tions for the observations reported here are: It is possible that B-3-TA does not inhibit antibody synthesis but merely inactivates the antibody in such manner as to make it non-reactive. This seems very unlikely since one would expect the antibody to be inactivated regardless of time of administration of analogue. Furthermore, the study of analogues has revealed a definite inhibitory action on the synthesis of certain proteins, which in many cases has a selectivity for one stage only of the synthetic mechanism, much as the one seen here. Novick(14) has observed that B-3-TA will inhibit the synthesis of permease and block concentration of inducer in *E. coli*, but not the synthesis of B-galactosidase once the inducer has been concentrated inside the organisms. On the other hand the inhibitory effect might be exercised at the level of antibody synthesis regardless of time of feeding of the analogue. The observation that antibody synthesis is not affected even by addition to an *in vitro* antibody forming system of 7 times the amount of drug normally used definitely contradicts this possibility(1). This lack of inhibition has recently been confirmed by Hruban(13).

Summary. The time of action of B-3-TA on antibody synthesis has been further narrowed to the first 2 days following antigen injection. A study of the histological reaction observed in the red pulp of the spleen following immunization has revealed only slight decrease in number of pyronin positive cells at 5 days after administration of antigen.

1. La Via, M. F., Uriu, S. A., Barber, N. D., Warren, A. E., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 562.

2. Wissler, R. W., Frazier, L. E., Soules, K., Barker, P., Bristow, E. C., III, *A.M.A. Arch. Path.*, 1956, v62, 62.

3. Wissler, R. W., Fitch, F. W., LaVia, M. F., Gunderson, C. H., *J. Cell. and Comp. Physiol.*, 1957, Supp. 1, 50.

4. Hruban, Z., Wissler, R. W., *Cancer Res.*, 1960, v20, 1530.

5. Cannon, P. R., Wissler, R. W., Woolridge, R. L., Benditt, E. P., *Ann. Surg.*, 1944, v120, 514.

6. La Via, M. F., Barker, P. A., Wissler, R. W., *J. Lab. and Clin. Med.*, 1956, v48, 237.

7. Wilson, G. S., Miles, A. A., in Topley and Wilson's *Principles of Bacteriology and Immunity*, Vol. II, Williams and Wilkins Co., Baltimore, 1957, 1261.

8. Taliaferro, W. H., Talmage, D. W., *J. Infect. Dis.*, 1955, v97, 88.

9. Sterzl, J., *Folia Microbiol.*, 1959, v4, 91.

10. Taliaferro, W. H., Taliaferro, L. G., Janssen, E. F., *J. Infect. Dis.*, 1952, v91, 105.

11. Sterzl, J., *Nature*, 1960, v185, 256.

12. Nossal, G. J. V., Makela, O., *J. Exp. Med.*, 1962, v115, 209.

13. Hruban, Z., personal communication.

14. Novick, A., personal communication.

Received March 8, 1963. P.S.E.B.M., 1963, v114.

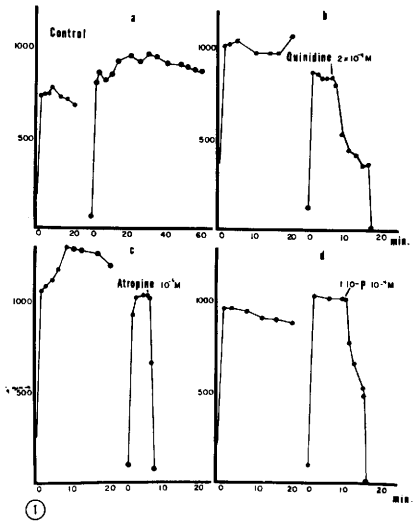
Comparative Actions of 1,10-Phenanthroline and Quinidine on Normal and Fibrillating Isolated Atria.* (28607)

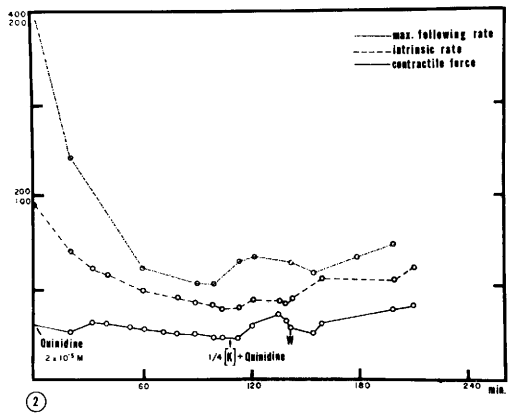
WILLIAM C. YANG, SOL ROTHMAN AND ALVARO GIMENO
Department of Pharmacology, School of Medicine, University of Southern California, Los Angeles

The chelating agent, 1,10-Phenanthroline (1,10-P), has been used successfully in experimentally induced atrial and ventricular fibrillation in rabbits(1). To study further the action of 1,10-P, the effects of 1,10-P and quinidine on fibrillating and normal rabbit atria were compared. The effects of equal concentrations of 1,10-P and quinidine on the action potential of rat atria were also compared.

Methods. The atrial preparation. Male albino rabbits weighing 2-3 kg were killed by a blow on the neck and bled, the heart quickly removed, and the atria dissected free from ventricles and surrounding tissues. The right atrial appendage was impaled on a platinum electrode and the left atrial appendage

* This work was supported by a grant from the Am. Heart Assn.





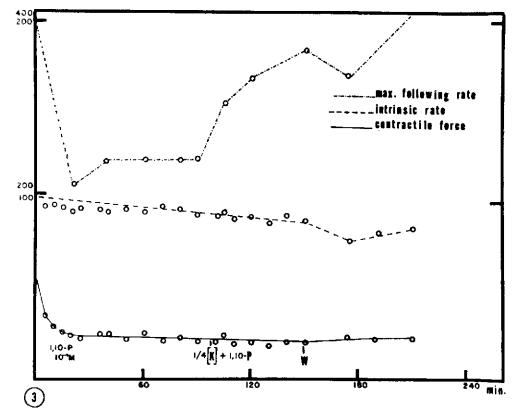


FIG. 1. Effects of quinidine, atropine and 1,10-Phenanthroline on induced rabbit atrial fibrillation. Two successive fibrillations were induced in each preparation. Complete recovery was observed before induction of second fibrillation. Ordinate: atrial rate per min. Abscissa: time in min, 0 time denotes time after completion of stimulation.

FIG. 2. Effect of quinidine on rate, contractile force and maximal following rate of normal rabbit atria. Ordinate: upper number, maximal following rate per min; lower number, intrinsic rate per min, lower number of 100 also represents 2 g for the contractile force. W indicates washing.

FIG. 3. Effect of 1,10-Phenanthroline on rate, contractile force and maximal following rate of normal rabbit atria. Ordinate: upper number, maximal following rate per min; lower number, intrinsic rate per min, lower number of 100 also represents 1 g for the contractile force.

TABLE II. Effects of 1,10-P, Quinidine and Atropine on Atrial Maximal Following Rate.

Drug	Maximal following rate: Initial	After drug addition	Low potassium + drug
1,10-P 10^{-4} M (6)	355	175	300
Quinidine 2×10^{-5} M (4)	400	96	145
Atropine 10^{-6} M (2)	390	390	—

Number in parentheses indicates number of experiments performed.

tion to which the atria can respond with corresponding contractile activity. Higher stimulatory rate would not elicit a one-to-one contractile response to the stimulation. The effect of 1,10-P on maximal following rate was compared to that of quinidine. A single stimulation strength of 8 volts and duration of 0.75 msec were used in all the experiments. It is evident from Table II that both 1,10-P and quinidine reduce the maximal following rate of rabbit atria, quinidine being the more effective. Fig. 2 and 3 also show that the maximal effects of 1,10-P and quinidine were reached after a long period of incubation (about 90 minutes). The effects persisted after quinidine or 1,10-P had been washed out.

The ability of low-potassium medium to counteract the action of quinidine is well known(6). Lowering the concentration of potassium in the medium to one-fourth the original concentration reduced the effect of quinidine and 1,10-P on the maximal following rate of the atria (Table II). The antagonism by low potassium of the reduction of maximal following rate by 1,10-P is more distinct. When 1,10-P and low potassium, or quinidine and low potassium, were replaced with normal medium, the maximal following rate decreased showing again the

hooked to a Statham strain gauge. Contractions of the atria were recorded on an Offner Dynograph through a strain gauge coupler. The unipolar electric potential was recorded simultaneously on the Dynograph through an A-C coupler. The atria were suspended in an oxygenated medium at 31°C containing 153 mM NaCl, 5.36 mM KCl, 1.86 mM $CaCl_2$, 5.95 mM $NaHCO_3$, and 11.1 mM glucose and oxygenated with 100% oxygen.

Induction of atrial fibrillation. Fibrillation was induced by the method of Holland and Burn(2). The atrial preparation was equilibrated in normal medium for one hour. Two washes with a medium containing 1.34 mM KCl (one-fourth of normal) and 0.23 mM $CaCl_2$ (one eighth of normal) were then employed and a further 15-minute equilibration period allowed. Acetylcholine was added to the medium to the final concentration of 2.2 mM. Fibrillation was initiated by square wave pulses of 0.75 msec and 8-10 V strength at a frequency of 1200 per minute for a period of 2-3 minutes. The first fibrillation thus produced was allowed to continue for a period of 20 minutes and then terminated by several washes of normal solution. A consistently high rate of electrical activity during the 20 minutes indicated that long periods of fibrillation can be induced. After a one-hour equilibration period, a second fibrillation was induced in the same manner and allowed to continue for a 5-10 minute period. If the rate of electrical activity of the atria remained high during this period, the drug was then added. The activity of the atria was observed to a period of one hour after addition of the drug. The method required use of a very high concentration of acetylcholine; however, an additional series of experiments with gradual reduction in concentration of acetylcholine to 0.005 mM led to successful induction of fibrillation. Intracellular membrane potential of the rat atrium was studied by the method previously described(3).

Results. Atrial fibrillation. Consistently high rates of electrical activity can be induced and maintained in 9 out of 10 preparations at both the high and low concentrations of acetylcholine employed. This is illustrated in Fig. 1a where the first fibrillation was allowed to continue for 20 minutes, the second for 60 minutes. There were always differences between the atrial rates of the first and the second fibrillations. Distinct fluctuation of the rate also occurred during each fibrillation period. Maintenance of high frequency without a steady decrease in rate was usually used as the criterion for successful induction. The induced fibrillation was observed for 90 minutes and was terminated by washing.

TABLE I. Effects of 1,10-P and Quinidine on Induced Atrial Fibrillation.

Drug	Concentration	Time to stop fibrillation (min) Avg	Range
1,10-P	2×10^{-5} M (3)	16.0	7-24
"	10^{-4} M (3)	7.3	2-14
"	10^{-4} M (3)*	11.0	7-16
Quinidine	4×10^{-5} M (2)		one in 34 min; one did not stop after 45 min
"	2×10^{-4} M (4)	12.9	10.5-15.5
"	2×10^{-4} M (4)*	8.3	1.5-21

* Fibrillation induced in lower concentration (5×10^{-6} M) of acetylcholine. Number in parentheses indicates number of experiments performed.

Following addition of quinidine 2×10^{-4} M (Fig. 1b), there was a period of gradual decrease in frequency, followed by a sudden change to a very slow rhythm or standstill. The effect of 1,10-P was similar to that of quinidine (Fig. 1d). There was an initial gradual decrease followed by a sharp drop in rate. This was in contrast to the action of atropine (Fig. 1e), which, at a concentration of 10^{-6} M, produced an immediate reversion to a slow regular rhythm of 70 per minute.

The results of experiments using different concentrations of 1,10-P and quinidine are summarized in Table I. 1,10-P at 10^{-4} M and quinidine at 2×10^{-4} M were both effective in stopping the fibrillation. At lower concentration (2×10^{-5} M), 1,10-P was effective in stopping fibrillation while quinidine at 4×10^{-5} M, was not as effective.

Normal atria. The maximal following rate of the rabbit atria was employed by Dawes (4) and Vaughan Williams and Szekeres(5) to test the antifibrillatory activities of various drugs. The maximal following rate is defined as the highest rate of electrical stimula-

TABLE III. Effects of 1,10-P, Quinidine and Atropine on Autonomous Atrial Rate.

Drug	Initial	After drug addition			
		30 min	% change	60 min	% change
1,10-P 10^{-4} M	100.4/min	92.0/min	− 8.6	89.5/min	−11.1
Quinidine 2×10^{-5} M	86.4/min	60.5/min	−29.5	51.6/min	−41.3
Atropine 10^{-6} M	93.0/min	90.0/min	− 3.2	90.0/min	− 3.2

TABLE IV. Effects of Quinidine and 1,10-Phenanthroline on the Electrical and Mechanical Activity of Isolated Rat Atria.

Parameter	% change			
	Quinidine (1.6×10^{-5} M)		1,10-Phenanthroline (1.6×10^{-5} M)	
	0-30′	30-60′	0-30′	30-60′
Resting potential (mv)	+ .5	+ .2	+ 2.2	+ 2.5
Action potential magnitude (mv)	− .2	− 1.8	− 4.2	− 7.0
Depolarization rate (v/sec)	−46.2	−63.0	−51.1	−70.4
Repolarization " (v/sec)	−21.4	−30.3	−21.7	−29.4
Conduction rate (cm/sec)	−27.9	−39.3	−21.3	−27.2
Developed tension magnitude (mg)	+ 7.0	− 8.7	−10.0	−13.1
No. of penetrations	100	105	80	75
" " atria	4	4	3	3

persistent effect of 1,10-P and quinidine after the drug is removed (Fig. 2 and 3).

The effects of 1,10-P and quinidine on the rate and force of contraction of rabbit atria were also compared. As 10^{-4} M quinidine readily produced atrial arrest, concentrations of 4×10^{-5} M of quinidine were employed. Quinidine at 4×10^{-5} M greatly reduced the atrial rate, while 1,10-P at a higher concentration of 10^{-4} M produced only a relatively small reduction in atrial rate (Table III). Quinidine at 4×10^{-5} M did not reduce the contractility of the atria. No appreciable effect on the force of contraction was produced by 1,10-P at 2×10^{-5} M. A distinct reduction of contractile force was effected by 1,10-P at 10^{-4} M.

As shown in Table IV, 1,10-P and quinidine produced very similar effects on the action potential of the rat atria. Both rate of depolarization and rate of repolarization were reduced.

Discussion. Quantitative estimation of the effect of an antifibrillatory drug was measured indirectly by its effect on the maximal following rate, conduction velocity, flutter and fibrillation during high frequency stimulation(5). It is important that such a drug should also be tested on fibrillation induced directly in experimental animals both *in vivo* and *in vitro*. The successful induction of atrial fibrillation in heart-lung preparations(7) and in atrial preparations(2) requires the presence of acetylcholine. In the latter preparation, a high concentration of 2.2 mM was employed. We have found that the concentration of acetylcholine can be reduced from 2.2 mM to 0.005 mM. In the use of such a preparation to study the antifibrillatory effect of an unknown compound, even when a low concentration of acetylcholine is employed, it is necessary to test the anticholinergic activity of the compound. We have found that 1,10-P at 10^{-4} M does not block the effect of acetylcholine on atria(1). The effectiveness of 1,10-P in stopping induced atrial fibrillation thus cannot be referred to its anticholinergic activity.

The possibility of spontaneous reversion of an induced fibrillation to a slower rate adds to the uncertainty in direct testing of the antifibrillatory efficacy of a drug. Every precaution should be taken to prevent spontaneous reversion to normal rate. In the present series of experiments in comparing antifibrillatory activities of quinidine and 1,10-P, the conditions for inducing fibrillation were standardized and a control period of successfully induced and maintained fibrillation was always employed before a second

induced fibrillatory period and addition of the drug.

The mechanism of the antifibrillatory action of quinidine has been extensively studied. One of the most consistent findings is the lengthening of the effective refractory period by therapeutic concentrations of quinidine. Intracellular membrane potential studies have shown that quinidine lengthened both the depolarization period(8) and the repolarization period(9). The lengthening of depolarization and repolarization periods of the rat atrial action potential by quinidine was also illustrated in our experiments. The depressant effect of quinidine on Na influx(10) and on K efflux(11) can be related to its effect on the lengthening of depolarization and repolarization of the action potential. Thus, the change of membrane permeability to K and Na ions by quinidine can be the basis of its effect in lengthening the effective refractory period. The effect of quinidine on rabbit atria, however, may be 2-fold. It has a distinct effect on the effective refractory period, thus slowing the conduction rate. Any rapid firing of a focus cannot be followed. It must also have an effect on the S-A node, producing a decrease in atrial rate and eventual atrial arrest at higher concentrations.

The chelator 1,10-P shows similar antifibrillatory activity to that of quinidine. It further shares many properties with quinidine, such as reduction of maximal following rate and reversal of the reduction of the maximal following rate by low potassium. At identical concentrations, 1,10-P lengthens the depolarization and repolarization periods of the atrial action potential to the same extent as that of quinidine. Unlike quinidine, 1,10-P has distinctly less effect on the intrinsic sinus rate of the isolated rabbit atria. This raises the possibility that the antifibrillatory effect of quinidine may be dissociated from its undesirable cardiac chronotropic inhibitory effects. The effectiveness of 1,10-P as an antifibrillatory agent indicates the possibility of finding usable drugs which are antifibrillatory but do not greatly influence the intrinsic sinus cardiac rhythm.

Summary. 1. A method of inducing fibrillation in isolated rabbit atria was explored. Atrial fibrillation can be readily produced and maintained under the conditions described by Holland and Burn(2). The method is improved here by the use of much lower concentration of acetylcholine. 2. 1,10-Phenanthroline is as effective as quinidine in stopping induced fibrillation in isolated rabbit atria. 3. 1,10-Phenanthroline, like quinidine, reduces the maximal following rate of isolated rabbit atria, but to a lesser extent. 4. The lowering of maximal following rate by 1,10-Phenanthroline and quinidine can be lessened by reduction of potassium in the medium. 5. At comparable concentrations, 1,10-Phenanthroline does not affect the intrinsic sinus rate of isolated rabbit atria, while quinidine reduces the intrinsic rate markedly. 6. 1,10-Phenanthroline, like quinidine, produces distinct reduction of the rate of depolarization and repolarization of the action potential of the rat atria.

1. Rothman, S., Yang, W. C., Webb, J. L., *J. Pharm. Exp. Therap.*, 1962, v137, 1.

2. Holland, W. C., Burn, J. H., *Br. Med. J.*, 1957, v1, 1031.

3. Gimeno, A., Gimeno, M., Webb, J. L., *Am. J. Physiol.*, 1962, v203, 104.

4. Dawes, G. S., *Brit. J. Pharmacol.*, 1946, v1, 90.

5. Vaughan Williams, E. M., Szekeres, L., *ibid.*, 1961, v17, 424.

6. Holland, W. C., *Am. J. Physiol.*, 1957, v190, 492.

7. Burn, J. H., Vaughan Williams, E. M., Walker, J. M., *J. Physiol.*, 1955, v128, 277.

8. Vaughan Williams, E. M., *Brit. J. Pharmacol.*, 1958, v13, 276.

9. West, T. C., Amory, D. W., *J. Pharm. Exp. Therap.*, 1960, v130, 183.

10. Klein, R. L., Holland, W. C., Tinsley, B., *Circulation Res.*, 1960, v8, 246.

11. Holland, W. C., Klein, R. L., *Circulation Res.*, 1958, v6, 516.

Received April 25, 1963. P.S.E.B.M., 1963, v114.