

## SYNTHETIC SUBSTITUTES FOR QUINIDINE

BY

G. S. DAWES

*From the Department of Pharmacology, Oxford*

(Received January 29, 1946)

A preliminary account (Dawes, 1946) has already been given of a new method of measuring the activity of drugs as substitutes for quinidine, using the isolated auricles of the rabbit. It was found that many of the local anaesthetics and spasmolytics in common use had quinidine-like properties, and that some of them were intrinsically much more active than quinidine. This paper gives a fuller description of the method, and of the relation between chemical structure and quinidine-like activity.

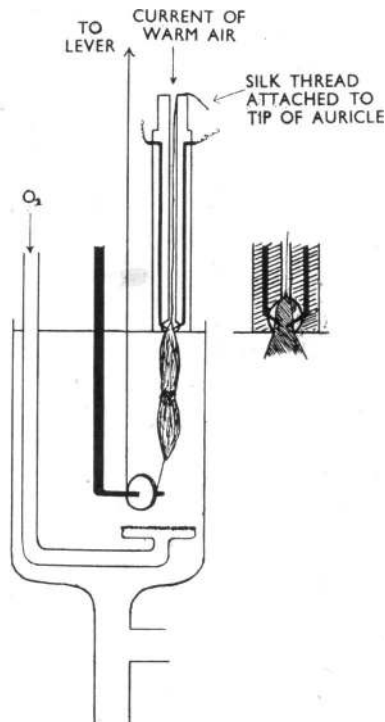


FIG. 1.—Preparation of rabbit auricles suspended in oxygenated Ringer-Locke at 29° C. The electrode holder is made of perspex, and the inset shows a closer view of its lower end.

## METHOD

Fig. 1 is a diagrammatic illustration of the preparation. The auricles are dissected from the heart of a rabbit and suspended in a bath of oxygenated Ringer-Locke at 29° C.; at their upper end they are fixed in a pair of platinum electrodes just above the surface of the bath. The sharpened tips of the electrodes project into a tiny chamber at the bottom of a perspex rod. This chamber tapers at its lower end to form an oval opening; a silk thread is tied through the tip of one auricle, which is then drawn into the chamber so that the electrodes penetrate its substance, and so that the auricle itself completely seals the oval opening. At its upper end the chamber is continuous with a circular channel which runs through the perspex rod; a gentle current of warm air is blown down this channel at constant pressure, and serves the double purpose of oxygenating the tissue in the chamber and of preventing Ringer-Locke entering the chamber by capillary attraction, and so short-circuiting the electrodes. The object of this device is to ensure that, while the main part

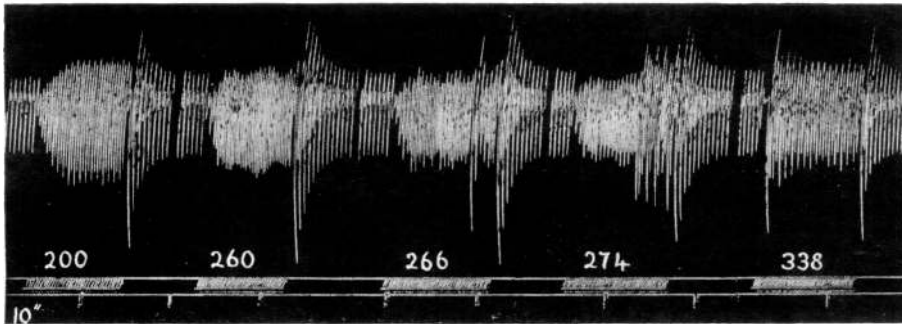


FIG. 2.—Record obtained from preparation shown in Fig. 1. The upper signal-marker indicates the duration of stimulation by break induction shocks; the number of stimuli per minute is recorded above this. Up to 260/min. the auricle responds to each stimulus; at 266 and 274/min. it just fails to follow; at 338/min. it adopts a 2 : 1 rhythm.

of the muscle is immersed in the bath, the electrodes are outside it; when methyl violet was added to the bath, the tip of the auricle drawn up into the electrode chamber was scarcely stained at all.

The contraction of the muscle is recorded by a lever writing on a smoked drum, and attached to the lower end of the auricles by a silk thread running round a pulley immersed in the bath. The auricles contract spontaneously (at a rate of 80–120 per minute), and they can also be stimulated by break-shocks from an induction coil at any desired speed, using a Lewis rotary contact-breaker. The coil separation is adjusted so that the peak voltage in the secondary is about ten times that necessary to cause extrasystoles at the beginning of the experiment; this ensures that stimuli are so far above threshold that the notorious irregularity of induction shocks will not be of practical significance. As the rate of electrical stimulation is increased the auricle follows each stimulus up to a certain point (between 250 and 350 per minute) at which it begins to drop beats (because the interval between shocks is less than the absolute refractory period; cf. Mines, 1913). It is easy to distinguish these dropped beats since the next auricular contraction is more powerful. Thus in the experiment shown in Fig. 2 the auricle followed each stimulus at 200 and 260 per minute; at 266 per minute it dropped a single beat, and at 274 per minute the response soon became very irregular; at 338 per minute it had adopted a 2 : 1 rhythm. In this instance the maximal rate at which the auricle could respond would be recorded as 260 per minute. The method is based upon the observation that quinidine reduces this maximal rate.

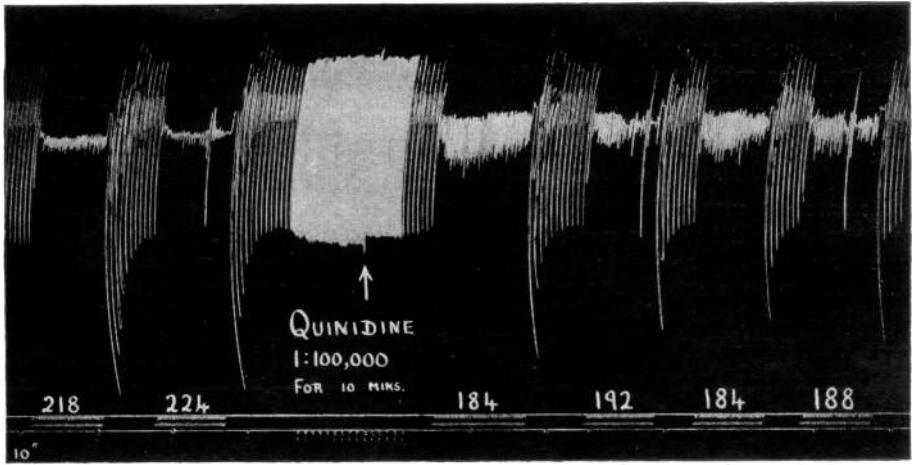


FIG. 3.—Preparation as in Fig. 2. The maximal rate at which the auricle responds to electrical stimuli is 218/min.; after quinidine 1:100,000 for 10 minutes this is reduced to 184/min. (The smoked drum was stopped for 9 minutes at the arrow, and was run at reduced speed immediately before and after it.)

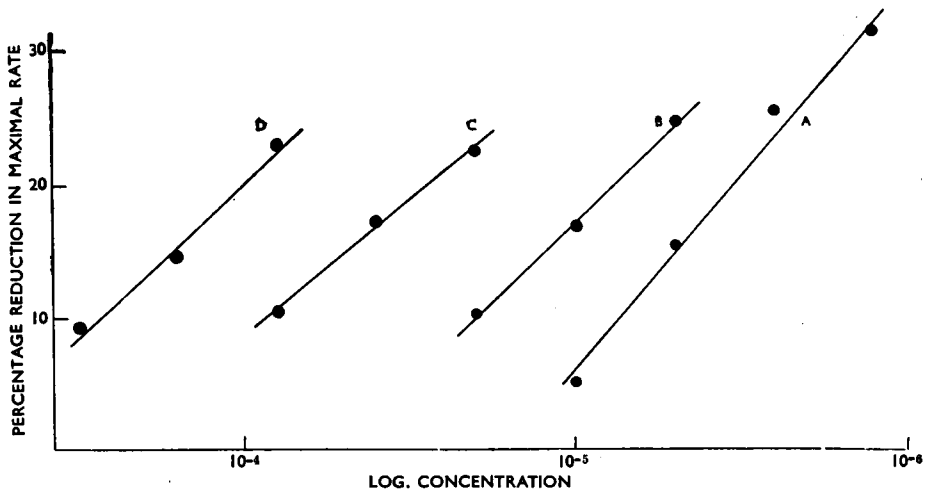


FIG. 4.—Ordinates: per cent reduction in maximal rate at which the auricle responds to electrical stimuli. Abscissae: concentration on a logarithmic scale. The points are the mean figures taken from (a) Quinine, 10<sup>4</sup> auricles; (b) Quinidine, 65<sup>4</sup> auricles; (c) compound 25, 10<sup>4</sup> auricles; and (d) Butethanol, 10<sup>4</sup> auricles.

The maximal rate is measured by applying stimuli at varying rates, each for 10–15 seconds (the maximal rate remains steady over long periods of time, provided no drug is added to the bath). Quinidine is then added and the maximal rate measured again at the end of 10 minutes. Fig. 3 illustrates this procedure. Before the quinidine was added to the bath the maximal rate at which the auricles would follow electrical stimulation was 218 per minute; ten minutes after the addition of quinidine, the auricles followed stimuli

at 184 per minute, but not at 188 per minute; i.e., the maximal rate was reduced by 34 per minute or 15.6 per cent. Preliminary experiments with quinine and quinidine showed that the percentage reduction in the maximal rate bore a linear relationship to the logarithm of the concentration. As Fig. 4 shows, the slope of this line for various drugs is sufficiently parallel to allow their relative activity to be expressed in a single figure. Quinidine is used as the standard for comparison on each preparation.

The auricle returns to its initial maximum rate after the use of quinidine and closely related compounds rather slowly; some cumulation of effect inevitably occurs during the course of the assay, although an interval of 45–60 minutes is normally allowed between each determination. This cumulation does not interfere seriously with the assay, since the percentage reduction of the maximal rate caused by a given dose is, within wide limits, independent of the initial maximal rate. However, in order to reduce this source of error to a minimum it is expedient to use concentrations of quinidine (or of quinidine substitutes) which cause not less than 10 per cent and not more than 30 per cent reduction of the maximal rate.

Some idea of the error of the method may be obtained by a consideration of the standard deviations recorded in Table I for those compounds which were tested upon 5 or more auricles each.

## RESULTS

The relative activities of a number of substances are presented in Table I.

TABLE I

Q=4 quinolyl N=α-naphthyl Ph=phenyl

Name or Number	Formula	Activity (Quinidine=1)	Number of Auricles used	Molar Weight	Activity per mol.	LD 50 mg./kg. mice IP	Therapeutic Index
Quinidine	$  \begin{array}{c}  \text{CH} \\  \diagup \quad \diagdown \\  \text{CH}_2 \quad \text{CH}_2 \quad \text{CH} \cdot \text{CH} : \text{CH}_2 \\  \diagdown \quad \diagup \\  \text{CH} \quad \text{CH}_2 \quad \text{CH}_2 \\    \\  \text{N}  \end{array}  $ 6-MeO.Q.CHOH.CH $2\text{H}_2\text{O}$	1.0	—	360	1.0	135	1.0
1	$  \begin{array}{c}  \text{CH} \\  \diagup \quad \diagdown \\  \text{CH}_2 \quad \text{CH}_2 \quad \text{CH} \cdot \text{CHOH} \cdot \text{CH}_3 \\  \diagdown \quad \diagup \\  \text{CH} \quad \text{CH}_2 \quad \text{CH}_2 \\    \\  \text{N}  \end{array}  $ 6-MeO.Q.CHOH.CH $2\text{HBr}$	0.12	2	504	0.17	—	—
2	$  \begin{array}{c}  \text{CH} \\  \diagup \quad \diagdown \\  \text{CH}_2 \quad \text{CH}_2 \quad \text{CH} \cdot \text{CH}_2\text{OH} \\  \diagdown \quad \diagup \\  \text{CH} \quad \text{CH}_2 \quad \text{CH}_2 \\    \\  \text{N}  \end{array}  $ 6-MeO.Q.CHOH.CH $2\text{HBr}$	0.04	1	490	0.06	—	—

TABLE I (continued)

Q=4-quinolyl

N=α-naphthyl

Ph=phenyl

Name or Number	Formula	Activity (Quinine = 1)	Number of Auricles used	Molar Weight	Activity per mol.	LD50 mg./kg. mice IP	Therapeutic Index
Niquidine	$  \begin{array}{c}  \text{CH:CH.CH}_3 \\    \\  \text{CH} \\  / \quad \backslash \\  \text{CH}_2 \quad \text{CH}_2 \\    \quad   \\  \text{6-MeO.Q.CHOH.CH} \quad \text{CH}_2 \\  \backslash \quad / \\  \text{NH}  \end{array}  $ 2HCl	0.35	2	371	0.36	—	—
3	$  \begin{array}{c}  \text{CH}_2 \\  / \quad \backslash \\  \text{CH}_2 \quad \text{CH}_2 \\    \quad   \\  \text{6-MeO.Q.CHOH.CH} \quad \text{CH}_2 \\  \backslash \quad / \\  \text{NH}  \end{array}  $ HCl	0.14	2	308	0.12	—	—
4	$  \begin{array}{c}  \text{CH}_2 \quad \text{CH}_2 \\  / \quad \backslash \quad / \quad \backslash \\  \text{CH}_2 \quad \text{CH} \quad \text{CH}_2 \\    \quad   \quad   \\  \text{6-MeO.Q.CHOH.CH}_2\text{N} \quad \text{CH} \quad \text{CH}_2 \\  \backslash \quad / \quad \backslash \quad / \\  \text{CH}_2 \quad \text{CH}_2  \end{array}  $ 2HCl	0.82	2	412	0.94	190	1.2
5	$  \begin{array}{c}  \text{CH}_2 \\  / \quad \backslash \\  \text{CH}_2 \quad \text{CH.C}_3\text{H}_7 \\    \quad   \\  \text{6-MeO.Q.CHOH.CH}_2\text{N} \quad \text{CH}_2 \\  \backslash \quad / \\  \text{CH}_2  \end{array}  $ 2HCl	1.05	2	400	1.16	—	—
6	$  \begin{array}{c}  \text{CH}_2\text{—CH}_2 \\  / \quad \backslash \\  \text{CH}_2\text{—N} \quad \text{O} \\  \backslash \quad / \\  \text{CH}_2\text{—CH}_2  \end{array}  $ 2HCl	0.10	2	360	0.10	—	—
7	$  \begin{array}{c}  \text{CH}_2\text{—CH}_2 \quad \text{CH}_2\text{—CH}_2 \\  / \quad \backslash \quad / \quad \backslash \\  \text{CH}_2\text{—N} \quad \text{CH.CH} \quad \text{NH} \\  \backslash \quad / \quad \backslash \quad / \\  \text{CH}_2\text{—CH}_2 \quad \text{CH}_2\text{—CH}_2  \end{array}  $ 3HCl	0.10	1	478	0.13	—	—

TABLE I (continued)  
 Q=4-quinoyl    N=α-naphthyl    Ph=phenyl


Name or Number	Formula	Activity (Quimidine=1)	Number of Auricles used	Molar Weight	Activity per mol.	LD50 mg./kg. mice IP	Therapeutic Index
8	$  \begin{array}{c}  \text{C}_3\text{H}_7 \\    \\  \text{CH}-\text{CH}_2 \\    \quad   \\  \text{CH} \quad \text{NH} \\    \quad   \\  \text{CH}_2-\text{CH}_2 \\  \text{HNO}_3  \end{array}  $ 6-MeO.Q.CHOH.CH <sub>2</sub> .CH <sub>2</sub> .CH	0.96	2	391	1.05	150	1.1
9	$  \begin{array}{c}  \text{C}_3\text{H}_7 \\    \\  \text{CH.NH}_2 \\  \text{2HBr}  \end{array}  $ 6-MeO.Q.CHOH.CH.NH <sub>2</sub>	0.35	3	422	0.41	—	—
10	6-MeO.Q.CHOH.CH <sub>2</sub> .N(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> 2HCl	0.46	2	347	0.46	—	—
11	6-MeO.Q.CHOH.CH <sub>2</sub> .N(C <sub>4</sub> H <sub>9</sub> ) <sub>2</sub> 2HCl	1.9	3	403	2.14	175	2.5
12	6-MeO.Q.CHOH.CH <sub>2</sub> .N(C <sub>6</sub> H <sub>11</sub> ) <sub>2</sub> 2HCl	0.49	2	431	0.59	—	—
13	Q.CHOH.CH <sub>2</sub> .N(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> 2HCl	0.43	1	317	0.39	—	—
14	$  \begin{array}{c}  \text{CH}_2-\text{CH}_2 \\    \quad   \\  \text{CH}_2 \quad \text{CH}_2 \\  \text{HCl}  \end{array}  $ 6-MeO.Q.CHOH.CH <sub>2</sub> .N	0.55	3	322	0.50	260	1.0
15	$  \begin{array}{c}  \text{CH}_2-\text{CH}_2 \\    \quad   \\  \text{CH}_2 \quad \text{CH}_2 \\  \text{HCl}  \end{array}  $ 7-MeO.N.CHOH.CH <sub>2</sub> .N	3.4	4	321	3.0	(175)	4.4
16	$  \begin{array}{c}  \text{CH}_2-\text{CH}_2 \\    \quad   \\  \text{CH}_2 \quad \text{CH}_2 \\  \text{HCl}  \end{array}  $ N.CHOH.CH <sub>2</sub> .N	2.8±0.11	5	291	2.3	200 (250)	4.1
17	$  \begin{array}{c}  \text{CH}_2-\text{CH}_2 \\    \quad   \\  \text{CH}_2 \quad \text{CH}_2 \\  \text{HCl}  \end{array}  $ Ph.  .CHOH.CH <sub>2</sub> .N	2.4	4	317	2.1	170 (175)	3.0
18	$  \begin{array}{c}  \text{CH}_2-\text{CH}_2 \\    \quad   \\  \text{CH}_2 \quad \text{CH}_2 \\  \text{HCl}  \end{array}  $ Ph.CHOH.CH <sub>2</sub> .N	1.4	2	241	0.94	(175)	1.8

TABLE I (continued)

Q=4-quinolyl N=*a*-naphthyl Ph=phenyl

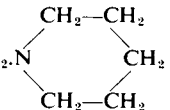
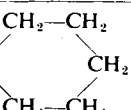
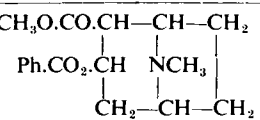
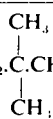
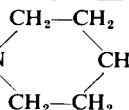
Name or Number	Formula	Activity (Quinidine=1)	Number of Auricles used	Molar Weight	Activity per mol.	LD50 mg./kg. mice IP	Therapeutic Index
19	$n\text{-C}_{11}\text{H}_{23}\cdot\text{CHOH}\cdot\text{CH}_2\cdot\text{N}$  HCl	0.18	3	319	0.15	(400)	0.53
20	Ph.CO.CH <sub>2</sub> .N(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> HBr	0.32	2	272	0.24	—	—
21	Ph.CO.CH <sub>2</sub> .N  HBr	0.90	2	284	0.71	65	0.43
Cocaine	 HCl	6.2	5	325	5.6	—	—
Procaine	<i>p</i> -NH <sub>2</sub> .Ph.CO <sub>2</sub> .CH <sub>2</sub> .CH <sub>2</sub> .N(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> . HCl	0.8	3	272	0.6	180 (250)	1.1
Butethanol	<i>p</i> -C <sub>4</sub> H <sub>9</sub> NH.Ph.CO <sub>2</sub> .CH <sub>2</sub> .CH <sub>2</sub> .N(CH <sub>3</sub> ) <sub>2</sub> . HCl	13.8 ± 4.6	10	300	11.5	70	6.4
Butyn	<i>p</i> -NH <sub>2</sub> .Ph.CO <sub>2</sub> .CH <sub>2</sub> .CH <sub>2</sub> .CH <sub>2</sub> .N(C <sub>4</sub> H <sub>9</sub> ) <sub>2</sub> ½H <sub>2</sub> SO <sub>4</sub>	5.5	4	355	5.3	80	3.3
Syntropan	Ph.CH.CO <sub>2</sub> .CH <sub>2</sub> .C  .N(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> H <sub>3</sub> PO <sub>4</sub>	1.3	2	405	1.5	—	—
Trasentin	Ph <sub>2</sub> CH.CO <sub>2</sub> .CH <sub>2</sub> .CH <sub>2</sub> .N(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> HCl	0.63	2	347	0.59	—	—
22	Ph <sub>2</sub> C(OH).CO <sub>2</sub> .CH <sub>2</sub> .CH <sub>2</sub> .N(CH <sub>3</sub> ) <sub>2</sub> . HCl	2.1	2	335	2.0	—	—
23	Ph <sub>2</sub> C(OH).CO <sub>2</sub> .CH <sub>2</sub> .CH <sub>2</sub> .N(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> HCl	3.0	4	363	3.0	160	3.5
24	Ph <sub>2</sub> C(OH).CO <sub>2</sub> .CH <sub>2</sub> .N(CHMe <sub>2</sub> ) <sub>2</sub> HCl	6.8 ± 1.1	6	391	7.4	155	7.8
25	Ph <sub>2</sub> C(OH).CO <sub>2</sub> .CH <sub>2</sub> .CH <sub>2</sub> .N  HCl	5.4 ± 1.9	10	375	5.6	150	6.0

TABLE I (continued)

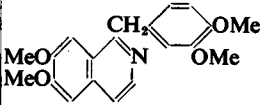
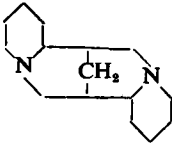
Q=4-quinoyl N= $\alpha$ -naphthyl Ph=phenyl

Name or Number	Formula	Activity (Quinidine = 1)	Number of Auricles used	Molar Weight	Activity per mol.	LD50 mg./kg. mice IP	Therapeutic Index
26		HCl 4.6	4	403	5.1	75	2.6
F933		HCl 3.2	4	269	2.4	180	4.3
F1262		HCl 4.7 ± 0.67	5	305	3.9	125	4.4
27		HCl 4.4 ± 1.4	5	321	4.0	150	4.8
28		2HCl 0.17	1	481	0.23	—	—
lower m.p. 29		2HCl 3.2	4	459	4.4	140	4.1
higher m.p.		2.1	2	459	2.9	—	—
30		2HCl 0.63	2	471	0.82	—	—
31		2HCl < 0.10	1	531	—	—	—
Pethidine		HCl 0.83	2	283	0.65	—	—



TABLE I (continued)

Q=4-quinoyl N= $\alpha$ -naphthyl Ph=phenyl

Name or Number	Formula	Activity (Quinidine=1)	Number of Auricles used	Molar Weight	Activity per mol.	LD50 mg./kg. mice IP	Therapeutic Index
Phenacaine	$p\text{-EtO.Ph.NH} \begin{array}{l} \diagup \\ \diagdown \end{array} \text{C.CH}_3$ $p\text{-EtO.Ph.N} \begin{array}{l} \diagup \\ \diagdown \end{array} \text{C.CH}_3$ HCl	4.5	4	334	4.2	80	2.7
Papaverine	 HCl	0.50	3	375	0.52	—	—
Sparteine	 H <sub>2</sub> SO <sub>4</sub>	0.34	2	422	0.40	—	—

The toxicity figures in parentheses are taken from MacIntosh and Work (1941) for substances in short supply. Their figures for compounds 16 and 17 and procaine afford a comparison with results obtained in this laboratory.

The base of compound 29 exists in two, probably stereoisomeric, forms: ( $\alpha$ ) melts to an opaque liquid at 89° which becomes clear at 102° C.; hydrochloride, m.p. 238° efferv.; ( $\beta$ ) melts to an opaque liquid at 136° which becomes clear at 144° C.; hydrochloride, m.p. 247° efferv. (personal communication from Dr. H. R. Ing).

The third column records their activity in terms of quinidine=1.0, weight for weight. In all these experiments quinidine (Howards) was weighed as base, dissolved in dilute hydrochloric acid and neutralized. All other substances were weighed as crystalline salts. The fourth column records the number of auricles on which the substance has been assayed against quinidine. The sixth column records the activity per molecule, and the seventh column the LD50 on intraperitoneal injection into mice. The index of therapeutic efficiency in the eighth column is calculated as

$$\frac{\text{Activity} \times \text{LD50 of new compound}}{\text{Activity} \times \text{LD50 of quinidine}}$$

The choice of substances tested may require some explanation. The investigation began with a series of compounds (Nos. 1-19 and 28) which Drs. Harold King and T. S. Work had made in a search for new anti-malarials. Later the work was extended to include local anaesthetics and the benzilic ester series

(Nos. 22-26), partly because of previous work on the prevention of experimental fibrillation by local anaesthetics and partly because of the structural resemblance between alkamine esters of aromatic acids and the anti-malarials prepared by King and Work. Finally some other compounds which were reputed to prevent experimental fibrillation (such as F 993, F 1262, papaverine and sparteine) were included in order to cover as wide a field as possible. The omission of some compounds was unavoidable (for references see Bijlsma and van Dongen, 1939; Jack, 1942-3; Deulofen *et al.*, 1945), and as most of the synthetic drugs in Table I were made with very different purposes in mind, there are numerous gaps in homologous series which it would have been interesting to have filled, if the compounds had been at my disposal.

Inspection of Table I shows that quinidine-like activity is displayed by a very large range of compounds. The majority of these contain an aromatic group joined to a basic group by a carbinol, keto, ester or ether linkage. Atropine also satisfies these conditions (cf. syntropan, which is also an ester of tropic acid) and in a concentration greater than 1:25,000 has a quinidine-like action upon the auricle. This concentration is of course many times greater than that required to antagonize the action of acetylcholine. Pethidine, phenacaine and papaverine possess both aromatic and basic groups and have a quinidine-like action; similarly the rosaniline dyes, methyl violet and ethyl violet, which also possess these two groups, have a quinidine-like action in very high concentrations (1:10,000 or more).

Compounds 22-26, tertiary amino-alkyl esters of benzoic acid, were originally made during a search for atropine substitutes (Ing, Dawes and Wajda, 1945). Several analogous quaternary salts were available, three of which were tested upon the rabbit auricle. These were benzilylcholine chloride and benzilyloxyethyl-dimethylethylammonium chloride (Lachesine, until recently known as E3), the metho- and etho-chlorides respectively of compound 22; and benzilyloxyethyl-diethylmethylammonium chloride, the metho-chloride of compound 23. These three quaternary salts were quite inactive when tested on the auricle in concentrations of up to 1:5,000.

Of the forty-four compounds in Table I twenty have a therapeutic efficiency index of 1.0 or more (i.e., equal to or greater than quinidine). Since intraperitoneal toxicity figures conceal such factors as rates of absorption and excretion, intravenous toxicity tests were also made on the seven compounds which had an index greater than 4.0. The results are shown in Table II, arranged in order of ascending therapeutic efficiency as judged by the intraperitoneal tests. The two benzoic ester derivatives (24 and 25) are outstanding in that they are still nearly three times as good as quinidine, even when injected intravenously. No. 25 has an LD<sub>50</sub> on oral administration to mice of 440 mg./kg., compared with 630 mg./kg. for quinidine; this gives it an oral therapeutic efficiency index of 3.7. It is suggested that this compound might be worth clinical trial as a substitute for quinidine in auricular fibrillation.

TABLE II

Name	Activity	LD50 Mice i.p. mg./kg.	Therapeutic Index	LD50 Mice i.v. mg./kg.	Therapeutic Index
Quinidine	1.0	135	1.0	65	1.0
No. 16	2.8	200	4.1	35	1.5
F 933	3.2	180	4.3	35	1.7
F 1262	4.7	125	4.4	27	2.0
No. 27	4.4	150	4.9	25	1.7
No. 25	5.4	150	6.0	40	3.3
Butethanol	13.8	70	6.4	7.5	1.6
No. 24	6.8	155	7.8	28	2.9

#### *Sympathomimetic Amines*

Compound No. 18 ( $\beta$ -phenyl  $\beta$ -hydroxyethylpiperidine) has a close structural resemblance to many sympathomimetic amines. MacIntosh and Work (1941) found that it had a diphasic effect on blood pressure and pulse rate, occasionally producing a purely pressor and accelerator response. Like cocaine, procaine (MacGregor, 1939, *b*), butyn and stovaine (Tripod, 1940), compound No. 18 (as well as Nos. 15, 16, 17, 19 and 28) potentiated the pressor action of small doses of adrenaline. When tested on the rabbit auricle it was found to be a little more active than quinidine and consequently a few sympathomimetic amines were tested on this preparation.

Adrenaline in concentrations of from 1:1,000,000 to 1:25,000 caused an *increase* in the maximal rate at which the auricle would respond to electrical stimuli. This is yet another instance of the stimulant action of adrenaline upon the heart.

The depressant action of large concentrations of ephedrine is well known (Chen and Schmidt, 1930). In one out of four auricles ephedrine in a concentration of 1:100,000 caused an increase in the maximal rate; in the other three experiments and in higher concentrations it caused a decrease (with a mean figure of 0.30 of the activity of quinidine). This quinidine-like action was accompanied by an increase in the spontaneous rate at which the auricles beat with low concentrations, but with higher concentrations (1:25,000 or more) there was a decrease both in spontaneous rate and amplitude. Methedrine and amphetamine had about the same quinidine-like activity as ephedrine upon the auricle;  $\beta$ -phenylethylamine was only half as active.

We may therefore conclude that, while many local anaesthetics which may *in some respects* be regarded as sympathomimetic possess a quinidine-like action

upon the auricle, yet other drugs which are usually thought of as predominantly sympathomimetic may in higher concentrations also act like quinidine. In this respect they are the very reverse of sympathomimetic.

*Action of Quinidine Substitutes on Pacemaker and Amplitude of Contraction*

For a given reduction in the maximal rate, butethanol caused a notably smaller decrease in the rate at which the auricles beat spontaneously than did quinidine. In ten experiments quinidine 1:100,000 caused a mean reduction in the maximal rate of 16.3 per cent and in the spontaneous rate of 15.2 per cent; butethanol 1:1,600,000 caused a mean reduction in the maximal rate of 14.6 per cent and in the spontaneous rate of only 3.0 per cent. None of the other quinidine substitutes with a high therapeutic efficiency index showed a similar difference. Similarly, while adrenaline increased both the spontaneous rate and the maximal rate of the auricles, and quinidine decreased both, acetylcholine in concentrations which almost stopped the auricle increased the maximal rate. On the other hand, ephedrine, and in some experiments cocaine, in low concentrations accelerated the spontaneous rhythm but decreased the maximal rate, while in high concentrations they decreased both. The action of these drugs upon the pulse-rate cannot therefore be relied upon as an indication of their effect upon the heart muscle.

The majority of the quinidine-substitutes listed in Table I cause a considerable decrease in the maximal rate of the auricles before they affect the amplitude of each contraction. Quinidine usually causes a 20 per cent decrease in the maximal rate before the amplitude is much reduced (see Fig. 3). Since it is inexpedient to use concentrations of a drug which cause more than a 30 per cent reduction in the maximal rate during the assay, it is difficult to judge accurately the relative effect upon the amplitude. The majority are certainly no more depressant (relatively) than is quinidine. No. 31 is a striking exception, for it caused a very large depression in a concentration of 1:10,000 without affecting the maximal rate. Pentobarbitone (nembutal) behaved in the same way. These differences between the relative activity of drugs upon the maximal rate of the auricle, the pacemaker, and the amplitude of each contraction do not lend support to the view that these drugs are 'general tissue poisons'. On the contrary, the property required in the ideal quinidine-substitute, which some of these substances go a certain way towards fulfilling, is that it should act principally upon the heart muscle; like digitalis, therefore, it should cause death from heart failure by an extension of its therapeutic action. This is the best possible insurance against untoward reactions, e.g., on the central nervous system. It is interesting in this connection to observe that compound 25, which was considered the most suitable for clinical trial on account of its high therapeutic efficiency index (low relative toxicity), has one of the lowest local anaesthetic activity to quinidine-like activity ratios (Fig. 5).

*Local Anaesthetic Activity*

The local anaesthetic activity of these compounds has already been referred to. Quinine itself is reputed to possess mild local anaesthetic properties. MacIntosh and Work (1941) demonstrated the local anaesthetic activity of compounds 15, 16, 17, 18, 19 and 28. Gilman and his collaborators (1942) demonstrated the local anaesthetic activity of trasentin and compound 23. In this laboratory compounds 20 and 21 were found to have transient local anaesthetic activity on intradermal injection into the guinea-pig; compounds 23, 24, 25, 26, pethidine and F1262 were as active as, or more active than, procaine. With cocaine, procaine, butethanol, butyn and phenacaine this brings up to twenty-one the number of compounds in Table I which possess considerable

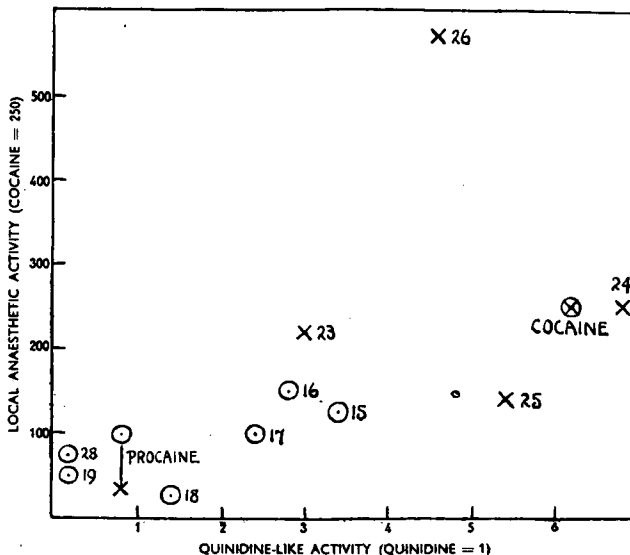


FIG. 5.—Ordinates: Local anaesthetic activity (cocaine=250). Abscissae: Quinidine-like activity (quinidine=1). Numbers refer to compounds in Table I. The data for local anaesthetic activity are taken from MacIntosh and Work (circles) and from Wajda (crosses).

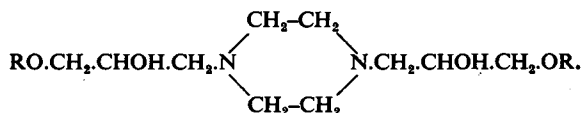
local anaesthetic activity. In addition there is nupercaine; the latter is difficult to assay upon the auricle since it causes a profound and prolonged depression of amplitude, as well as a reduction in the maximal rate at which the auricle will respond. Papaverine (Pal, 1914; Macht, Johnson and Bollinger, 1916; Reynolds, 1940) has also been shown to possess feeble local anaesthetic properties.

Saligenin (*o*-hydroxybenzylalcohol) is a moderately powerful local anaesthetic of very different structure (Hirschfelder, Lundholm and Norrgard, 1920). It possesses no basic group, and it has no action upon the auricle even in concentrations of 1:5,000. Quinidine-like activity is not therefore invariably associated with local anaesthetic activity; it is possible that saligenin produces

its local anaesthetic action in a different way from the local anaesthetics in common use, which are mostly dialkylaminoalkyl esters of aromatic acids.

Although among the compounds in Table I local anaesthetic activity does not run parallel with quinidine-like activity, there is a fair measure of agreement. This point is illustrated in Fig. 5, in which the quinidine-like activity estimated upon the rabbit auricle is plotted against the local anaesthetic activity estimated by intradermal injection into guinea-pigs of six piperidino-methyl carbinol derivatives (MacIntosh and Work, 1941) and four tertiary amino-alkyl esters of benzoic acid (Wajda, 1946). Similarly, if the familiar local anaesthetics in Table I are put in order of quinidine-like activity (butethanol, cocaine, phenacaine, butyn and procaine), that also is the order of their activity in infiltration anaesthesia, so far as can be judged by a study of the relevant literature.

The relative local anaesthetic activity estimated upon the guinea-pig's cornea may show a 100-fold difference from that estimated upon the guinea-pig's skin (MacIntosh and Work, 1941). This is probably due to the introduction of an additional factor into the assay, viz., the rate of penetration of the cornea by the drug. Quinidine-like activity as estimated on the rabbit auricle should therefore be compared with *intrinsic* local anaesthetic activity, estimated by applying the drug as directly as possible to the nerve. For instance, Fourneau and Samdahl (1930) examined a series of piperazine derivatives of the type:



When R was  $\text{C}_6\text{H}_{13}$  or  $\text{C}_7\text{H}_{15}$  they had respectively 8 and 22 times the activity of cocaine upon the rabbit's cornea. In compound 31 R is  $\text{C}_8\text{H}_{17}$ , yet it fails to reduce the maximal rate of the rabbit's auricle in a concentration of 1 : 10,000, and when tested for local anaesthetic properties by intradermal injection into the guinea-pig it was found to have less than half of the activity of procaine.

#### Spasmolytic Activity

Some of the compounds in Table I are well recognized as spasmolytics, e.g., syntropan, trasentin, pethidine and papaverine. Others undoubtedly possess the property of causing relaxation in isolated strips of intestine, though this has been described as a sympathomimetic action and may be preceded in low concentrations by a period of increased tone and amplitude, e.g., cocaine and procaine, (Roth, 1917 ; Macgregor, 1939, *b*), butyn and nupercaine (Tripod, 1940). Bovet, Fourneau, Tréfouël and Strickler (1939) found that F1262 had a spasmolytic action in the dog under chloralose, and antagonized the contraction produced by acetylcholine and barium chloride *in vitro*.

Quinidine and procaine (in a concentration of 1:100,000 to 1:25,000) also cause a reduction of tone in the isolated rabbit duodenum, suspended in oxygenated Ringer-Locke at 37°C. Both quinidine and procaine greatly reduce

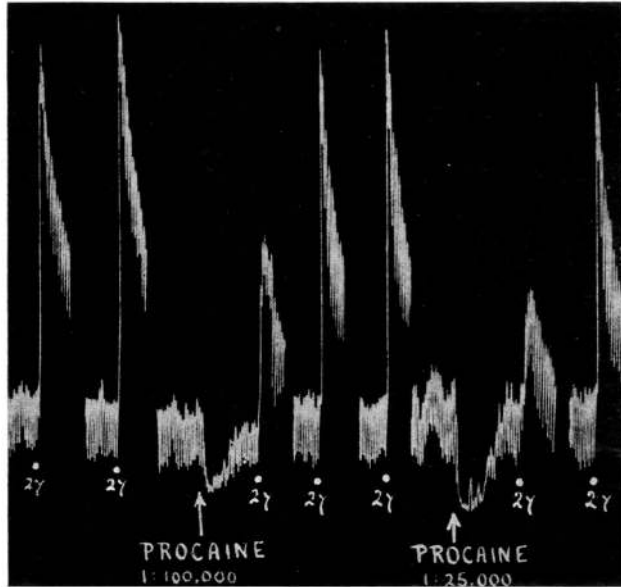


FIG. 6.—Isolated rabbit duodenum suspended in oxygenated Ringer-Locke at 37° C. Procaine hydrochloride in a concentration of 1:25-100,000 reduces the contraction caused by 2  $\mu$ g. acetylcholine (50 ml. bath).

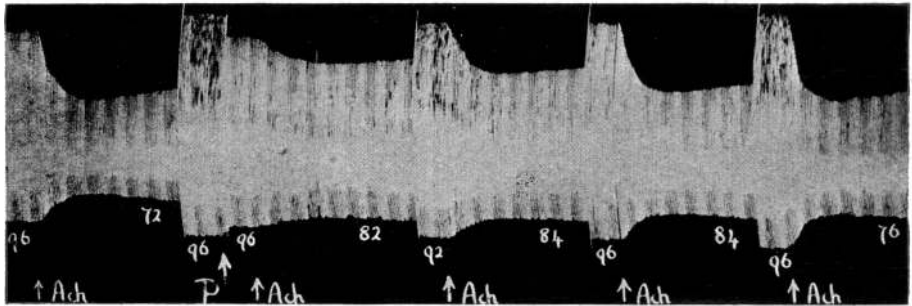


FIG. 7.—Isolated rabbit auricles suspended in oxygenated Ringer-Locke at 29° C. Acetylcholine  $10^{-6}$  (Ach) was added at 8-minute intervals; the initial rate and the subsequent slowest rate per minute are recorded on the tracing. Procaine (1:25,000) was added to the bath at P and the drum stopped for two minutes. The inhibitory action of acetylcholine was reduced.

the contraction produced by acetylcholine or potassium chloride in this preparation. Fig. 6 illustrates the antagonism of acetylcholine by procaine. Compound Nos. 16, 18, 20 and 21 also antagonized the action of acetylcholine; of these Nos. 16, 18 and 21, which contain a piperidine ring, in low concentrations stimulated the isolated intestine and in high concentrations depressed it, whereas No. 20, which has a diethylamino group (in place of the piperidine ring of No. 21), had a purely depressant action.

The fact that quinidine and procaine reduced the action of acetylcholine upon the isolated intestine suggested that they might do so upon the heart. Starr (1936) has shown that quinidine diminishes or abolishes the ability of acetyl- $\beta$ -methylcholine and of acetylcholine to slow the heart rate in anaesthetized cats and in isolated cats' and rabbits' hearts. His observation regarding acetylcholine has been confirmed in the isolated rabbit auricle. Not only quinidine, but also procaine (Fig. 7) reduce the ability of acetylcholine to slow the rate and depress the amplitude of contraction of the isolated rabbit's auricle.

#### DISCUSSION

The method described in this paper for measuring quinidine-like activity enables an estimate to be made of the reduction in the maximal rate at which isolated rabbit auricles will respond to electrical stimulation. This measurement was adopted as an index of quinidine-like activity because it was relatively simple, and because a direct comparison could be made of two or more substances upon the same piece of tissue. Quinidine is believed to stop auricular fibrillation because it prolongs the refractory period. Lewis (1922) emphasized the fact that quinidine not only prolongs the refractory period, but also slows the conduction of excitation, a change which would act in the opposite direction and tend to perpetuate circus movement. Strictly speaking, therefore, an ideal method of testing quinidine substitutes should take into account the action of a drug on conduction rate as well as on refractory period. The method described, which involves the measurement of the maximal rate, depends principally upon the refractory period. However, a number of substances, other than quinidine, which reduce the maximal rate of the rabbit auricle have already been shown to prevent or stop auricular or ventricular fibrillation induced by various methods in experimental animals. These include procaine (for references see Dawes, 1946), cocaine (Hermann and Jourdan, 1931), butethanol (under the name pantocaine, Hirschfelder and Tamcales, 1942), F1262 (Bovet, Fourneau, Tréfouël and Strickler, 1939), F933 (Shen, 1939; van Dongen, 1939), papaverine (Lindner and Katz, 1941; Elek and Katz, 1942; Wégria and Nickerson, 1942), and sparteine (Crawford, 1926). It may therefore be reasonably supposed that drugs which are intrinsically more active than quinidine upon the auricle will stop auricular fibrillation in human beings, provided that a sufficiently high concentration can be maintained in the blood-stream over an adequate period of time without untoward reactions. (There is, for instance, evidence that the protection afforded by procaine against cyclopropane-adrenaline ventricular tachycardia is more transient than that of quinidine, Meek, 1940-41). Clerc and Sterne (1939) have used F1262 in a dose of up to 0.2 gm. orally per day in a number of cases of angina pectoris and of disorders of rhythm with promising results.

There is only one outstanding exception to this agreement between results obtained on the rabbit auricle and in experimental fibrillation. Wégria and Nickerson found that adrenaline (in very large doses, 0.9 to 4.0 mg. for dogs



averaging 10 kg. in weight) increased the threshold to ventricular fibrillation induced by applying a short D.C. shock during the 'vulnerable period' of late systole. In my experiments adrenaline, even in a concentration of 1:25,000, increased the maximal rate of the auricle; this is in better agreement with the known physiological actions of adrenaline upon the heart, and its notorious effect (in quite small doses) of precipitating ventricular fibrillation in a heart which has been damaged by light chloroform, benzol or cyclopropane anaesthesia. In his reviews on the subject, Meek (1940-41) discussed the factors which may be involved in the latter phenomenon; he emphasized the evidence "that adrenaline strongly excites the automatic ventricular tissue of a heart already rendered highly irritable by chloroform". Orth, Leigh, Mellish and Stutzmann (1939) found that whereas sympathomimetic amines which contain a catechol nucleus (such as adrenaline, arterenol and cobefrin) produced multifocal ventricular tachycardia in dogs under light cyclopropane or chloroform anaesthesia, other amines such as ephedrine or neosynephrin were almost inactive in comparable doses. The presence of meta- or para-hydroxyl groups in the ring were not essential for the reaction, but they did very greatly increase its intensity. In the same way, while adrenaline would only increase the maximal rate of the auricle, the outstanding effect of ephedrine was to decrease it. The observation that adrenaline acts upon the heart muscle in a way directly contrary to the specific effect of quinidine, makes it easier to understand how adrenaline can precipitate ventricular fibrillation. Otto and Gold (1926) have described an interesting case in which adrenaline induced attacks of paroxysmal auricular tachycardia indistinguishable from those occurring spontaneously; under quinidine administration spontaneous attacks did not occur, nor could they be induced by adrenaline.

Acetylcholine also increased the maximal rate of the auricles. Acetylcholine, and particularly acetyl- $\beta$ -methylcholine, were observed to produce auricular or ventricular fibrillation on topical application or injection into experimental animals (Iglauer, Davis and Altschule, 1941; Smith and Wilson, 1944) and into human beings predisposed by thyrotoxicosis (Nahum and Hoff, 1940). Since large doses were used, adrenaline released from the adrenals or locally (Hoffmann, Hoffmann, Middleton and Talesnik, 1945) may have been an additional factor.

#### *Structure and Action*

Inspection of Table I shows that most of the substances tested contain both an aromatic and a basic group. Nos. 19 and 31 contain eleven and eight carbon aliphatic chains respectively in place of an aromatic ring, and they are both relatively inactive. No. 29 with two phenyl rings is considerably more active than No. 30, in which the rings are saturated. Sparteine is another example of a saturated ring compound which retains the characteristic activity. As to the nature of the aromatic rings, a comparison of Nos. 14, 15, 16, 17 and 18 suggests that naphthalene is as good as diphenyl, and better than phenyl or

methoxyquinoline. The benzoic ester series (22–26) is also particularly active, but trasantin, which also contains the diphenylmethane unit of structure, is relatively feeble.

An increase in the number of carbon atoms attached to the basic nitrogen group is accompanied by an increase in activity in the benzoic ester series (22–25); there is also an optimum in the series 10–14, of which the di-*n*-propyl member was unfortunately not available. Tréfouël, Strickler and Bovet (1939) studied the ability of various homologues of F1262 to protect the ventricle of anaesthetized rabbits against fibrillation induced by an alternating current. The method was not strictly quantitative, but there was evidently an increase in activity as the number of carbon atoms attached to the nitrogen was increased up to the diethylamino-derivative (F1262), which was a little more active than the dimethylamino and dipropylamino compounds. Chen, Wu and Henriksen (1929) also found, in a series of homologues of adrenaline and ephedrine, that an increase in the number of carbon atoms attached to the nitrogen or to the  $\alpha$ -carbon atom of the side chain caused an increase in the depressant action on the frog's heart and a change from pressor to depressor action in the pithed cat; their results also suggest a concomitant increase in toxicity on intravenous injection into rabbits. Similarly Lands, Lewis and Nash (1945), studying the comparative pharmacological actions of some phenyl-, cyclohexyl- and cyclopentyl-alkylamines, found that increasing the size of the alkyl groups on the nitrogen from dimethyl to diethyl produced compounds that were depressor instead of pressor and had less accelerator action upon the heart.

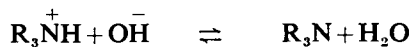
The nature of the linkage between the aromatic and basic groups does not appear to be of the first importance; it may be a carbinol, keto, ester or ether group or even a short alkyl chain as in methedrine, amphetamine and  $\beta$ -phenylethylamine. Tréfouël, Strickler and Bovet (1939) found that a two-carbon chain was better than a three-carbon chain in compounds of the F1262 type. Papaverine, pethidine and phenacaine provide yet more complex variants. The more active compounds contain an aromatic (hydrophobic) and a basic (hydrophilic) group, and there is evidence that increased lipoid-solubility is associated with increased quinidine-like activity (e.g., 14, 15, 16, 18), as it is with local anaesthetic activity (MacIntosh and Work, 1941). Similarly, Barger and Dale (1910–11) found in a series of aliphatic amines that in the higher members of the series (which are more lipoid soluble) the pressor action on the spinal cat was complicated by a depressant action on the heart. The view that the basicity of the common local anaesthetics is of considerable importance in determining their activity was confirmed by the observation of Trevan and Boock (1927) that there was a linear relationship between *pH* and the logarithm of the minimal effective concentration applied to the rabbit's cornea. This relationship supported the view that the active constituent of a solution of a local anaesthetic is the free base and not the ion or undissociated salt. This is probably true also for the quinidine-like action of drugs upon the heart; not only are the most powerful

local anaesthetics most active upon the rabbit's auricle, but conversion into the quaternary salts (thus stabilizing the cation) of compounds 22 and 23 abolished their quinidine-like activity, just as conversion of local anaesthetics into quaternary salts abolishes their local anaesthetic activity.

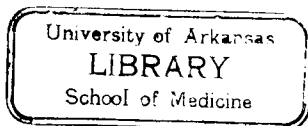
*The Pharmacological Actions of Quinine, Quinidine and Procaine*

It is very remarkable that quinine, quinidine and procaine antagonize the effect of acetylcholine on many different types of tissue. They reduce its effect upon the rate and amplitude of contraction of heart muscle, and upon the isolated intestine. Harvey (1939, *a, b*) showed that the response of normal and denervated mammalian striated muscle to injected acetylcholine was reduced or abolished by quinine and procaine; procaine also abolished the response of the superior cervical ganglion to acetylcholine. Oester and Maaske (1939) obtained similar results to Harvey on striated muscle, and Frank, Nothmann and Hirsch-Kauffmann (1920) and MacGregor (1939, *a*) found that cocaine and procaine reduced the contractures caused by acetylcholine or nicotine in denervated mammalian muscle. Cocaine and procaine also reduced the pressor response to acetylcholine or nicotine in atropinized cats (MacGregor, 1939, *b*). Quinine inhibited the secretory action of choline or acetylcholine upon the salivary gland (Stavraky, 1932).

It may be observed that large quantities of these drugs are required to antagonize acetylcholine. The more specific antagonism towards acetylcholine in highly selective sites manifested by curare-like substances is a common property of *quaternary* ammonium salts; similarly among both the belladonna alkaloids and synthetic atropine substitutes the *quaternary* metho-salts are more active than the tertiary bases (Ing, Dawes and Wajda, 1945; Bülbring and Dawes, 1945). Whereas local anaesthetic and quinidine-like properties appear to be characteristic of the free base (disappearing or being greatly reduced when the tertiary base is converted into the quaternary metho-salt), curare-like and atropine-like properties appear to be characteristic of the cation (increasing when the tertiary base is converted into the quaternary metho-salt). Any solution of a tertiary alkamine such as quinidine or procaine will contain both the tertiary cation  $R_3NH^+$  and the base  $R_3N$  in equilibrium:



so that it is not surprising to find that quinidine and procaine not only have local anaesthetic activity and a quinidine-like action upon the heart, but also a curare-like and atropine-like action in high concentrations. This conception of a solution of procaine as consisting of two dissimilar molecular species is of assistance in understanding its very complex action at neuro-muscular junctions. Its most striking action at this site is antagonism to acetylcholine, whether injected or released by stimulation of the motor nerve. This curariform action is, however, not sufficient to explain all the observed effects; Harvey (1939, *b*) showed that



procaine reduces the output of acetylcholine from the superior cervical ganglion on stimulation of the preganglionic nerve, and both Harvey (1939) and Jaco and Wood (1944) suggest that procaine also depresses the production of acetylcholine at the neuro-muscular junction by a 'local anaesthetic' action on the motor nerve endings. In addition, procaine has an action upon the muscle itself, in small doses occasionally causing increase, in larger doses decrease of directly excited twitches (Harvey, 1939, *b*; Macgregor, 1939, *a*). Of these effects the curariform is regarded as characteristic of the cation, and the direct depressant action upon nerve and muscle as characteristic of the free base. A further justification for this view is to be found in the analogous experiments of Harvey (1939, *a*) upon quinine. In this instance the direct action of the alkaloid upon the muscle was relatively greater; the prolongation of the refractory period, which was a prominent feature of this action, provides an obvious analogy with the action of these drugs upon cardiac muscle.

Although the typical properties of atropine appear to be a characteristic of the cation because they are more marked in atropine metho-salts, atropine itself is an ester of an aromatic acid and a tertiary alkamine, and might therefore be expected to show local anaesthetic and quinidine-like properties too. In high concentrations it was found to have a quinidine-like action upon the isolated rabbit auricle. It is also reputed to have a feeble local anaesthetic action. Brown (1937) has suggested that atropine may reduce the liberation of acetylcholine at the neuromuscular junction, since injection of 0.1 c.c. of 1:1,000 into the frog gastrocnemius abolished the response to nerve stimulation, but left a large part of the response to injected acetylcholine. Bülbiring (1946), working with the isolated phrenic nerve diaphragm preparation of the rat, was driven to a similar conclusion, and has pointed out the qualitative resemblance between the actions of atropine and procaine on the neuromuscular junction. This can be accounted for by the fact that both are tertiary alkamine esters, and will therefore possess the properties characteristic not only of the action but also of the free base.

The difference between the pharmacological actions of the cation and of the free base is probably due to the inability of the former to penetrate inside cells. Thus curariform and atropine-like effects would be expected to occur at the cell surface (*cf.* Cook, 1926), while local anaesthetic and quinidine-like properties would be dependent on the ability of the free base to penetrate the cell membrane. (Presumably quaternary compounds possessing atropine and curare-like properties act at the junction between nerve and effector tissue, because only there can they come into contact with the 'transmission process', whatever that may be). While this difference between cation and free base implies a considerable limitation of the site of action of the two molecular species, and so of their pharmacological properties, one cannot help being impressed by the broad structural similarity of drugs which on the one hand antagonize the action of acetylcholine at sites more or less strictly delimited, and

on the other hand depress the transmission of excitation in cardiac muscle and nerve. (It goes without saying that this discussion only applies to the broad outlines of structure and action in the series of alkamines under consideration; it remains to be seen why curare, for instance, does not affect the action of acetylcholine on the heart, and why atropine has such an inconsiderable action at the neuro-muscular junction).

There is one further consideration which may be mentioned. The free base of a tertiary alkamine, having once penetrated the nerve or muscle cell, will come into equilibrium with its cation again according to the reaction given above. In this way it is theoretically possible for cations of tertiary bases to reach the inside of nerve and muscle cells. Hitherto the suggestion has been made that it is the free base of local anaesthetics and of quinidine substitutes which is the active constituent; it is conceivable, however, that the free base only acts by facilitating the entrance of the cation.

#### SUMMARY

1. A number of compounds have been tested as substitutes for quinidine upon a preparation of isolated rabbit auricles. Many of the local anaesthetics and spasmolytics in common use possess quinidine-like properties when tested in this way.

2. The most promising synthetic quinidine substitute is the benzilic ester of piperidino-ethanol (No. 25), which is 5.4 times as active as quinidine and has a therapeutic efficiency index from three to six times that of quinidine, according to whether their toxicities are compared in mice after intravenous or intraperitoneal injection respectively. This compound is considered worthy of therapeutic trial in man.

3. The relation between structure and quinidine-like action is discussed. The most active compounds possess aromatic and basic groups joined by ester, ether, keto or carbinol linkages. Within certain limits increase in lipoid solubility and increase in the size of the alkyl group attached to the basic nitrogen atom are associated with increased activity.

The best local anaesthetics on the whole possess the greatest quinidine-like activity; and, as with local anaesthetics, the quaternary salts of very active tertiary compounds are quite inactive. This suggests that the active component of a solution is the free base rather than the cation.

4. While local anaesthetic and quinidine-like properties are characteristic of the free base (which can penetrate the cell-membrane), curariform and atropine-like properties appear to be characteristic of the cation (which, it is believed, acts at the cell surface). A solution of the aromatic ester of a tertiary alkamine such as procaine will contain both cation and free base in equilibrium. This conception of a solution of procaine as being composed of two dissimilar molecular

species is of some assistance in understanding its complex action upon the mammalian nerve-muscle preparation.

## ACKNOWLEDGEMENTS

This work was carried out during the tenure of a grant from the Medical Research Council.

I am most grateful to Professor J. H. Burn for the advice and encouragement which he has given throughout. The work was discussed in all its stages with Dr. H. R. Ing, and I am most deeply indebted to him, not only for his very helpful advice and criticism, but also for compounds 20-27 and 29-31.

I also wish to express my gratitude to Dr. H. King and Dr. T. S. Work for compounds 1-19 and 28 (with which the investigation was begun); to Dr. Daniel Bovet for F 933 and F 1262; to Professor G. R. Clemo for sparteine; and to Mr. P. B. Marshall for niquidine.

Dr. J. B. E. Baker completed the assays on six compounds, and I am very grateful to him for this assistance.

## REFERENCES

- Barger, G., and Dale, H. H. (1910-11). *J. Physiol.* **41**, 19.  
 Bijlsma, U. G., and van Dongen, K. (1939). *Ergebn. Physiol.* **41**, 1.  
 Bovet, D., Fourneau, E., Tréfouël, J., and Strickler, H. (1939). *Arch. int. Pharmacodyn.* **62**, 234.  
 Brown, G. L. (1937). *J. Physiol.* **89**, 438.  
 Bülbring, E. (1946). *Brit. J. Pharmacol.* **1**, 38.  
 Bülbring, E., and Dawes, G. S. (1945). *J. Pharmacol.* **84**, 177.  
 Chen, K. K., and Schmidt, C. F. (1930). *Ephedrine and Related Substances*. Medicine Monographs, Vol. XVII, London: Baillière, Tindall & Cox.  
 Chen, K. K., Wu, C., and Henriksen, E. (1929). *Proc. Soc. exp. Biol., N.Y.* **26**, 348; *J. Pharmacol.* **36**, 363.  
 Clerc, A., and Sterne, J. (1939). *Pr. Méd.* **85**, 1817.  
 Cook, R. P. (1926). *J. Physiol.* **62**, 160.  
 Crawford, J. Hamilton (1926). *J. Pharmacol.* **26**, 171, 181.  
 Dawes, G. S. (1946). *Brit. med. J.*, **i**, 43.  
 Deulofen, V., Labriola, R., Oriás, O., Moisset de Espanés, E., and Taquini, A. (1945) *Science*, **102**, 69.  
 van Dongen, K. (1939). *Arch. int. Pharmacodyn.* **63**, 88.  
 Elek, S. R., and Katz, L. N. (1942). *J. Pharmacol.* **74**, 335.  
 Fourneau, E., and Samdahl (1930). *Bull. Soc. Chim.* **47**, 1003.  
 Frank, E., Nothmann, M., and Hirsch-Kaufmann, H. (1923). *Pflüg. Arch. ges. Physiol.* **198**, 391.  
 Gilman, A., Goodman, L., Thomas, J. M., Hahn, G. A., and Prutting, J. M. (1942). *J. Pharmacol.* **74**, 290.  
 Harvey, A. M. (1939a). *J. Physiol.* **95**, 45.  
 Harvey, A. M. (1939b). *Bull. Johns Hopk. Univ.* **65**, 223.  
 Hermann, H., and Jourdan, F. (1931). *C. R. Soc. Biol., Paris*, **106**, 1153.  
 Hirschfelder, A. D., Lundholm, A., and Norrgard, H. (1920). *J. Pharmacol.* **15**, 261.  
 Hirschfelder, A. D., and Tamcales, G. (1942). *Proc. Soc. exp. Biol., N.Y.*, **50**, 272.  
 Hoffmann, F., Hoffmann, H., Middleton, S., and Talesnik, J. (1945). *Amer. J. Physiol.* **144**, 189.  
 Iglauer, A., Davis, D., and Altschule, M. D. (1941). *Amer. Heart. J.* **22**, 27.  
 Ing, H. R., Dawes, G. S., and Wajda, I. (1945). *J. Pharmacol.* **85**, 85.  
 Jack, W. (1942-3). *Arch. exp. Path. Pharmacol.* **200**, 528.  
 Jaco, N. T., and Wood, D. R. (1944). *J. Pharmacol.* **82**, 63.  
 Lands, A. M., Lewis, J. R., and Nash, V. L. (1945). *Ibid.* **83**, 253.  
 Lewis, T. (1922). *Amer. J. med. Sci.* **163**, 781; **164**, 1.  
 Lindner, E., and Katz, L. N. (1941). *Amer. J. Physiol.* **133**, 155.  
 Macgregor, D. F. (1939a). *J. Pharmacol.* **66**, 350.  
 Macgregor, D. F. (1939b). *Ibid.* **66**, 393.

- Macht, D. I., Johnson, S. L., and Bollinger, H. J. (1916). *Ibid.* **8**, 451.  
MacIntosh, F. C., and Work, T. S. (1941). *Quart. J. Pharm.* **14**, 17.  
Meek, W. J. (1940-1). *Harvey Lectures*, **36**, 188.  
Meek, W. J. (1941). *Physiol. Rev.* **21**, 324.  
Mines, G. R. (1913). *J. Physiol.* **46**, 349.  
Nahum, L. H., and Hoff, H. E. (1940). *Amer. J. Physiol.* **129**, P 428.  
Oester, Y. T., and Maaske, C. A. (1939). *J. Pharmacol.* **66**, 133.  
Orth, O. S., Leigh, M. D., Mellish, C. H., and Stutzmann, J. W. (1939). *Ibid.* **67**, 1.  
Otto, H. L., and Gold, H. (1926). *Amer. Heart J.* **2**, 1.  
Pal, J. (1914). *Dtsch. med. Wschr.* **40**, 164.  
Reynolds, A. K. (1940). *J. Pharmacol.* **69**, 112.  
Roth, G. B. (1917). *Ibid.* **9**, 352.  
Shen, T. C. R. (1939). *Arch. int. Pharmacodyn.* **61**, 43.  
Smith, J. R., and Wilson, K. S. (1944). *Amer. Heart J.* **22**, 47.  
Starr, I. (1936). *J. Pharmacol.* **56**, 77.  
Stavraky, G. W. (1932). *Ibid.* **67**, 321.  
Tréfouël, J., Strickler, H., and Bovet, D. (1939). *C. R. Soc. Biol., Paris*, **130**, 27.  
Trevan, J. W., and Boock, E. (1927). *Brit. J. exp. Path.* **8**, 307.  
Tripod, J. (1940). *J. Physiol.* **97**, 289.  
Wajda, I. (1946). Personal communication.  
Wégria, R., and Nickerson, N. D. (1942). *J. Pharmacol.* **75**, 50.