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QUININE AND QUINIDINE INHIBITION OF PENTOBARBITAL METABOLISM*

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Abstract—The influence of quinine hydrochloride pretreatment on pentobarbital metabolism was assessed by measurements of sleeping times, LD_{50} values, hepatic microsomal metabolism and relative rates of decay of plasma levels of pentobarbital. These studies were conducted in Swiss–Webster mice, Sprague–Dawley rats and Angora or Toggenburg goats. Quinine increased sleeping time in phenobarbital-stimulated and unstimulated mice and in rats, decreased the LD_{50} of pentobarbital in mice, and increased the plasma half-life of pentobarbital in goats. Quinine in various concentrations inhibited the hepatic metabolism of pentobarbital in 9000 *g* supernatant incubation mixtures ($K_I = 2{\cdot}9 \times 10^{-5}$ M), as did quinidine ($K_I = 7{\cdot}6 \times 10^{-6}$ M). Duration of barbital hypnosis was not affected by quinine pretreatment. The presence of quinine in plasma decreased the extent of protein binding of pentobarbital in plasma from humans, ponies, swine, goats and mice. The pentobarbital concentration in brains from quinine-pretreated mice was not significantly different from the concentration in brains from control animals at awakening. The results obtained from these experiments indicate that quinine and quinidine alter the duration of action of pentobarbital by inhibition of microsomal enzymes.

WE OBSERVED, during the course of another investigation, that goats pretreated with quinine and anesthetized with pentobarbital slept much longer than goats treated with pentobarbital alone. This observation suggested a possible interaction of the two drugs on microsomal drug metabolism or on the sensitivity of receptors in the central nervous system.

Pentobarbital is almost completely transformed by hepatic microsomes in mice,[1] dogs,[2] man,[3] rabbits[4] and rats,[3] while barbital is excreted largely unchanged in the urine.[5] Quinine is metabolized by the liver,[6] but the subcellular locus of this biotransformation has not been established.

Quinine may be administered over prolonged periods of time for treatment of malaria, night cramps or myotonia congenita, while quinidine is an important antiarrhythmic agent. It is apparent, on the basis of chronic administration and the fact that some of these conditions are frequently treated with combinations of drugs, that potential drug interactions might be an important consideration in quinine or quinidine therapy.

In the present investigation, the effects of quinine pretreatment on sleeping time,

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toxicity, elimination and microsomal metabolism of pentobarbital were examined. The influence of quinine on barbital sleeping time was studied because of the possibility of interaction at the receptors.

EXPERIMENTAL

Animals. Female, nonpregnant, Swiss–Webster mice, 22–34 g in weight, male, Sprague–Dawley rats, 220–290 g, and Angora or Toggenburg goats of both sexes, 22–55 kg, were used. The rats and mice were allowed free access to Purina lab chow and water. Goats were provided with alfalfa hay and water which they consumed *ad lib.*

Drug pretreatments. Quinine hydrochloride (K & K Laboratories) dissolved in isotonic saline was administered intraperitoneally to mice and rats (50 mg/kg, calculated as free base) and intravenously to goats (7 mg/kg). Quinidine hydrochloride in saline was injected intraperitoneally in mice (50 mg/kg). Control animals were injected intraperitoneally with isotonic saline. Positive control animals for sleeping time experiments were treated intraperitoneally (i.p.) with phenobarbital at a dose of 40 mg/kg twice a day for 3 consecutive days.

Sleeping time determination. Sleeping times were determined after intraperitoneal administration of pentobarbital sodium (100 mg/kg in mice and 30 mg/kg in rats) or barbital sodium (250 mg/kg). The barbiturates were administered 20 min after pretreatment with the cinchona alkaloids or saline. Control and experimental groups were studied side by side to obviate possible differences in environmental conditions or influences of normal circadian rhythm. Sleeping time was defined as the time interval from loss of reflexes to the reappearance of the righting reflex. Quinine or quinidine alone produced no observable neurologic effects.

Kinetics of plasma decay. Pentobarbital sodium was given intravenously at a dose of 30 mg/kg to control goats. Goats in the experimental group received pentobarbital (15·6 mg/kg) 20 min after pretreatment with quinine (7 mg/kg). The difference in dosage between the two groups was essential because it was observed in preliminary studies that the combination of quinine and pentobarbital at 30 mg/kg was lethal, while the lower dose was not sufficient to maintain sedation or produce anesthesia in control goats. Blood samples were collected in EDTA at 20, 40, 60, 120, 180 and 240 min after administration of pentobarbital. Pentobarbital concentrations in plasma were estimated by the method of Brodie *et al.*[7] Since plasma decay of pentobarbital in goats was known to follow first-order kinetics (L. E. Davis, unpublished data), a least squares method for fitting an exponential curve was employed in the computation of kinetic constants.

Determination of LD_{50}. The LD_{50} values were determined using the Reed–Muench method.[8] Six dosage levels of sodium pentobarbital ranging from 100 to 220 mg/kg were employed (ten mice per dosage group). Numbers surviving or dead at the end of 13 hr were used in the calculations.

Enzyme assays. Rats were sacrificed by decapitation between 8 and 9 a.m. The livers of three rats were pooled for each determination. Livers were removed, weighed and homogenized with 2 vol. of ice-cold 1·15% KCl in a glass tube homogenizer with a motor-driven Teflon pestle. A 9000 *g* supernatant fraction was prepared and used in the assays.

Incubation mixtures contained the following components: 9000 *g* supernatant

fraction equivalent to 600 mg liver, 0·4 μmole NADP, 25 μmoles $MgCl_2$, 20 μmoles glucose 6-phosphate, 20 μmoles nicotinamide and 500 μmoles phosphate buffer (pH 7·4). The final volume of the incubation mixtures was 5·0 ml. Pentobarbital was added to the reaction mixtures in various concentrations (0·25, 0·5, 1·0 or 2·0 μmoles/5 ml of incubation mixture). Quinine hydrochloride or quinidine hydrochloride was added to some of the mixtures to provide final concentrations of $2{\cdot}5 \times 10^{-5}$ M or $5{\cdot}0 \times 10^{-5}$ M of the base. Incubations were carried out with shaking in a Dubnoff incubator at 37° for 12 min in an atmosphere of O_2. The rate of disappearance of pentobarbital was measured by the method of Brodie *et al.*[7] The data obtained were presented by the method of Lineweaver and Burk.[9] The mean of at least six determinations was used to plot each point. The means of the two concentrations of quinine or quinidine were averaged and plotted as a single point.

Statistical comparisons were made by using Student's *t*-test; P values $<0{\cdot}05$ were considered to represent significant differences between means.

RESULTS

Sleeping times. Quinine significantly increased the pentobarbital sleeping time in normal and phenobarbital-stimulated mice (Table 1). The relative increase in sleeping time produced by quinine was greater in the stimulated group than in the normal mice. Quinidine produced almost identical effects on pentobarbital sleeping time as compared to quinine pretreatment (Table 2). Quinine also increased the duration of

TABLE 1. EFFECT OF QUININE* ON PENTOBARBITAL SLEEPING TIME IN UNSTIMULATED AND PHENOBARBITAL-STIMULATED* MICE

Pretreatment	Experimental group	
	Unstimulated	Phenobarbital-stimulated
Saline	76·6 ± 6·4 (10)†	18·0 ± 0·5 (9)
Quinine	125·6 ± 3·3 (9)‡	41·9 ± 2·9 (9)‡
Relative value§	1·6	2·3

* Dosage and dosage schedules were as listed in Experimental.
† Values are expressed as time in minutes (mean ± S. E.); number of animals is in parentheses.
‡ Significantly different ($P < 0{\cdot}001$) from the corresponding saline-treated group.
§ Sleeping time of respective control groups was considered to be 1·0.

TABLE 2. EFFECT OF QUINIDINE* ON PENTOBARBITAL* SLEEPING TIME IN MICE

Pretreatment			
Saline	Quinidine	P value	Relative value†
76·3 ± 1·0 (10)‡	126·8 ± 1·8 (10)	<0·001	1·7

* Dosage and dosage schedules were as described in Experimental.
† Sleeping time of control group was considered to be 1·0.
‡ Values are expressed as time in minutes (mean ± S. E.); number of animals is in parentheses.

pentobarbital-induced sleep in male rats (Table 3). The absolute values for sleeping time in rats were less than corresponding times measured in the mice because of lower dosage rates in rats; however, the relative increase in sleeping time was greater in rats. Barbital sleeping time was decreased by quinine pretreatment, but this effect was not statistically significant (Table 4).

TABLE 3. EFFECT OF QUININE* ON PENTOBARBITAL* SLEEPING TIME IN MALE RATS

Pretreatment			
Saline	Quinine	P value	Relative value†
29·0 ± 7·9 (6)‡	79·6 ± 7·1 (7)	< 0·001	2·7

* Dosage and dosage schedules were as described in Experimental.
† Sleeping time of control group was considered to be 1·0.
‡ Values are expressed as time in minutes (mean ± S. E.); number of animals is in parentheses.

TABLE 4. EFFECT OF QUININE* ON BARBITAL* SLEEPING TIME IN MICE

Pretreatment			
Saline	Quinine	P value	Relative value†
34·0 ± 4·8 (9)‡	21·8 ± 5·1 (10)	> 0·05	0·64

* Dosage and dosage schedules were as described in Experimental.
† Sleeping time of control group was considered to be 1·0.
‡ Values are expressed as time in minutes (mean ± S. E.); number of animals is in parentheses.

TABLE 5. KINETIC CONSTANTS FOR THE DISAPPEARANCE OF PENTOBARBITAL FROM THE PLASMA OF GOATS*

Pretreatment	N	$T_{\frac{1}{2}}$ (min)	kd (min^{-1})	V'd† (L/kg)
Saline	10	43·5 ± 4·9	−0·0182 ± 0·0023	0·81 ± 0·07
Quinine	6	81·2 ± 13·4	−0·0097 ± 0·0014	0·79 ± 0·08
P value		< 0·02	< 0·001	N. S.

* Dosage and dosage schedules were as listed in Experimental. N. S. = not significant.
† V'd = apparent specific volume of distribution.

Toxicity of pentobarbital. The LD_{50} of sodium pentobarbital was found to be 182 mg/kg in saline-treated control mice. Quinine pretreatment lowered the LD_{50} to 157 mg/kg. The dose–response curves illustrating the influence of quinine on mortality in mice due to pentobarbital are shown in Fig. 1.

Quinine also increased pentobarbital toxicity in goats. When pentobarbital (30 mg/kg) was administered intravenously (i.v.) to goats pretreated with quinine, it promptly induced fatal respiratory depression. Quinine alone produced no signs of

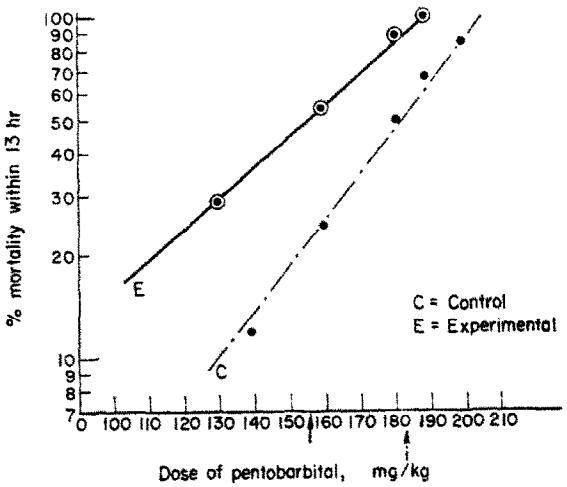

FIG. 1. Dose–response curves showing the increase in toxicity of pentobarbital produced by pretreatment with quinine hydrochloride (50 mg/kg). The respective LD_{50} values are denoted by the arrows.

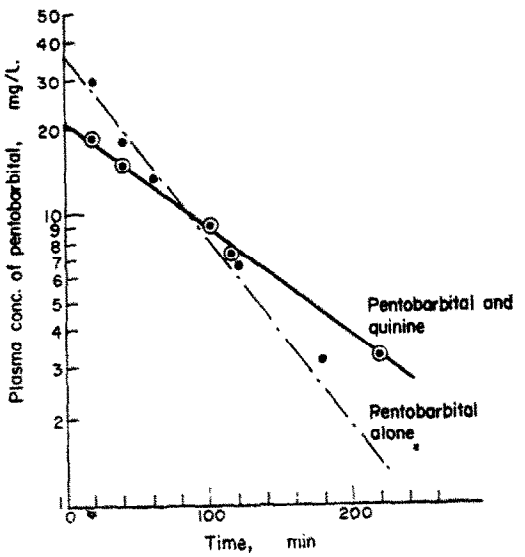

FIG. 2. Disappearance of pentobarbital from the plasma of goats. Control animals were given sodium pentobarbital (30 mg/kg) intravenously. The experimental group was pretreated with quinine (7 mg/kg, i.v.) followed in 20 min with sodium pentobarbital (15·6 mg/kg, i.v.). The points represent the mean values obtained from 10 animals (control) and 6 animals (experimental). The lines were fixed by the method of least squares.

hypnotic action in goats and quinine administered i.v. to goats at the time of recovery of the righting reflex failed to increase the level of depression.

Disappearance of pentobarbital from plasma. Quinine pretreatment decreased the rate of decay of pentobarbital concentrations in plasma of goats (Fig. 2). The half-life of pentobarbital in plasma was nearly doubled by quinine pretreatment, while the apparent specific volume of distribution was unchanged (Table 5).

Rat liver homogenates. The effects of quinine and quinidine on the rate of pentobarbital metabolism *in vitro* are illustrated in Figs. 3 and 4. The following values were obtained from the Lineweaver–Burk plots of the data obtained for quinine: $V_{max} = 2{\cdot}0$ μmoles per g per hr; $K_m = 4{\cdot}0 \times 10^{-4}$ M; and $K_I = 2{\cdot}9 \times 10^{-5}$ M. Similar values obtained for quinidine were: $V_{max} = 1{\cdot}4$ μmoles per g per hr; $K_m = 2{\cdot}9 \times 10^{-4}$ M; and $K_I = 7{\cdot}6 \times 10^{-6}$ M. It is evident that both of these cinchona alkaloids were potent inhibitors of pentobarbital metabolism. In both cases the inhibition appeared to be competitive, and quinidine appeared to produce a greater degree of inhibition than quinine.

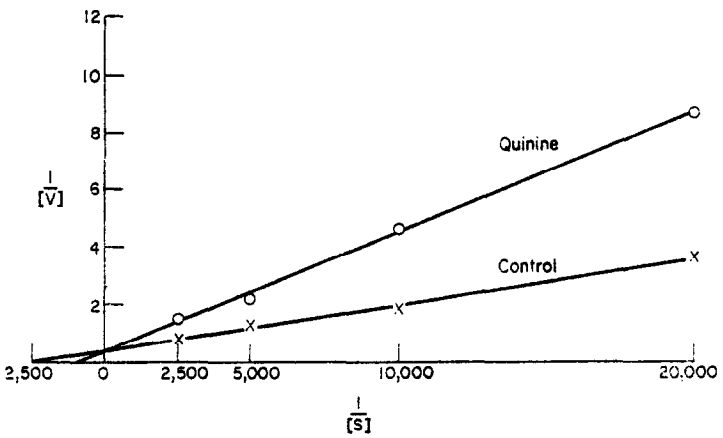

FIG. 3. Lineweaver–Burk plot of the metabolism of pentobarbital and its inhibition by quinine. Values for the kinetic constants were: $V_{max} = 2{\cdot}0$ μmoles/g/hr, $K_m = 4{\cdot}0 \times 10^{-4}$ M, and $K_I = 2{\cdot}9 \times 10^{-5}$ M. S = moles/liter and V = μmoles pentobarbital metabolized/g of liver/hr.

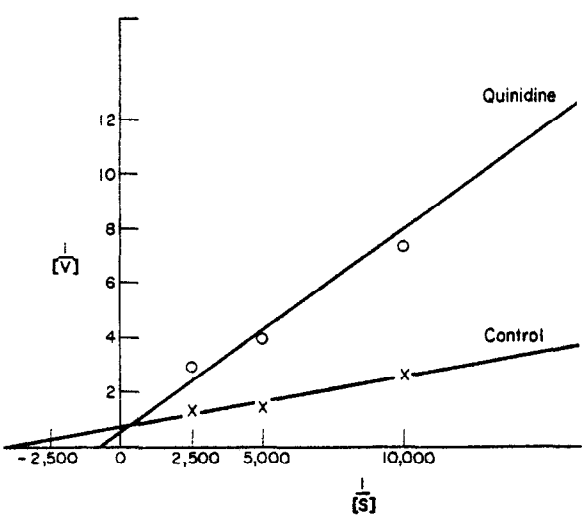

FIG. 4. Lineweaver–Burk plot of the metabolism of pentobarbital and its inhibition by quinidine. Values for the kinetic constants were: $V_{max} = 1{\cdot}4$ μmoles/g/hr, $K_m = 2{\cdot}9 \times 10^{-4}$ M, and $K_I = 7{\cdot}6 \times 10^{-6}$ M. S = moles/liter and V = μmoles pentobarbital metabolized/g of liver/hr.

Brain levels of pentobarbital. The increased toxicity of pentobarbital by pretreatment with quinine could not be explained on the basis of enzyme inhibition alone. Accordingly, brain concentrations of pentobarbital were measured in mice 5 min after intravenous administration and at awakening. These data are shown in Tables 6 and 7. Unchanged pentobarbital is represented by the ethereal fraction of extracted brain homogenates. The pentobarbital levels were slightly increased both at 5 min and at awakening in the quinine-treated animals, but these increases were not statistically

TABLE 6. RADIOACTIVITY OF MOUSE BRAIN 5 MIN AFTER ADMINISTRATION OF ^{14}C-PENTOBARBITAL*

Measured parameters	Control	Experimental†
Animal wt. (g)	28·4 ± 1·4	28·0 ± 1·3
Brain wt. (g)	0·41 ± 0·02	0·42 ± 0·02
Specific activity (nc/g)		
Ethereal fraction	39·5 ± 2·6	41·9 ± 0·02
Aqueous fraction	28·6 ± 2·1	30·8 ± 3·4

* ^{14}C-pentobarbital with carrier was injected intraperitoneally at a dosage of 50 mg/kg (57·8 μc/kg).

† Quinine hydrochloride (50 mg/kg, i.p.) was administered to the experimental group 20 min before the administration of pentobarbital. Brains were homogenized with 0·1 N HCl and extracted with petroleum ether containing 1·5% isoamyl alcohol. The two phases were separated and assayed for radioactivity.

TABLE 7. RADIOACTIVITY OF MOUSE BRAIN AT TIME OF AWAKENING FROM ^{14}C-PENTOBARBITAL-INDUCED SLEEP*

Measured parameters	Control	Experimental
Animal wt. (g)	31·1 ± 0·92	30·9 ± 1·1
Brain wt. (g)	0·45 ± 0·01	0·46 ± 0·01
Sleeping time (min)	19·2 ± 3·2	86·9 ± 12·8†
Specific activity (nc/g)		
Ethereal fraction	15·7 ± 1·42	20·0 ± 2·19
Aqueous fraction	8·2 ± 0·75	8·3 ± 1·5

* Dosages and conditions were as described in Table 6.

† Significantly different ($P < 0·001$) from the corresponding saline-treated group.

TABLE 8. INFLUENCE OF QUININE ON THE EXTENT OF PLASMA PROTEIN BINDING OF PENTOBARBITAL*

Species	Control (% bound)	Experimental (% bound)	Change (%)
Human	71·2	62·7	−12
Pony	67·9	60·6	−11
Swine	69·7	64·3	− 8
Mouse	58·0	44·5	−23
Goat	67·5	60·9	−10

* Quinine hydrochloride (12·5 μg/ml) was added to experimental plasmas; equivalent volumes of distilled water were added to control plasmas. Pentobarbital sodium (100 μg/ml as free acid) was added to all plasma samples. Extent of protein binding was determined by ultrafiltration.

significant. The aqueous fractions apparently consisted of radioactivity derived from polar metabolites of pentobarbital. There were no differences in concentration of metabolites in brain between control and experimental groups. The sleeping times reported in Table 7 are less than those in Table 1 because of differences in dosage of pentobarbital.

Plasma protein binding. Results of the study *in vitro* of the effects of quinine on the extent of protein binding of pentobarbital are shown in Table 8. The extent of binding was greatest in human plasma and least in murine plasma. Quinine interacted to decrease the extent of binding of pentobarbital. This effect was greatest in murine plasma where the percentage of pentobarbital bound was reduced by 23 per cent.

DISCUSSION

Our results show that quinine or quinidine pretreatment increases the duration of action and the toxicity of pentobarbital. These effects were not species specific, since similar results were obtained from mice, rats and goats, nor were they stereospecific, since the *d*- and *l*-isomers produced similar effects. The duration of action of drugs in the body is largely controlled by the efficiency of mechanisms for biotransformation, excretion and sequestration. An alternative hypothesis for explaining the observed increases in duration and toxicity of pentobarbital is that quinine may affect the receptors in the central nervous system in some manner such that their sensitivity is increased.

The inhibition of pentobarbital metabolism by quinine and quinidine explains the mechanism by which the duration of action and half-life of pentobarbital are increased. Duration of action and plasma concentrations of several drugs have been shown to be related to the activity of drug-metabolizing enzymes.[10] Evidence which tends to rule out impairment of excretory processes was presented in the experiment on barbital sleeping time. Barbital is largely excreted unchanged by mammals.[5] Quinine did not alter the barbital sleeping time in mice. This experiment also rules out the possibility of an increase in barbiturate receptor sensitivity. If receptor sensitivity were increased, one would expect that quinine pretreatment would increase both pentobarbital and barbital sleeping times.[11] Further evidence against an alteration in receptor sensitivity is that the brain levels of pentobarbital at awakening were not significantly different in the quinine-treated group even though the sleeping time was approximately four times as great as that in the control mice.

The increase in toxicity of pentobarbital produced by quinine pretreatment could be due to the decreased rate of biotransformation, since this would allow a given concentration of the barbiturate to persist longer at the receptor site. The reduction in protein binding of pentobarbital by quinine could also explain the increase in toxicity, as this would increase the amount of free diffusible drug in the plasma at a given dosage. Table 6 would tend to argue against the latter explanation, however, since the brain levels of pentobarbital at 5 min after the intraperitoneal administration of pentobarbital were not significantly different in the experimental group as compared to the control group. The amount of pentobarbital found in the brains of control mice and quinine-treated mice at 5 min were 2·4 and 2·6 per cent respectively, of the administered dose. According to Gillette's criteria,[12] displacement of pentobarbital from binding sites by quinine would not be expected to be pharmacologically important. Pentobarbital is

rapidly eliminated by the species studied, it is not extensively bound (Table 8), and its apparent volume of distribution exceeds the extracellular fluid volume.

Another possibility which may be considered is that of displacement of barbiturate from CNS binding sites by quinine. Quinine is known to cross freely the blood–brain barrier.[13] Such a mechanism would be compatible with the facts which are available at the present time; inability to demonstrate increased brain levels of pentobarbital and potentiation of depressant effect of pentobarbital by quinine. If bound inactive pentobarbital were displaced from CNS proteins, this could permit redistribution within the brain with no change in total barbiturate content of the brain.

Quinine and quinidine appear to belong to the class of drugs which increase the duration of action of barbiturates by interfering with the rate of biotransformation but which do not reinduce sleep. Other drugs which exert these effects are iproniazid,[14] β-diethylaminoethyl diphenylpropylacetate hydrochloride (SKF 525-A),[15] 2,4-dichloro-6-phenoxyethyldiethylamine hydrobromide (Lilly 18947)[16] and dimercaprol.[17] Chlorpromazine and reserpine prolong the action of barbiturates by true potentiation, i.e. they don't alter the uptake of drugs but they do reinduce sleep.[18]

The inhibition of pentobarbital metabolism by quinine and quinidine was shown to be of the competitive type. Rubin *et al.*[19] showed that several chemically unrelated drugs inhibited the biotransformation of each other competitively. Both quinine and quinidine were potent inhibitors of pentobarbital metabolism *in vitro*. The K_I values were of similar orders of magnitude to those reported for SKF 525-A ($6{\cdot}0 \times 10^{-6}$ M),[20] SKF 8742-A ($3{\cdot}6 \times 10^{-6}$ M)[20] and chlorpromazine ($5{\cdot}6 \times 10^{-5}$ M)[19] inhibition of ethylmorphine metabolism.

Quinine and quinidine are oxidized in the quinuclidine nucleus with the principal metabolite being the 2-hydroxy derivative.[21] The quantitative aspects of quinine and quinidine metabolism have not been investigated. The reason that quinidine was a more potent inhibitor *in vitro* than quinine was not apparent and this difference between the isomers was not observed in the effects *in vivo* of the alkaloids.

The doses of quinine and quinidine employed in the rodent studies were probably excessive as compared to usual doses employed in human patients. The dosage of quinine in goats was nearer to the human oral dose, but it would be untenable to extrapolate the meaning of our experimental observations to therapeutic usage.

The inhibitory effects of quinine and quinidine on the biotransformation of other substrates should be investigated and the practice of administering cinchona alkaloids in combination with other drugs should be reviewed.

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REFERENCES

1. L. J. Roth, E. Leiper, J. R. Hogness and W. H. Langham, *J. biol. Chem.* **178**, 963 (1949).
2. E. Titus and H. Weiss, *J. biol. Chem.* **214**, 807 (1955).
3. E. J. Algeri and A. J. McBay, *New Engl. J. Med.* **248**, 423 (1953).
4. E. W. Maynert and J. M. Dawson, *J. biol. Chem.* **195**, 389 (1952).
5. J. J. Burns, C. Evans and N. Trousof, *J. biol. Chem.* **227**, 785 (1957).
6. W. E. Knox, *J. biol. Chem.* **163**, 699 (1946).
7. B. B. Brodie, J. J. Burns, L. C. Mark, P. A. Lief, E. Bernstein and E. M. Papper, *J. Pharmac. exp. Ther.* **109**, 26 (1953).
8. L. J. Reed and H. Muench, *Am. J. Hyg.* **27**, 493 (1938).

9. H. LINEWEAVER and D. BURK, *J. Am. chem. Soc.* **56**, 658 (1934).
10. G. P. QUINN, J. AXELROD and B. B. BRODIE, *Biochem. Pharmac.* **1**, 152 (1958).
11. J. R. BOISSIER and P. SIMON, in *Importance of Fundamental Principles in Drug Evaluation* (Eds. D. H. TEDESCHI and R. E. TEDESCHI), p. 318. Raven Press, New York (1968).
12. J. R. GILLETTE, in *Importance of Fundamental Principles in Drug Evaluation* (Eds. D. H. TEDESCHI and R. E. TEDESCHI), p. 79. Raven Press, New York (1968).
13. B. B. BRODIE, H. KURZ and L. S. SCHANKER, *J. Pharmac. exp. Ther.* **130**, 20 (1960).
14. J. R. FOUTS and B. B. BRODIE, *J. Pharmac. exp. Ther.* **116**, 480 (1956).
15. J. AXELROD, J. REICHENTHAL and B. B. BRODIE, *J. Pharmac. exp. Ther.* **112**, 49 (1954).
16. J. R. FOUTS and B. B. BRODIE, *J. Pharmac. exp. Ther.* **115**, 68 (1955).
17. J. B. KAHN JR., *J. Pharmac. exp. Ther.* **109**, 292 (1953).
18. B. B. BRODIE, P. A. SHORE, S. L. SILVER and R. PULVER, *Nature, Lond.* **175**, 1133 (1955).
19. A. RUBIN, T. R. TEPHLY and G. J. MANNERING, *Biochem. Pharmac.* **13**, 1007 (1964).
20. M. W. ANDERS and G. J. MANNERING, *Biochem. Pharmac.* **15**, 319 (1966).
21. B. B. BRODIE, J. E. BAER and L. C. CRAIG, *J. biol. Chem.* **188**, 567 (1951).