stances. Neither reserpine nor meprobamate produced significant change in level of brain amino acids. The analgesic, morphine, though it produced a lowering in aspartic and glutamic acid levels when an excessively high dose was used, did not exert any effect at a dose level which was still quite adequate to produce analgesia.

Thus, the only correlation between pharmacological activity and biochemical action which emerges from this work is that between the rise in brain alanine and convulsant or excitant activity. Even this is not clear cut because the two phenothiazine tranquilizers tested both produced a rise in alanine levels in the brain although they are not excitants. The rise in glutamine produced by chlorpromazine and methoxypromazine may be characteristic of phenothiazines in general but can hardly be correlated with tranquilizing activity as neither meprobamate nor reserpine exerted this effect.

Summary. 1. Effect of electroshock and 18 chemical agents (pentylenetetrazole, semicarbazide, strychnine, picrotoxin, caffeine, pheniprazine, amphetamine, mescaline, LSD, phenobarbital, chloral hydrate, acetazolamide, diphenylhydantoin, meprobamate, chlorpromazine, methoxypromazine, reserpine and morphine) was determined on the level of 10 ninhydrin-reacting compounds in the rat brain (glutathione, phosphoethanolamine, aspartic acid, glutamic acid, GABA, glutamine, glycine +- serine, alanine, taurine and ethanolamine). 2. The convulsant agents, pentylenetetrazole, semicarbazide, strychnine, picrotoxin and electroshock as well as the excitants, pheniprazine, amphetamine and mescaline, brought about significant rises in free alanine in the brain. Lowering of the GABA levels to 60% of that of the controls was brought about by semicarbazide but not by any of the other convulsant agents. 3. Two phenothiazine tranquilizers chlorpromazine and methoxypromazine, brought about a significant rise in level of brain glutamine.

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Effects of Quinidine on Triacetin Utilizing Activity of Pancreas.* (26447)

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Rona and Reinicke(1) demonstrated that serum lipase, using tributyrin as substrate, was inhibited by quinine such that the logarithm of the inhibitor concentration plotted against the velocity of the reaction produced a straight line. A protective effect of tributyrin was not observed. Later, Hellerman *et al.*(2) mentioned that both quinine and atabrine inhibited pancreatic lipase reversibly, although no data were presented.

No reports have been seen in the literature which describe the action of quinidine upon triacetin utilizing activity (TUA). Quinidine, a stereosomer of quinine, appears to differ from quinine only in the spatial arrangement of the asymmetric carbon 3, the arrangements of the asymmetric centers, carbons 1, 2 and 4 apparently being identical (3),

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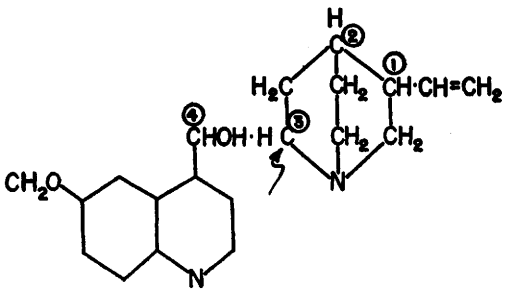

Quinidine is dextrorotatory compared to the levorotatory character of quinine. Quinidine is of special importance in treatment of cardiac arrhythmias and is administered in levels which feasibly could result in inhibiton of intestinal triglyceride breakdown, presumably a beneficial side effect in cardiac patients.

Quinine is generally known to act as a competitive inhibitor of flavineadeninedinucleotide coenzymes in a fashion similar to quinolines and auramines(2).

The present paper reports upon the nature of the interaction of quinidine and pancreatic TUA. Some of the features of this interaction have been reproduced with wheat germ lipase.

Experimental. A dried hog pancreatic preparation was obtained commercially from Nutritional Biochemicals Corp. (Cleveland, Ohio) and wheat germ lipase, prepared according to the specifications of Singer(4), was obtained from the same commercial source. *TUA Assay.* Essentially, the manometric method of Singer and Hofstee(5) for lipase was used. A nitrogen atmosphere was used throughout the studies. Linearity of enzyme concentration and carbon dioxide production occurred over a given range and all studies were conducted within these limits. Data obtained with wheat germ lipase also reflected enzyme levels which produced a linear relationship to carbon dioxide evolution. Triacetin was used as the substrate in all cases and was added from the side arm after 10 minutes incubation at 37°C under nitrogen unless otherwise indicated. Readings were made every 10 minutes for one hour or longer.

Results and discussion. The effect of increasing concentrations of quinidine sulfate upon TUA is shown by Fig. 1. A marked inhibition of TUA is not observed until the level of quinidine sulfate is greater than 10^{-3} *M*. However, some depression of TUA activity (Fig. 1) occurred in the region of 10^{-5} *M* which appeared to be statistically significant. The level of 5.10^{-3} *M* quinidine was therefore selected to determine the kinetic behavior of this interaction.

The results of a typical Ackermann-Potter type inhibition curve(6) suggested a reversible inhibition. This was confirmed using double reciprocal plots of the Lineweaver-Burk type(7). Since the inhibition was thought to be of the reversible type, an experiment was designed to test the possible reversal of quinidine sulfate inhibition by substrate. A typical result of such a study, shown in Fig. 2, shows the possibility for at least partial reversal of inhibition by substrate addition. Curve D has a greater slope than the reversal curve B, which indicates that the reversal is incomplete and may be calculated to show that the reversal under these conditions amounts to about 70%.

Because the inhibition was reversible by substrate an experiment was designed to test the possibility of substrate protection. A typical result is demonstrated in Fig. 3. Little or no substrate protection is afforded under these conditions, and curiously enough, a similar result was obtained by Rona and Reinicke(1) who used human serum lipase, tributyrin substrate, and quinine as the inhibitor.

Fig. 4 illustrates the effect of heparin upon TUA activity in presence or absence of quinidine sulfate. Ten mg heparin per vessel produced about 30% inhibition of TUA activity and in the presence of quinidine, inhibition is increased by heparin by about 20%. This suggests that heparin and quinidine may operate at different loci. Inhibition of TUA with Tween 60 as substrate, by 5.10^{-4} *M* heparin has been reported by Katz(8).

The effects of various metals upon TUA activity are represented in Table I. Fe^{+3} is practically without effect and does not influence quinidine inhibition. On the other hand, both Cu^{+2} and Mg^{+2} are inhibitory. The presence of Cu^{+2} ion slightly increases

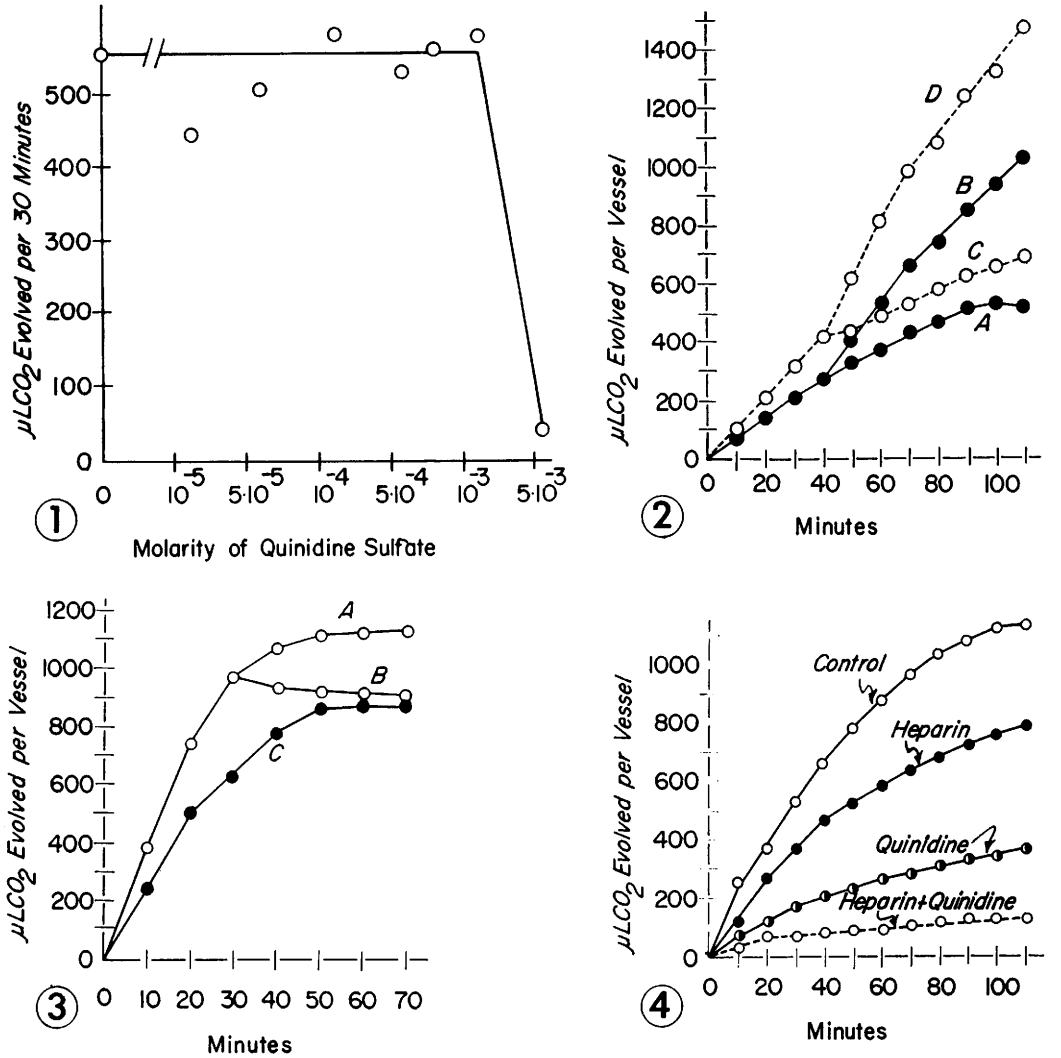

FIG. 1. Effect of quinidine sulfate on TUA activity. Illustrates one of 21 similar experiments.

FIG. 2. Partial reversal of quinidine sulfate inhibition of TUA activity by triacetin substrate. Curve A, 5.10^{-3} M quinidine sulfate in suspension was incubated with the test system using 265 µmoles triacetin in the standard system; Curve B, same system as in A except that 530 µmoles triacetin are added after 40 min. of incubation. Curve C, standard system incubated with 265 µmoles triacetin in absence of quinidine sulfate. Curve D, same system as in C except that 530 µmoles triacetin are added after 40 min. of incubation. Illustrates one of 6 similar experiments.

FIG. 3. Attempt to protect from quinidine inhibition by substrate. Curve A, standard system with 795 µmoles triacetin. Curve B, same system as in A except that 5.10^{-3} M quinidine sulfate is added after 30 min. incubation. Curve C, standard system as in A except that 5.10^{-3} M quinidine sulfate is added at time zero. Illustrates one of 5 similar experiments.

FIG. 4. Effects of heparin upon TUA activity in presence or absence of 5.10^{-3} M quinidine sulfate. Ten mg heparin was used per system. Illustrates one of 6 similar experiments.

the inhibition by quinidine, however, Mg^{+2} ions do not appear to elicit a similar response. It is assumed that the metals are probably not forming significant quantities of soaps with the released fatty acids, in which case it would be expected that the reaction would be stimulated by a Mass Action effect. The possibility, however, cannot be completely ruled out. These studies were extended to determine what effect, if any, ethylenedia-

TABLE I. Effects of Metals upon Pancreatic TUA in Presence or Absence of Quinidine.

Exp.	No. of exp.	TUA activity (μl CO_2/30 min.)	% of control exp.
Control	6	556 ± 26†	100
" + quinidine sulfate*	6	178 ± 5	32
" + $FeCl_3$	3	500 ± 25	90
" + quinidine sulfate + $FeCl_3$	3	175 ± 11	32
" + $CuCl_2$	6	175 ± 6	32
" + quinidine sulfate + $CuCl_2$	6	102 ± 9	18
" + $MgCl_2$	3	420 ± 56	76
" + quinidine sulfate + $MgCl_2$	3	167 ± 8	30

* Concentrations used: Quinidine sulfate, 5.10^{-3} M suspension; $FeCl_3$, or $CuCl_2$, or $MgCl_2$, 10^{-3} M; all solutions adjusted to pH 7.0 before use.
† ± stand. error of mean.

minetetraacetic acid (EDTA) would have upon TUA inhibition by Cu^{+2}. The results are presented in Table II. Cu^{+2} again enhances inhibition by quinidine or vice versa. EDTA itself inhibits TUA activity, which suggests that a metal component may occur as an activator or stabilizer of TUA or that EDTA may prevent an hypothetical salt formation of the released free fatty acids which could produce an inhibition. The former hypothesis has received considerable support (9,10) and Wills(10) has recently confirmed

TABLE II. Effect of EDTA on Pancreatic TUA and Its Inhibition by Cu^{+2} and Its Stimulation by Ca^{+2} in Presence or Absence of Quinidine.

Exp.	No. of exp.	Relative TUA activity (μl CO_2/30 min.), % of control
Control	4	100
" + quinidine SO_4†	4	27 (23–31)*
" + Cu acetate	4	28 (24–37)
" + quinidine SO_4 + Cu acetate	4	15 (10–18)
" + EDTA	4	31 (27–40)
" + quinidine SO_4 + EDTA	4	21 (14–41)
" + Cu acetate + EDTA	4	52 (39–64)
" + quinidine SO_4 + Cu acetate + EDTA	4	24 (13–40)
" + $CaCl_2$	4	145 (125–160)
" + $CaCl_2$ + EDTA	4	54 (49–67)
" + quinidine SO_4 + $CaCl_2$	4	34 (30–41)
" + quinidine SO_4 + $CaCl_2$ + EDTA	4	7 (4–12)

* Range.
† Concentrations used: Quinidine SO_4, 5.10^{-3} M in suspension; cupric acetate, 10^{-3} M; EDTA, 10^{-3} M; $CaCl_2$, 10^{-3} M; all solutions adjusted to pH 7.0 before use.

that Ca^{+2} acts as a stabilizing metal for lipase. The data shown in Table II indicate a stimulation of TUA activity by Ca^{+2} and more specifically that inhibition of TUA activity by EDTA is in part reversed by Ca^{+2} ions. TUA is only slightly stimulated even in the presence of quinidine which may suggest that the action of quinidine may not involve the Ca^{+2} ion site on the enzyme.

Comparisons of kinetic studies show that quinidine gluconate is 2 to 3 times more effective as an inhibitor of TUA than quinidine sulfate, possibly because of its greater solubility. Quinine sulfate is shown by the inhibitor constant to be more effective than quinidine. A similar study was carried out with wheat germ lipase (WGL) and the apparent relationship between quinidine and quinine is also reflected in these experiments (Table III). The Michaelis-Menten constant for WGL is about one-half that for TUA using triacetin as substrate. Because triacetin is a low-molecular weight triglyceride it cannot be stated that the results with the pancreatic preparation are exclusively due to pancreatic lipase; on the other hand, many of the results have been reproduced with wheat germ lipase.

Summary. Pancreatic TUA is reversibly inhibited by quinidine and quinine. Quinidine inhibition can be reversed by triacetin to about 70%; however, protection by triacetin could not be demonstrated. Quinidine and related compounds are effective as inhibitors of pancreatic triacetin utilizing activity in the following order: quinine sulfate >quinidine sulfate; quinidine gluconate>

TABLE III. *In Vitro* Inhibition of Wheat Germ Lipase by Suspensions of Quinidine Sulfate or Quinine Sulfate.*

Inhibitor	No. of exp.	K_s (moles triacetin/l) ± SEM	K_i (± SEM)
Quinidine sulfate	5	$2.3 \pm .2 \times 10^{-1}$	$1.0 \pm .5 \times 10^{-3}$
Quinine sulfate	3	$1.9 \pm .3 \times 10^{-1}$	$5.7 \pm .5 \times 10^{-4}$

* Performed at 37°C under N_2 with a 10 min. preincubation.

quinidine sulfate. To the extent tested, the same relationship holds for wheat germ lipase using triacetin as substrate. In the wheat germ lipase system the K_i for quinidine sulfate $= 1 \times 10^{-3}$, and the K_i for quinine sulfate $= 5.7 \times 10^{-4}$. Heparin inhibits the pancreatic TUA in presence or absence of quinidine. Metals, such as Cu^{+2} and Mg^{+2} inhibit this system and Cu^{+2} increases quinidine inhibition but Mg^{+2} does not. Fe^{+3} does not inhibit the system or interfere with quinidine inhibition. EDTA inhibits this system by 70% at a level of 10^{-3} *M*. This inhibition is partially reversed by Ca^{+2} ions which also markedly stimulate TUA activity. EDTA does not greatly influence quinidine inhibition. Cu^{+2} inhibition is partially reversed by EDTA in spite of the fact that EDTA is an inhibitor. EDTA reduces the stimulatory effect of Ca^{+2}. Quinidine inhibition is only slightly lessened by Ca^{+2} so that it appears that the site of action of quinidine may not involve a Ca^{+2} ion stabilizer.

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Oxidation of Ergosterol by Rat and Mouse Liver Mitochondria.*† (26448)

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That plant sterols may be absorbed by mammals was first suggested by Ellis and Gardner(1), who carried out experiments involving feeding of ordinary or ether-extracted feedstuffs to rabbits and found the liver sterol content of the group fed the untreated diet to be the greater. Since then, other workers, using more direct means, have demonstrated absorption of phytosterols such as sitosterol (2,3) and ergosterol(4,5) in a number of experimental animals. The metabolic pathway of plant sterols is of interest since they comprise a significant proportion of the sterol

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