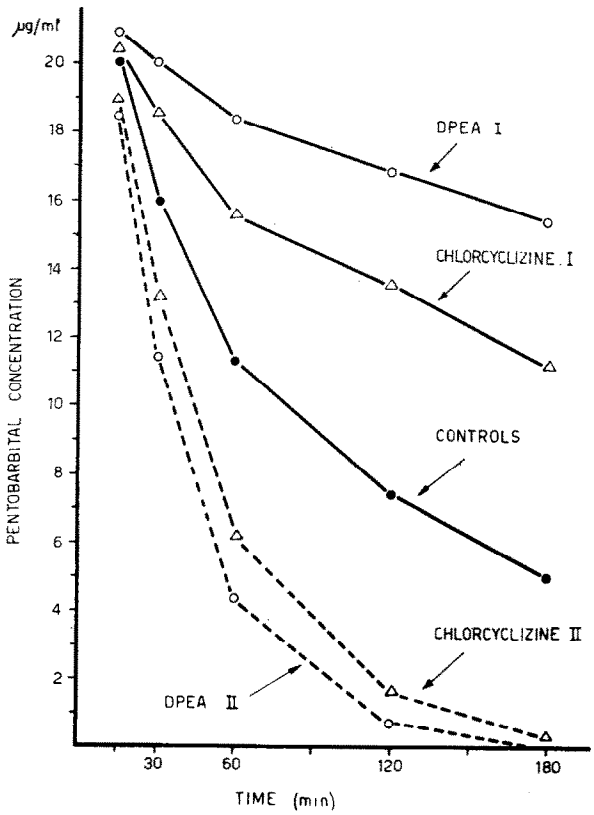

FIG. 3. Inhibitory and stimulatory action of DPEA and chlorcyclizine on the *in vivo* metabolism of pentobarbital.

Pentobarbital (20 mg/kg, i.p.) were administered 30 min or 48 hr after the pretreatment with DPEA (50 mg/kg) or chlorcyclizine (25 mg/kg). The serum concentrations were determined 15, 30, 60, 120 and 180 min later. I and II after the compounds indicate types of the pretreatments (I, 30 min before; II, 48 hr before). The female rats, weighing about 110 g were used. The values given represent averages obtained from at least four animals.

525A, glutethimide, MG 3062 or phenaglycodol. The inhibitory and stimulatory actions of other compounds on the *in vivo* metabolism of pentobarbital and meprobamate are given in Table 1.

Well known inhibitors, such as Lilly 18947, CFT 1201[24] (diethylaminoethyl-phenyldiallyl acetate), U-320 (4,5-dihydro-6-methyl-2-(2-(4-piperidyl))-3-pyridazine) markedly inhibited the metabolisms of pentobarbital and meprobamate 30 min after the administration, and they also markedly stimulated the metabolism 48 hr after the administration.

On the contrary, imipramine and phenylisopropylhydrazine inhibited the metabolisms of the drugs 30 min after the administration, but they did not increase the metabolisms 48 hr later. Although phenobarbital, the most potent stimulator, has no inhibitory action, it had marked stimulatory action. Triparanol, chlorpromazine did not significantly inhibit the *in vivo* metabolism, but they significantly stimulate it.

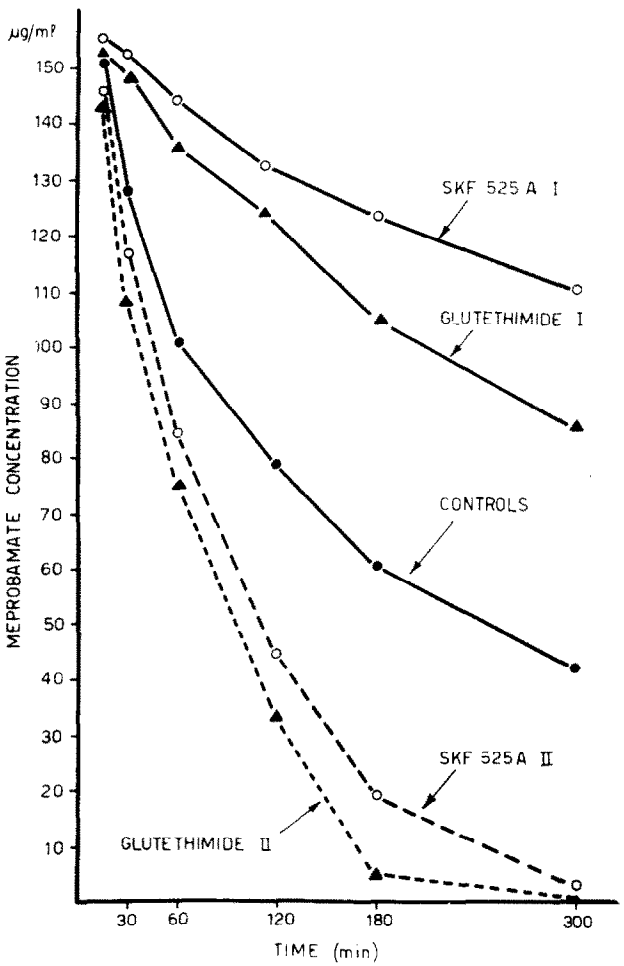

FIG. 4. Inhibitory and stimulatory action of SKF 525 A and glutethimide on the *in vivo* metabolism of meprobamate.

Meprobamate (150 mg/kg, i.p.) were administered 30 min or 48 hr after the pretreatment with SKF 525 A (50 mg/kg) or glutethimide (40 mg/kg). The values given represent averages obtained from at least four animals.

INHIBITION AND STIMULATION OF THE *IN VITRO* METABOLISM OF THE HEXOBARBITAL, MERPROBAMATE, CARISOPRODOL AND STRYCHNINE BY VARIOUS COMPOUNDS

The ability of a compound to inhibit the drug microsomal enzymes either when it is added to incubation mixtures or when it is administered to animals does not appear to be related to its ability to enhance the activity of the drug metabolizing enzymes during the second phase.

The compounds in Group A markedly inhibit these enzymes when added to the incubation mixture, but markedly stimulated the activity of the enzymes when administered to rats 48 hr before the enzymes were assayed (Table 2a and 2b). The compounds in Group B have a marked inhibitory action, but little if any stimulatory action (Table 3a and 3b). The compounds in Group C elicit little or no inhibitory

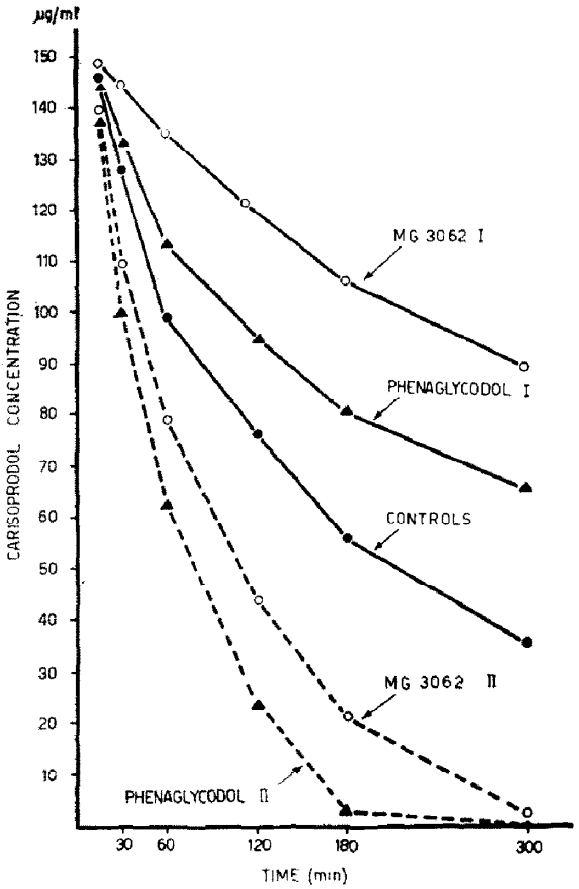

FIG. 5. Inhibitory and stimulatory action of MG 3062 and phenaglycodol on *in vivo* metabolism of carisoprodol.

Carisoprodol (150 mg/kg i.p.) were administered 30 min or 48 hr after the pretreatment with MG 3062 (50 mg/kg) or phenaglycodol (60 mg/kg). The values given represent averages obtained from at least four animals.

action but produce a marked stimulatory effect (Table 4a and 4b). Finally a number of compounds cause neither an inhibitory effect nor a stimulatory action; these include morphine (20 mg/kg), amphetamine (40 mg/kg), methylphenidate (25 mg/kg), bemegride (10 mg/kg), thalidomide (300 mg/kg), lidocaine (40 mg/kg), procainamide (50 mg/kg), alcohol (2500 mg/kg), chloralhydrate (150 mg/kg), myanesine (140 mg/kg), strychnine sulphate (1 mg/kg), caffeine (100 mg/kg), aspirine (300 mg/kg) and adrenaline hydrochloride (1 mg/kg) (The doses used in the treatment given in the brackets.)

These results are well corresponding with that obtained in the *in-vivo* experiments. Phenobarbital, barbital, primidone and meprobamate have strong stimulatory action but they have only a weak inhibitory action on the *in-vitro* drug metabolism. On the contrary, hydrazine derivatives, such as W 1927, U 16392A and JB 516 and iproniazide, imipramine and azacyclonol have strong inhibitory action but they have no stimulatory action on the *in-vitro* metabolism of the drugs.* It is also observed that the inhibitory action of the compounds on the drug-metabolism in the liver slice preparation were

TABLE 1. INHIBITORY AND STIMULATORY ACTIONS OF LILLY 18947, CFT 1201, U-320, HYDROXIZINE THIOPENTAL, TRIPARANOL, BENADRYL, PHENYLISOPROPYLHYDRAZINE, IMIPRAMINE, CHLORPROMAZINE AND PHENOBARBITAL ON *in-vivo* METABOLISMS OF PENTOBARBITAL AND MEPROBAMATE.

Pretreatment	Dose, mg/kg	Serum pentobarbital concentration, μg/ml Interval between pretreatment and pentobarbital injection		Serum meprobamate concentration, μg/ml Interval between pretreatment and meprobamate injection	
		30 min	48 hr	30 min	48 hr
(1) Controls	—	11·4±0·3	11·6±0·2	59±3·8	63±2·9
(2) Lilly 18947	40	17·2±0·5*	6·2±0·2*	108±5·9*	22±1·3*
(3) CFT 1201	50	17·9±0·7*	7·6±0·3*	107±5·3*	31±2·9*
(4) U-320	400	15·8±0·6*	8·2±0·1*	82±6·1*	32±2·4*
(5) Hydroxyzine	80	14·3±0·5*	7·2±0·4*	86±6·0*	23±2·6*
(6) Thiopental	25	—	3·3±0·1*	74±4·3*	8±0·5*
(7) Triparanol	50	12·4±0·3*	9·5±0·4*	67±3·1	45±3·1*
(8) Benadryl	25	13·7±0·2*	7·3±0·2*	76±4·4*	30±2·4*
(9) Chlorpromazine	10	12·1±0·4	7·6±0·2*	71±3·8	36±3·0*
(10) Phenylisopropyl-hydrazine (JB516)	15	17·2±0·9*	12·6±0·5	101±6·1*	74±6·5
(11) Imipramine	20	13·8±0·4*	11·4±0·3	88±5·8*	65±4·9
(12) Phenobarbital	60	—	0·9±0·1*	64±6·9	0*

Female rats, weighing about 100 gr were used.
Serum pentobarbital concentration was determined 1 hr after the administration (20 mg/kg), while serum meprobamate concentration was determined 3 hr after the administration (150 mg/kg). Pentobarbital and meprobamate were administered 30 min after the treatment with various compounds to investigate the inhibitory activity while they were administered 48 hr after the treatment to investigate the stimulating action. The values given represent averages ± standard errors obtained from at least four determinations.
* = Significative difference ($p < 0.05$) to the controls.

generally 2–3 times weaker than that in the liver microsomal preparation. For example, 1.7×10^{-5} M of SKF 525A produce 50 per cent inhibition of meprobamate metabolism, in the liver microsomal preparation, while in the liver slice preparation 4.6×10^{-5} M of the inhibitor is required for causing 50 per cent inhibition.

EFFECT OF COMBINED ADMINISTRATION OF THE TWO COMPOUNDS ON THE STIMULATION OF THE *IN-VITRO* METABOLISM OF HEXOBARBITAL AND LIVER ENLARGEMENT

Effect of combined administration of the inhibitor and the stimulator of the drug metabolism on the stimulation of the *in-vitro* metabolism of hexobarbital was investigated.

* Phenylpropylcyclamine have strong inhibitory action on the *in-vitro* metabolism of the drugs, but they have no inhibitory action on the *in-vivo* drug metabolism and also have no stimulatory action.[26]

In all cases of the combined administration of two drugs the increased effect of the stimulation of the *in vitro* metabolism of hexobarbital was observed. The effect of phenobarbital was, especially, markedly potentiated by DPEA and SKF 525A. For example, 30 mg/kg of phenobarbital induced an increase of 88 per cent of hexobarbital metabolism and 25 mg/kg of DPEA and SKF induced an increase of 45 and 31 per cent, but the combined administration of DPEA and phenobarbital induced an increase of 176 per cent and the combined administration of SKF 525A + phenobarbital induced an increase of 147 per cent (Table 5).

These potentiating effects may be partially due to the direct stimulatory effect of DPEA and SKF 525A on the microsomes and also partially due to the inhibition of phenobarbital metabolism.

TABLE 2a. INHIBITION AND STIMULATION OF PENTOBARBITAL, HEXOBARBITAL, MEPROBAMATE, CARISOPRODOL AND STRYCHNINE BY GROUP A COMPOUNDS. (Part a, Inhibition).

	Inhibition of drug metabolism (IC^{50})				
Compound	Pentobarbital	Hexobarbital	Meprobamate	Carisoprodol	Strychnine
(1) DPEA	$5{\cdot}8\times10^{-6}$	$1{\cdot}2\times10^{-5}$	$9{\cdot}3\times10^{-6}$	$3{\cdot}5\times10^{-5}$	—
(2) MG 3062	$7{\cdot}8\times10^{-6}$	$1{\cdot}2\times10^{-5}$	$1{\cdot}2\times10^{-5}$	$1{\cdot}9\times10^{-5}$	$3{\cdot}2\times10^{-5}$
(3) SKF 525A	$1{\cdot}8\times10^{-5}$	$3{\cdot}4\times10^{-5}$	$1{\cdot}7\times10^{-5}$	$2{\cdot}6\times10^{-5}$	$6{\cdot}7\times10^{-5}$
(4) Chlorcyclizine	$3{\cdot}1\times10^{-5}$	$4{\cdot}3\times10^{-5}$	$2{\cdot}2\times10^{-5}$	$2{\cdot}7\times10^{-5}$	—
(5) Cyclizine	$2{\cdot}4\times10^{-5}$	$6{\cdot}2\times10^{-5}$	$2{\cdot}9\times10^{-5}$	$2{\cdot}8\times10^{-5}$	—
(6) CFT 1201	$2{\cdot}2\times10^{-5}$	$5{\cdot}4\times10^{-5}$	$2{\cdot}1\times10^{-5}$	$2{\cdot}4\times10^{-5}$	—
(7) Lilly 18947	$4{\cdot}4\times10^{-5}$	$1{\cdot}9\times10^{-4}$	$5{\cdot}2\times10^{-5}$	—	—
(8) Benadryl	$7{\cdot}3\times10^{-5}$	$4{\cdot}3\times10^{-5}$	$6{\cdot}7\times10^{-5}$	$9{\cdot}7\times10^{-5}$	—
(9) Thiopental	—	—	$5{\cdot}1\times10^{-5}$	$4{\cdot}8\times10^{-5}$	$1{\cdot}9\times10^{-4}$
(10) Benzemalacene	—	—	$6{\cdot}1\times10^{-5}$	$8{\cdot}0\times10^{-5}$	$6{\cdot}8\times10^{-4}$
(11) Glutethimide	$8{\cdot}8\times10^{-5}$	$3{\cdot}6\times10^{-4}$	$6{\cdot}5\times10^{-5}$	$7{\cdot}5\times10^{-5}$	$1{\cdot}8\times10^{-4}$
(12) Phenaglycodol	$1{\cdot}3\times10^{-4}$	$1{\cdot}8\times10^{-4}$	$9{\cdot}8\times10^{-5}$	$8{\cdot}7\times10^{-5}$	$1{\cdot}7\times10^{-4}$
(13) U-320	$1{\cdot}2\times10^{-4}$	$3{\cdot}8\times10^{-4}$	$1{\cdot}0\times10^{-4}$	$1{\cdot}8\times10^{-4}$	—
(14) Meclizine	$9{\cdot}0\times10^{-5}$	$5{\cdot}6\times10^{-4}$	$2{\cdot}2\times10^{-4}$	$2{\cdot}4\times10^{-4}$	—
(15) Hydroxyzine	$1{\cdot}0\times10^{-4}$	$5{\cdot}8\times10^{-4}$	$2{\cdot}1\times10^{-5}$	$1{\cdot}8\times10^{-4}$	—
(16) Zoxazolamine	$2{\cdot}6\times10^{-4}$	$4{\cdot}9\times10^{-4}$	$7{\cdot}0\times10^{-5}$	$9{\cdot}8\times10^{-5}$	$6{\cdot}6\times10^{-4}$
(17) Phenylbutazone	—	—	$2{\cdot}8\times10^{-4}$	$1{\cdot}9\times10^{-4}$	$3{\cdot}1\times10^{-4}$
(18) Mer 29	$1{\cdot}9\times10^{-4}$	$8{\cdot}1\times10^{-4}$	$1{\cdot}8\times10^{-4}$	$1{\cdot}6\times10^{-4}$	—
(19) Pentobarbital	—	—	$2{\cdot}1\times10^{-4}$	$1{\cdot}8\times10^{-4}$	$5{\cdot}1\times10^{-4}$
(20) Chlorproapamide	—	—	$2{\cdot}2\times10^{-4}$	$2{\cdot}9\times10^{-4}$	$<2\times10^{-3}$
(21) Chlorpromazine	$1{\cdot}6\times10^{-4}$	$2{\cdot}9\times10^{-4}$	$2{\cdot}3\times10^{-4}$	$3{\cdot}1\times10^{-4}$	—
(22) Perphenazine	$1{\cdot}6\times10^{-4}$	$6{\cdot}8\times10^{-4}$	$2{\cdot}3\times10^{-4}$	—	—
(23) Triflupromazine	$2{\cdot}8\times10^{-4}$	$7{\cdot}9\times10^{-4}$	$3{\cdot}0\times10^{-4}$	—	—
(24) Chloretone	$2{\cdot}4\times10^{-4}$	$7{\cdot}3\times10^{-4}$	$3{\cdot}4\times10^{-4}$	$3{\cdot}6\times10^{-4}$	—
(25) Niketamide	$5{\cdot}2\times10^{-4}$	$9{\cdot}8\times10^{-4}$	$7{\cdot}8\times10^{-4}$	$7{\cdot}8\times10^{-4}$	—

Male rats weighing about 80 g were used. The inhibitory actions were expressed by the concentration causing inhibition of 50 per cent.

The stimulatory effect produced by the combined administration of two inhibitors (DPEA + SKF 525A) was markedly greater than that produced by the administration of either. A marked increase of liver weight was observed in the rats pretreated with the combination of the two compounds (Table 6). It is surprising that the liver weight is increased 33 per cent and 25 per cent within 48 hr in rats pretreated with DPEA + nikethamide and SKF 525A + phenobarbital respectively. Thus the whole liver enzyme activity was markedly increased in the rats pretreated with the combination of two drugs. For example, whole liver enzyme activity was increased 121 per cent and 257 per cent by treatment with phenobarbital and phenobarbital + DPEA

TABLE 2b. INHIBITION AND STIMULATION OF PENTOBARBITAL, HEXOBARBITAL, MEPROBAMATE, CARISOPRODOL AND STRYCHNINE BY GROUP A COMPOUNDS. (Part b, Stimulation).

		Stimulation of drug metabolism (Control-100)				
Compound	Dose mg/kg	Pento-barbital	Hexo-barbital	Mepro-bamate	Cariso-podol	Strychnine
(1) DPEA	40	163	174	165	175	163
(2) MG 3062	40	163	158	172	165	158
(3) SKF 525A	40	159	145	150	159	164
(4) Chlorcyclizine	20	169	178	152	—	140
(5) Cyclizine	20	163	155	158	—	171
(6) CFT 1201	60	153	144	143	137	151
(7) Lilly 18947	40	151	138	141	135	146
(8) Benadryl	25	163	149	158	172	164
(9) Thiopental	28	179	183	183	171	169
(10) Benzmalacene	50	119	128	121	107	123
(11) Glutethimide	60	208	196	201	189	193
(12) Phenaglycodol	100	216	209	221	213	198
(13) U-320	300	152	139	141	145	137
(14) Meclizine	20	145	158	—	149	—
(15) Hydroxyzine	50	137	145	149	135	151
(16) Zoxazolamine	100	168	173	185	162	155
(17) Phenylbutazone	100	181	193	183	178	171
(18) Mer 29	70	131	129	143	131	135
(19) Pentobarbital	25	170	178	163	162	172
(20) Chlorpropamide	100	155	142	153	—	139
(21) Chlorpromazine	12	143	146	152	—	140
(22) Perphenazine	12	148	152	149	—	158
(28) Triflupromazine	12	158	149	163	149	152
(24) Chloretone	50	149	155	149	158	157
(25) Niketamide	150	158	164	159	163	148

Male rats weighing about 80 g were used.
The stimulatory actions were determined 48 hr after the administrations of compounds.
The values given represent averages obtained from at least three determinations.

TABLE 3a. INHIBITION AND STIMULATION OF PENTOBARBITAL, HEXOBARBITAL, MEPROBAMATE, CARISOPRODOL AND STRYCHNINE BY GROUP B COMPOUNDS. (Part a, Inhibition).

	Inhibition of drug metabolism (IC^{50})				
Compound	Pentobarbital	Hexobarbital	Meprobamate	Carisoprodol	Strychnine
(1) W 1927*	$1{\cdot}8\times10^{-5}$	$3{\cdot}9\times10^{-5}$	$3{\cdot}9\times10^{-5}$	$2{\cdot}1\times10^{-5}$	—
(2) Phenylpropyl-cyclamine	$1{\cdot}7\times10^{-5}$	$5{\cdot}2\times10^{-5}$	$1{\cdot}3\times10^{-5}$	$5{\cdot}2\times10^{-5}$	$3{\cdot}1\times10^{-4}$
(3) U 16392A†	$2{\cdot}2\times10^{-5}$	—	$5{\cdot}3\times10^{-5}$	—	—
(4) JB 516	$4{\cdot}3\times10^{-5}$	$1{\cdot}2\times10^{-4}$	$1{\cdot}6\times10^{-4}$	$1{\cdot}1\times10^{-4}$	$7{\cdot}1\times10^{-4}$
(5) Imipramine	$5{\cdot}3\times10^{-5}$	$3{\cdot}1\times10^{-4}$	$1{\cdot}9\times10^{-4}$	$1{\cdot}3\times10^{-4}$	—
(6) Azacylonol	$7{\cdot}9\times10^{-5}$	$7{\cdot}4\times10^{-4}$	$9{\cdot}3\times10^{-5}$	$1{\cdot}1\times10^{-4}$	$4{\cdot}8\times10^{-4}$
(7) Parathion	$3{\cdot}6\times10^{-4}$	$6{\cdot}4\times10^{-4}$	$3{\cdot}4\times10^{-4}$	$3{\cdot}5\times10^{-4}$	$4{\cdot}8\times10^{-4}$
(8) Iproniazide	$3{\cdot}9\times10^{-4}$	$7{\cdot}8\times10^{-4}$	$2{\cdot}6\times10^{-4}$	$1{\cdot}8\times10^{-4}$	$9{\cdot}8\times10^{-4}$
(9) Chlorpheniramine	$3{\cdot}6\times10^{-4}$	$1{\cdot}8\times10^{-3}$	$4{\cdot}0\times10^{-4}$	$4{\cdot}2\times10^{-4}$	—
(10) Pipradol	$3{\cdot}1\times10^{-4}$	$1{\cdot}5\times10^{-3}$	$5{\cdot}6\times10^{-4}$	$5{\cdot}6\times10^{-4}$	—
(11) Isoniazide	$3{\cdot}9\times10^{-4}$	$4{\cdot}5\times10^{-3}$	$7{\cdot}8\times10^{-5}$	$9{\cdot}8\times10^{-5}$	—

* W 1927 = *p*-chlorbenzylhydrazine.
† U 16392A = *o*-chloro-α-methylphenethylhydrazine.
The experimental conditions are same in Table 2.

TABLE 3b. INHIBITION AND STIMULATION OF PENTOBARBITAL, HEXOBARBITAL, MEPROBAMATE, CARISOPRODOL AND STRYCHNINE BY GROUP B COMPOUNDS. (Part b, Stimulation).

	Stimulation of drug metabolism (Control = 100)					
Compound	Dose mg/kg	Pento-barbital	Hexo-barbital	Mepro-bamate	Cariso-prodol	Strychnine
(1) W 1927	15	93	97	95	90	97
(2) Phenylpropyl-cyclamine	10	103	94	97	103	93
(3) U 16392A	15	105	97	—	—	—
(4) JB 516	15	93	98	94	83	102
(5) Imipramine	20	104	93	108	91	98
(6) Azacyclonol	100	103	110	113	97	106
(7) Parathion	1·5	95	92	103	—	—
(8) Iproniazide	60	106	101	103	96	99
(9) Chlorpheniramine	25	105	98	104	—	90
(10) Pipradol	20	95	99	94	—	—
(11) Isoniazide	100	105	112	100	105	96

TABLE 4a. INHIBITION AND STIMULATION OF PENTOBARBITAL, HEXOBARBITAL, CARISOPRODOL, AND STRYCHNINE BY GROUP C COMPOUNDS. (Part a, Inhibition).

	Inhibition of drug metabolism, per cent									
Compound	Pentobarbital		Hexobarbital		Meprobamate		Carisoprodol		Strychnine	
	1	2	1	2	1	2	1	2	1	2
(1) Phenobarbital	—		—		28	49	23	45	10	15
(2) Barbital	—		—		0	15	5	13	7	19
(3) Primidone	—		—		23	51	—		18	42
(4) Diphenyl-hydantoine	—		—		20	41	—		23	44
(5) Tolubutamide	—		—		24	59	25	41	4	12
(6) Aminopyrine	10	27	4	13	21	38	14	31	—	
(7) Meprobamate	7	19	3	12	—		—		10	21
(8) Carisoprodol	13	27	0	17	—		—		13	29
(9) Urethan	3	7	0	3	—		—		3	0
(10) Methylpentynol-carbamate	14	26	4	3	—		—		4	12

The inhibitory actions expressed in percentage of the inhibition with concentration of 2×10^{-4} and 5×10^{-4} respectively. The values given represent two results obtained from each experiment.

respectively. Slightly increased liver microsomal protein was observed in the rats pretreated with two drugs, but it was not significant. On the other hand, liver microsomal TPNH oxidase was increased markedly in the rats pretreated with the two compounds. This result confirms the observation made by Conny *et al.*[9] that a repeated administration of phenobarbital to rats increases microsomal TPNH-oxidase activity.

DISCUSSION

In the present studies, it is demonstrated that a variety of drugs cause biphasic responses on the liver microsomal enzyme activity. They block the oxidation of drugs in the first phase and stimulate the oxidation in the second phase.

For example, many inhibitors of the drug metabolism, such as SKF 525A, Lilly 18947, DPEA, MG 3062, CFT 1201 and U-320, induce an increased activity of the microsomal drug-metabolizing enzyme 48 hr after the administration; on the other hand, many inducers of the microsomal drug-metabolizing enzyme, such as glutethimide, phenaglycodol, chlorcyclizine, phenylbutazone, thiopental, chlorpromazine, zoxazolamine, chloretone and nikethamide produce an inhibition of the drug metabolisms if they are added to the incubation medium or administered to rats 30 min before the assay of the enzymes.

TABLE 4b. INHIBITION AND STIMULATION OF PENTOBARBITAL, HEXOBARBITAL, CARISOPRODOL AND STRYCHNINE BY GROUP C COMPOUNDS. (Part b, Stimulation).

		Stimulation of drug metabolism (controls = 100)				
Compounds	Dose mg/kg	Pento-barbital	Hexo-barbital	Mepro-bamate	Cariso-prodol	Strychnine
(1) Phenobarbital	70	259	272	263	281	271
(2) Barbital	130	195	184	178	202	189
(3) Primidone	130	158	164	169	—	181
(4) Diphenyl-hydantoine	90	145	131	146	—	152
(5) Tolubutamide	100	139	143	143	154	133
(6) Aminopryine	100	143	135	135	—	143
(7) Meprobamate	160	151	145	159	159	145
(8) Carisoprodol	160	158	163	158	155	139
(9) Urethan	600	135	139	143	133	145
(10) Methylpentynol-carbamate	120	121	129	134	—	122

The values given represent averages obtained from at least three determinations.

TABLE 5. EFFECT OF THE COMBINED ADMINISTRATION OF TWO COMPOUNDS ON THE STIMULATION OF THE *in vitro* METABOLISM OF HEXOBARBITAL.

	Number of determination	Metabolism of Hexobarbital (μg/g/h)	Variation, per cent
(1) Controls	8	251 ± 5	
(2) DPEA	5	364 ± 9	+45
(3) MG 3062	5	371 ± 7	+48
(4) SKF 525A	5	325 ± 12	+31
(5) Chlorcyclizine	3	326 ± 8	+31
(6) Glutethimide	3	379 ± 12	+51
(7) Phenobarbital	6	472 ± 7	+88
(8) DPEA + Chlorcyclizine	4	467 ± 14	+80
(9) DPEA + Glutethimide	4	573 ± 15	+128
(10) DPEA + Phenobarbital	4	693 ± 12	+176
(11) SKF 525A + Glutethimide	4	538 ± 14	+114
(12) SKF 525A + Phenobarbital	4	621 ± 10	+147
(13) Chlorcyclizine + Glutethimide	3	531 ± 16	+112
(14) Chlorcyclizine + Phenobarbital	4	598 ± 10	+138
(15) Phenobarbital + Glutethimide	4	609 ± 9	+143
(16) DPEA + MG 3062	3	583 ± 13	133
(17) MG 3062 + SKF 525A	3	579 ± 13	131
(18) SKF 525A + DPEA	3	501 ± 16	100

25 mg/kg of DPEA, MG 3062 and SKF 525A, 12 mg/kg of chlorcyclizine, 30 mg/kg of phenobarbital and 40 mg/kg of glutethimide were administered alone or in combination 48 hr before the determination of hexobarbital metabolism.

The biphasic effects of drug on the microsomal drug-metabolizing enzyme was first reported by Serrone and Fujimoto in connection with the action of N-methyl-3-piperidyl-(N′,N′)-diphenylcarbamate HCl(MPDC). The present work not only confirms that MPDC caused a biphasic action on the microsomal drug-metabolizing enzymes but also shows that many other substances cause the biphasic effects on the

TABLE 6. EFFECT OF COMBINED ADMINISTRATION OF THE TWO COMPOUNDS ON THE STIMULATION OF THE GROWTH OF LIVER SIZE AND MICROSOMAL TPNH OXIDASE.

	Body weight, g	Liver weight, g	Liver weight, Body weight per cent	Variation, per cent	Variation of TPNH oxidase activity per cent
(1) Controls	75·3±1·2	3·31±0·18	4·53±0·15	—	—
(2) Phenobarbital	76·8±0·9	3·90±0·23	5·08±0·24	+12	+69
(3) Nikethamide	74·4±1·3	3·70±0·15	4·99±0·18	+10	+24
(4) DPEA	75·1±1·0	3·67±0·24	4·89±0·13	+8	+43
(5) SKF 525A	74·5±0·6	3·57±0·26	4·80±0·23	+6	+32
(6) MG 3062	76·2±1·4	3·76±0·19	4·94±0·18	+9	+29
(7) DPEA + Phenobarbital	75·5±1·9	4·29±0·34	5·68±0·21	+25	+95
(8) DPEA + Nikethamide	74·8±1·0	4·49±0·29	6·01±0·30	+33	+52
(9) SKF 525A + Phenobarbital	75·9±0·8	4·29±0·15	5·65±0·19	+25	+107
(10) SKF 525A + Nikethamide	75·5±1·3	4·16±0·24	5·25±0·23	+22	+49
(11) MG 3062 + Phenobarbital	76·3±0·9	4·36±0·29	5·72±0·24	+26	+85
(12) Phenobarbital + Nikethamide	74·6±1·0	3·80±0·30	5·09±0·18	+13	+89
(13) DPEA + MG 3062	75·1±1·5	4·10±0·25	5·46±0·15	+21	+83
(14) SKF 525A + MG 3062	75·8±1·1	4·04±0·17	5·33±0·17	+18	+79

50 mg/kg of phenobarbital, 90 mg/kg of nikethamide, 25 mg/kg of DPEA, SKF 525A and MG 3062 were given alone or in combination 48 hr before the sacrifice. Microsomal TPNH oxidase activity is expressed by the variation to the controls.

microsomal drug-metabolizing enzymes. Moreover, many compounds which have no marked inhibitory action; for example, morphine, alcohol, chloralhydrate, caffein, methylphenidate, thalidomide, megimide, aspirine, amphetamine, adrenaline have no stimulatory action. Although these findings suggested the possibility that the two effects are related, there are a number of compounds which do only one of the effects and not the other. For example, phenobarbital, barbital and urethan have little if any inhibitory action on the drug enzyme systems either in *in vivo* or *in vitro*,* but on the *in vitro* metabolisms these compounds are very effective in stimulating the activity of the microsomal enzymes. Moreover, meprobamate and carisoprodol have only very weak inhibitory action, but they have a marked stimulatory action. On the other hand, imipramine and azacyclonol have strong inhibitory action on the *in vitro* and *in vivo* metabolism, but they have no significant stimulatory action. Some hydrazine derivatives, such as W-1927, U-16392A, JB 516 and iproniazide also have only

* Remmer[27] observed a decrease in enzyme activity for hexobarbital metabolism in the liver microsomal preparation obtained from the rat pretreated with phenobarbital 2 hr or 4 hr before, but this result was not confirmed by Kato and Gillette[28].

inhibitory action. These results thus indicate that there is no clear relationship between ability for the inhibitory and the stimulatory. In general, however, compounds like SKF525A, DPEA, MG 3062 which are very potent inhibitors, accelerate drug metabolism only slightly during the second phases, whereas compound like phenaglycodol and glutethimide which have a potent stimulating action, inhibit the microsomal enzymes only slightly; Although there is no apparent relationship between the pharmacological activity of structure, and inhibitory or stimulatory activity of the compounds, there appears to be a relationship between the lipid solubility of a compound and its ability to inhibit or stimulate the microsomal enzymes. For example, MG 3062, glutethimide and phenaglycodol, are relative high lipo-soluble compounds and either inhibit or stimulate drug metabolism markedly, whereas the compounds such as alcohol, chloralhydrate, caffeine and aspirine are relative lipid insoluble compounds cause neither inhibition nor enhancement of drug metabolisms. On the other hand, in the present work, it is demonstrated that many pharmacological interesting compounds act as inhibitor and stimulator of the microsomal drug-metabolizing enzymes. These results are indicating that some observed pharmacological actions of the two drugs is not only a summary of actions at pharmacological level, but it must be a summary of actions at pharmacological level plus metabolic interference. Therefore, these result also give practical importance for the evaluation of the combined effect of two drugs in laboratory experiment and also probably in clinical using.

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REFERENCES

1. D. M. SERRONE and J. M. FUJIMOTO, *J. Pharmacol.* **133**, 12 (1961).
2. J. AXELROD, J. REICHENTHAL and B. B. BRODIE, *J. Pharmacol.* **112**, 49 (1954).
3. R. KATO and P. VASSANELLI, *Biochem. Pharmacol.* **11**, 779 (1962).
4. R. KATO, E. CHIESARA and P. VASSANELLI, *Biochem. Pharmacol.* **11**, 913 (1962).
5. J. R. FOUTS and B. B. BRODIE, *J. pharmacol.* **115**, 68 (1954).
6. R. KATO, P. VASSANELLI and E. CHIESARA, *Biochem. Pharmacol.* **12**, 353 (1963).
7. R. KATO, and E. CHIESARA, unpublished observation.
8. R. KATO, E. CHIESARO and P. VASSANELLI, *Med. Exp.* **6**, 254 (1962).
9. A. H. CONNEY, I. A. MAICHELSON and J. J. BURNS, *J. Pharmacol.* **132**, 202 (1961).
10. R. KATO, Atti Soc. *Lombarda Med.* **14**, 777 (1959).
11. R. KATO, *Med. Exp.* **3**, 95 (1960).
12. R. KATO, E. CHIESARA and G. FRONTINO, *Jap. J. Pharmacol.* **11**, 31 (1961).
13. R. KATO, and E. CHIESARA, *Brit. J. Pharmacol.* **18**, 29 (1962).
14. R. KATO, E. CHIESARA and P. VASSANELLI, *Biochem. Pharmacol.* **11**, 211 (1962).
15. R. KATO, P. VASSANELLI and E. CHIESARA, *Experimentia.* **18**, 452 (1962).
16. J. R. GILLETTE, B. B. BRODIE and B. N. LADU, *J. Pharmacol.* **119**, 532 (1957).
17. J. R. COOPER and B. B. BRODIE, *J. Pharmacol.* **114**, 409 (1955).

18. B. B. BRODIE, J. J. BURNS, L. C. MARK, P. A. LIEF, E. BERNSTEIN and E. M. PAPPER, *J. Pharmacol.* **109**, 266 (1953).
19. A. J. HOFFMAN and B. J. LUDWIG, *J. Amer. Pharm. Ass.* **68**, 740 (1959).
20. R. KATO, P. VASSANELLI, G. FRONTINO and A. BOLEGO, *Med. Exp.* **6**, 149 (1962).
21. R. KATO, E. CHIESARA and P. VASSANELLI, *Jap. J. Pharmacol.* **12**, 26 (1962).
22. W. C. Schneider, *J. biol. Chem.* **161**, 293 (1945).
23. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. Biol. Chem.* **193**, 265 (1951).
24. D. NEUBERT and H. HERKEN, *Arch. exp. Path. Pharmakol.* **225**, 453 (1955).
25. R. H. BULLER, W. T. ROCKHOLD, J. A. BUZARD and J. J. STERN, *J. Pharmacol.* **134**, 95 (1961).
26. R. KATO, E. CHIESARA and P. VASSANELLI, *Chemotherapia.* **4**, 611 (1962).
27. H. REMMER, *Metabolic factors controlling duration of drug action*, p. 235, Ed. B. B. Brodie, Pergamon Press (1962).
28. R. KATO and J. GILLETTE, unpublished observations.