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THE VARIATION IN THE TOXICITY OF PHOSGENE FOR SMALL ANIMALS WITH THE DURATION OF EXPOSURE

BY

E. BOYLAND*, F. F. McDONALD AND M. J. RUMENS

(Received January 18, 1946)

In so far as the toxic effects of a lethal inhalant depend upon the amount of material absorbed these effects must depend upon the dosage (Ct) to which animals are exposed, where C = the concentration of the gas in mg. per cubic metre and t = the time of exposure in minutes. It must also depend upon the rate of inhalation and the efficiency of absorption of the gas in the respiratory tract. The present experiments show quantitatively how the median lethal dose of phosgene expressed as the $L(Ct)50$ varies with the exposure time (t). From the data it is possible to deduce the probable extent to which different species reduce their breathing rate in an irritant gas.

The expression for toxicity or lethal index (L) advanced by Haber (cf. Prentiss, 1937) was

$$L = Ctv/G \quad \ldots\ldots (1)$$

where C = concentration of toxic substance, t = time of exposure, v = volume of air breathed in per minute, and G = body weight in Kg. In general, for any one species under the same conditions v/G is constant, so that for any one species Ct would be constant. Thus the lethal index, or the median lethal dose, $L(Ct)50$, should be constant for any one species if the breathing rate is unchanged. American work on the toxicity of phosgene for dogs (Geiling, 1944; Prentiss, 1937) showed that the "lethal index" rose from 4,500 mg. min./m.^{3} when t was 2 min. to 12,000 mg. min./m.^{3} when the exposure time was 75 min. This increase in the lethal dose with increase in exposure time can be corrected for on the assumption that the organism can eliminate a constant amount of the toxic agent. By introducing an elimination factor C_0 which may be assumed to be equal to the just harmless concentration, the formula becomes

$$(C - C_0)t = L \quad \ldots\ldots (2)$$

which is more consistent for long exposure time. The American data on dogs

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referred to above fit in with the function $(C - 100)t = L$ fairly well. This suggests that under the condition of the experiments a concentration of 100 mg./m.^{3} would not be fatal except after exposure for an infinite time.

With short exposure times the lethal concentration of phosgene increases considerably as the time is reduced. With exposure times of less than one minute the $L(Ct)50$ is very high probably because the animals breathe less and possibly because with the high concentrations required the phosgene is less completely absorbed by the tissues of the respiratory tract.

EXPERIMENTAL PROCEDURE

The apparatus used is shown in Fig. 1. Air was sucked through the Bruhl jar containing the animals placed on a stand of wire mesh, and phosgene was introduced for a short period of predetermined duration, at a rate measured on the flowmeter. The contents of the chamber were sampled by sucking through absorption bubblers at a measured rate. The bubblers, which contained hexamine and caustic soda solution, were turned on prior to introduction of the phosgene and were allowed to run for half to one minute after the

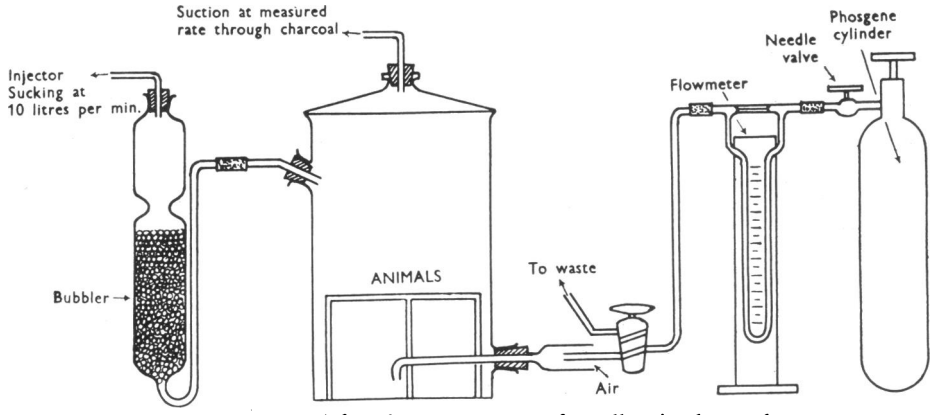

FIG. 1.—Apparatus used for short exposures of small animals to phosgene.

phosgene was turned off. The dosage (Ct) to which the animals were subjected was calculated by dividing the weight of the phosgene collected (in mg.) by the rate of sampling (expressed in m^3 per min.). Extra air was sucked through the jar and a charcoal filter and allowed to go to waste so that during exposures the total flow was 30 l./min. Exposures of 4 min. or longer were carried out in a 20 litre chamber with the air passing through at 200 l./min.

RESULTS

Guinea-pigs.—The results obtained with guinea-pigs are given in Table I, and the relationship between the $L(Ct)50$ and the time of exposure is plotted in Fig. 2. The $L(Ct)50$ is not constant, but is minimal at 8 min., and increases considerably with the shortening or lengthening of the exposure time. The corresponding curve for a constant concentration-time product on Fig. 2 would be a straight horizontal line.

TABLE I

MORTALITY OF GUINEA-PIGS EXPOSED TO PHOSGENE

Exposure time (t) min.	Dosage Ct mg. min./m.^{3}	Mortality	$L(Ct)50$ (approx.) mg. min./m.^{3}	Value for K, $\frac{L(Ct)\ 50-C_0t}{1+t_0/t}$ $t_0 = 0.8$ min. $C_0 = 20$ mg./m.^{3}
$\frac{1}{4}$	7400 6400 4200 2700	6/6 2/3 1/6 0/6	5500	1310
$\frac{1}{2}$	7700 5100 4200 2350	6/6 4/6 2/3 3/6	3000	1130
1	8900 7500 4550 4000 3100 1700	3/3 3/3 5/6 3/3 8/9 2/6	2000	1190
2	8500 4400 1800 1575	6/6 6/6 3/6 2/7	1800	1260
4	2480 1915 1675 1425	4/6 9/10 8/10 4/10	1500	1180
8	2410 1760 1700 1620 1200	6/6 9/10 13/16 8/10 2/10	1400	1130
16	1900 1740 1660 1375	11/16 4/6 5/10 2/10	1660	1270
32	2275 1835 1800 1775 1700 1575	6/6 8/10 8/10 11/16 6/10 2/10	1700	1090
64	3480 3425 3125 2560	5/6 9/10 6/10 2/6	2900	1570

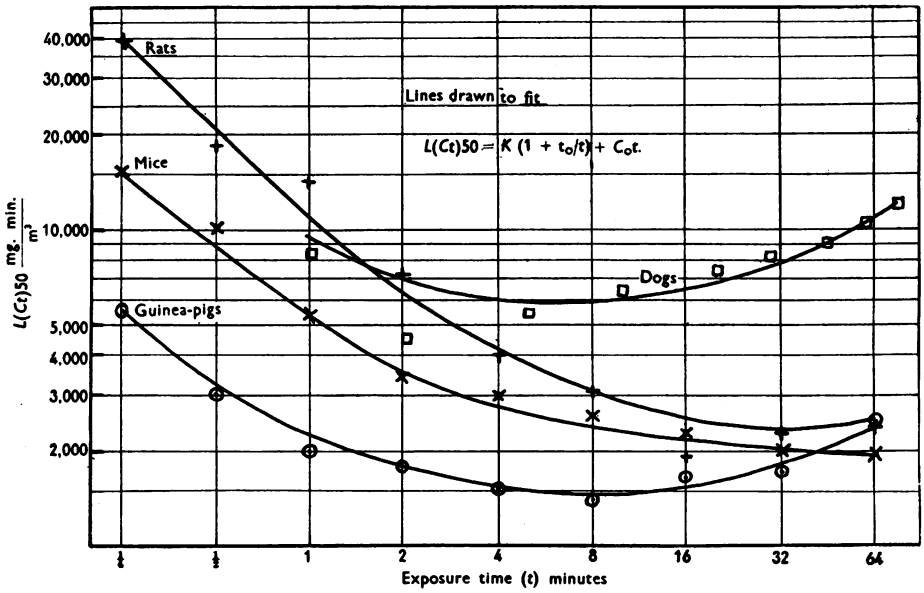

FIG. 2.—The $L(Ct)50$ values for animals exposed to phosgene.

Rats and Mice.—The data in Tables II and III and Fig. 2 show that the dosages necessary for 50 per cent mortality are high. The $L(Ct)50$ for rats rises with shortening exposure time to a greater extent than with the other species examined.

Dogs.—Mortality figures for dogs from American experiments are given in Table IV and are also indicated on Fig. 2. Dogs are less susceptible to phosgene than are the other species with long exposure times, but no figures are available of exposure times less than 1 min.

THE VARIATION IN $L(Ct)50$ WITH EXPOSURE TIME

The data summarized in Fig. 2 show how enormously the $L(Ct)50$ for most animals increases as the duration of exposure to phosgene is reduced. It is almost certain that this effect is due largely to reduction and modification of breathing in the presence of the gas, although it is possible that other factors may play a part, for example, the efficiency of absorption may be influenced by the concentration.

By inspection and empirical trial it was found that the data could best be fitted by an equation of the form

$$(C - C_0)t = K(1 + t_0/t) \quad \ldots\ldots (3)$$

where C is the $LC50$ corresponding to a time t, and C_0 and t_0 are constants. The values of K for different times are seen in Tables I–IV, and mean values are

TABLE II

MORTALITY OF RATS EXPOSED TO PHOSGENE

Exposure time (t) min.	Dosage Ct mg. min./m.^{3}	Mortality	$L(Ct)50$ (approx.) mg. min./m.^{3}	Value for K, $\frac{L(Ct)50-C_0t}{1+t_0/t}$ $t_0 = 4$ min. $C_0 = 10$ mg./m.^{3}
¼	41,500	7/12	39,000	2300
	29,000	3/12		
	20,000	5/16		
	16,000	2/8		
	10,000	1/4		
	6,200	0/4		
	3,200	0/4		
½	32,000	7/8	18,000	2000
	25,700	13/20		
	14,000	8/20		
	9,600	4/12		
	5,800	0/4		
1	56,000	8/8	14,000	2800
	32,000	3/4		
	27,000	7/8		
	21,700	10/12		
	12,500	3/12		
	5,700	0/4		
	2,900	0/4		
2	11,700	6/8	7,000	2320
	5,900	3/8		
	3,350	1/4		
	2,500	1/4		
4	6,100	10/12	4,000	1980
	5,200	3/4		
	2,900	3/10		
	2,475	1/10		
8	4,075	7/10	3,000	1950
	3,710	24/40		
	3,625	8/10		
	3,450	1/10		
	2,880	12/30		
	2,410	2/10		
16	2,930	14/20	2,400	1740
	1,900	3/10		
	1,740	0/10		
	1,400	1/10		
32	2,600	7/10	2,200	1670
	2,275	7/10		
	2,225	5/10		
	2,000	2/10		
	1,775	2/10		
	1,700	4/10		
64	3,500	10/10	2,800	2040
	3,480	10/10		
	2,975	21/30		
	2,875	2/10		
	2,560	2/10		
	2,500	4/10		

TABLE III

MORTALITY OF MICE EXPOSED TO PHOSGENE

Exposure time (t) min.	Dosage Ct mg. min./m.^{3}	Mortality	$L(Ct)$ 50 mg. min./m.^{3}	Value for K, $\frac{L(Ct)\ 50}{1 + t_0/t}$ $t_0 = 1.6$ min.
$\frac{1}{4}$	19700	20/20	15000	2020
	11500	7/20		
$\frac{1}{2}$	17000	20/20	10000	2380
	11200	15/20		
	7900	3/20		
1	9500	20/20	5400	2080
	9200	19/20		
	4200	5/20		
2	5700	20/20	3400	1900
	5000	19/20		
	3700	13/20		
	3450	24/40		
	3100	6/20		
	2600	3/20		
	1925	2/20		
4	3525	17/20	3000	2140
	2900	8/20		
	2475	1/10		
8	3625	16/20	2500	2080
	3450	17/20		
	2935	14/20		
	2410	1/10		
	1675	4/10		
16	3450	14/20	2200	2000
	3050	20/20		
	3025	10/20		
	2930	20/20		
	2300	12/20		
	2035	8/20		
	1950	8/20		
	1900	9/10		
	1740	2/10		
	1500	2/10		
	1400	0/10		
32	2275	8/10	2000	1900
	1800	5/20		
	1775	3/10		
	1700	7/20		
64	3480	10/10	1900	1850
	2560	10/10		
	2085	15/20		
	1775	7/20		
	1650	3/20		

indicated in Table V. The value of K is approximately equal to the minimum value of the $L(Ct)50$. In discussing the significance of these constants it is convenient to consider short exposures and long exposures separately.

TABLE IV

MORTALITY OF DOGS EXPOSED TO PHOSGENE

(Data for 1 and 2 min. exposures : Geiling, 1944. Data for longer exposures: Prentiss, 1937)

Exposure time (t) min.	$L(Ct)50$ mg. min./m.^{3}	Value for K, $\frac{L(Ct)\ 50-C_0t}{1+t_0/t}$ $t_0 = 1$ min. $C_0 = 100$ mg./m.^{3}
1	8400	4100
2	4500	2800
5	5500	4160
10	6500	5000
15	6900	5000
20	7400	5150
30	8100	4950
45	9000	4400
60	10,200	4130
75	12,000	4430

Short Exposures.—In this case C_0 is negligible compared with C and the expression (3) becomes approximately

$$Ct = K(1 + t_0/t) \quad (4)$$

Although the values of t_0 used to fit these data were originally obtained by inspection and trial, Professor Gaddum has pointed out that they can also be determined by plotting Ct against $1/t$; this should give a straight line, and K is equal to the value of Ct when $1/t$ is zero. The constant t_0 is the value of t when $Ct = 2K$ (when C_0 is neglected) and K is the value to which Ct approaches when t is infinite. Theoretically it must be less than the minimum value of Ct. Experimentally it was approximately equal to this quantity.

The constant t_0 is the time at which Ct is double this theoretical lower limit ; it corresponds approximately to points on the curves shown in Fig. 2 at a fixed height (log 2) above their minima. If the increase of Ct with short times is entirely due to inhibition of the respiration, then the animals breathe, in a time t_0, half what they would have breathed under threshold conditions with prolonged exposures. In many cases this is likely to be equal to half what they would have breathed in the absence of the gas.

The observed values of t_0 are given in Table V. The values for rats and mice were larger than those for larger animals ; this presumably indicates that rats and mice hold their breath longer than the other animals.

TABLE V

CONSTANTS OBTAINED FOR THE DIFFERENT SPECIES EXAMINED

Species	t_0 (min.)	Minimal values of $L(Ct)$ 50 (mg. min./m.^{3}) observed (K)	theoretical	Value of t at minimal value of $L(Ct)$ 50 (min.)	C_0 safe concentration (mg./m.^{3})
Rats	4	2200	2000	30	10
Mice ..	1.6	1900	2000	64	0
Guinea-pigs..	0.8	1400	1250	8	20
Dogs.. ..	1	4500	4500	5	100

The argument given above leads to the conclusion that K is an index of the toxicity of the gas, and that t_0 is an index of the time for which the animals reduce their breathing to a mean value of half their normal rate.

Long Exposures.—For long exposures Ct was found to increase in some cases, presumably owing to detoxication of the phosgene. It has in fact been found that when C falls below a threshold concentration, death does not result, however long the exposure.

When t is large the formula (3) given above becomes

$$(C - C_0)t = K \quad \ldots\ldots (5)$$

and the correction involving t_0 can be neglected.

DISCUSSION

With the data given, the expected $L(Ct)50$ at any exposure time can be calculated from the equation

$$L(Ct)50 = K(1 + t_0/t) + C_0 t \quad \ldots\ldots (6)$$

using the constants given in Table V. The curves in Fig. 2 are drawn to this equation.

No true minimum value of the $L(Ct)50$ was found for mice, which indicates that C_0 or the safe concentration is very low. Low concentrations which might be safe for other animals would probably cause death in mice on long exposure.

The variations in toxicity with exposure time thus give an indirect indication of the extent to which animals hold their breath in clouds of phosgene. The times (t_0) of reduced breathing may be measures of ability to hold the breath, or of reactions to the irritant gas or of combined effects of several factors.

SUMMARY

The lethal dose expressed as the $L(Ct)50$ of phosgene for small animals rises enormously as the exposure time is shortened. The extent of the rise varies with different species.

The expression $L(Ct)50(1 + t_0/t)$, where t_0 is a species constant, is reasonably constant for short exposures. Assuming that the increase of dosage (Ct) with short time is entirely due to inhibition of respiration, t_0 is an index of the time

for which the animals reduce their breathing by half, in the presence of the toxic gas. Thus the variation of toxicity with time gives an indication of the extent to which different species reduce breathing in the presence of an irritant gas.

We are indebted to Professor Gaddum for his interest in the treatment of the data and to the Chief Scientific Officer of the Ministry of Supply for permission to publish the results.

REFERENCES

Geiling, E. M. K. *Personal Communication*, March, 1944.
Prentiss, A. M. (1937). *Chemicals in War*. McGraw Hill, New York and London.

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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.

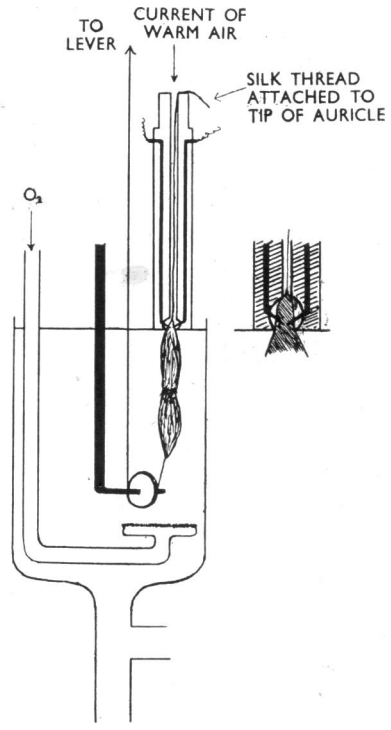


FIG. 1.—Preparation of rabbit auricles suspended in oxygenated Ringer-Locke at 29° C. The electrode holder is made of perspex, and the inset shows a closer view of its lower end.

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

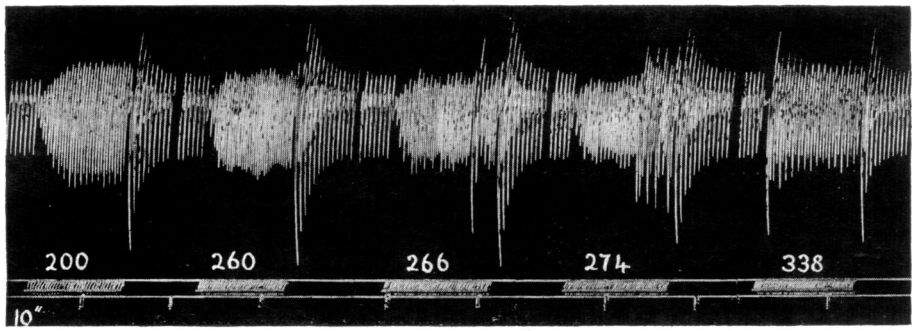


FIG. 2.—Record obtained from preparation shown in Fig. 1. The upper signal-marker indicates the duration of stimulation by break induction shocks; the number of stimuli per minute is recorded above this. Up to 260/min. the auricle responds to each stimulus; at 266 and 274/min. it just fails to follow;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

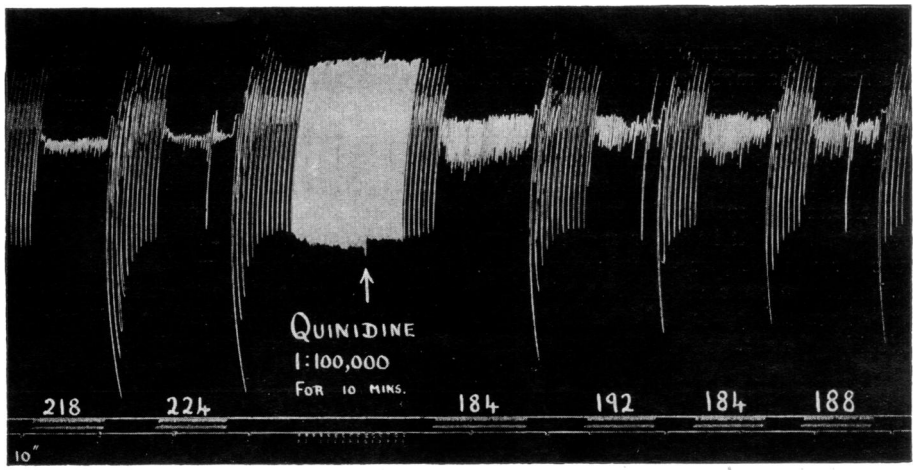


FIG. 3.—Preparation as in Fig. 2. The maximal rate at which the auricle responds to electrical stimuli is 218/min.; after quinidine 1 :100,000 for 10 minutes this is reduced to 184/min. (The smoked drum was stopped for 9 minutes at the arrow, and was run at reduced speed immediately before and after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

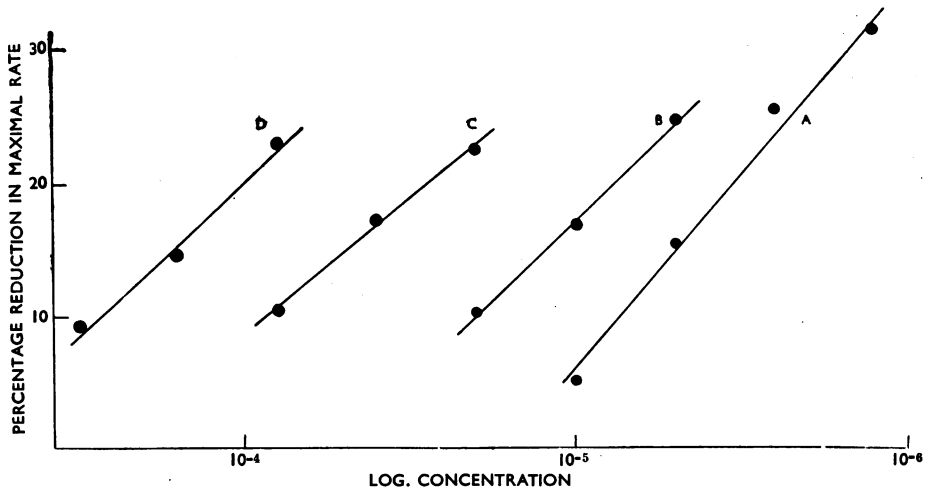


FIG. 4.—Ordinates: per cent reduction in maximal rate at which the auricle responds to electrical stimuli. Abscissae: concentration on a logarithmic scale. The points are the mean figures taken from *(a)* Quinine, 10^{-6} auricles; *(b)* Quinidine, 65 auricles; *(c)* compound 25, 10^{-6} auricles; and *(d)* Butethanol, 10^{-6} 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

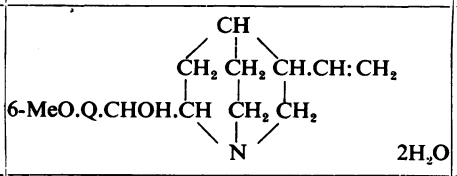
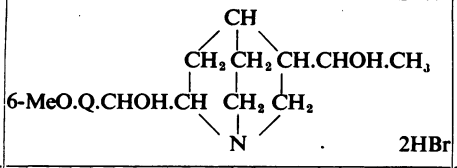
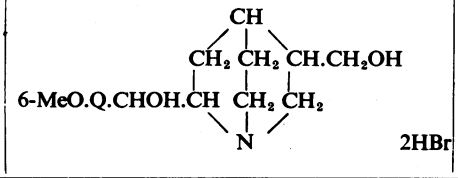
Name or Number	Formula	Activity (Quinidine = 1)	Number of Auricles used	Molar Weight	Activity per mol.	LD 50 mg./kg. mice IP	Therapeutic Index
Quinidine		1·0	—	360	1·0	135	1·0
1		0·12	2	504	0·17	—	—
2		0·04	1	490	0·06	—	—

TABLE I (*continued*)

Q=4-quinolyl N=α-naphthyl Ph=phenyl

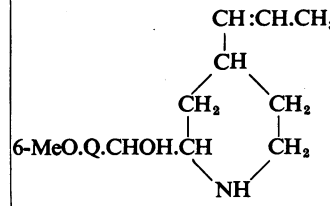
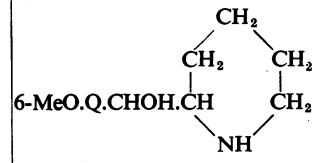
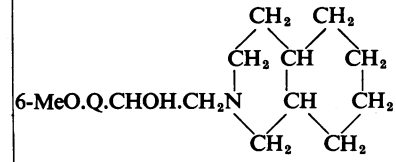
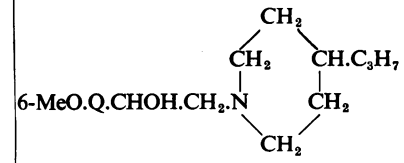
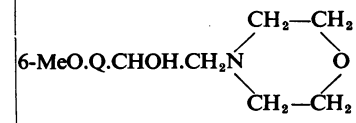
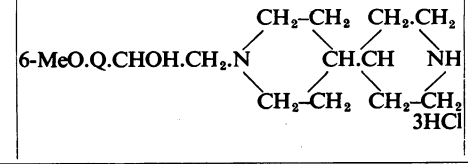
Name or Number	Formula	Activity (Quinidine=1)	Number of Auricles used	Molar Weight	Activity per mol.	LD50 mg./kg. mice IP	Therapeutic Index
Niquidine	 6-MeO.Q.CHOH.CH 2HCl	0·35	2	371	0·36	—	—
3	 6-MeO.Q.CHOH.CH HCl	0·14	2	308	0·12	—	—
4	 6-MeO.Q.CHOH.CH$_2$N 2HCl	0·82	2	412	0·94	190	1·2
5	 6-MeO.Q.CHOH.CH$_2$.N 2HCl	1·05	2	400	1·16	—	—
6	 6-MeO.Q.CHOH.CH$_2$N 2HCl	0·10	2	360	0·10	—	—
7	 6-MeO.Q.CHOH.CH$_2$.N 3HCl	0·10	1	478	0·13	—	—

TABLE I (*continued*)

Q=4-quinolyl N=α-naphthyl Ph=phenyl

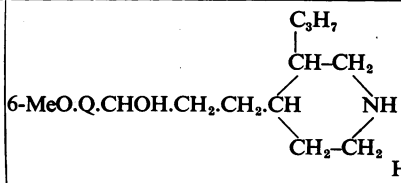
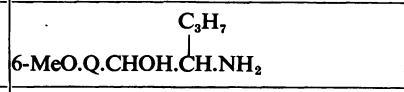
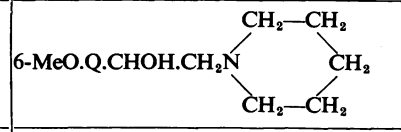
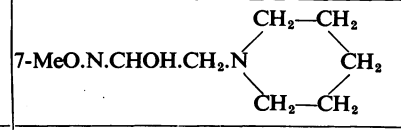
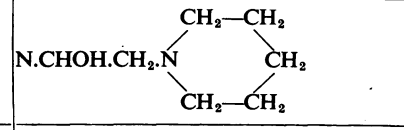
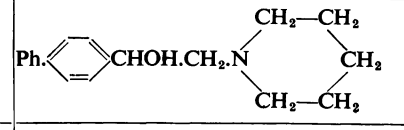
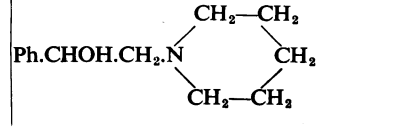
Name or Number	Formula	Activity (Quinidine=1)	Number of Auricles used	Molar Weight	Activity per mol.	LD50 mg./kg. mice IP	Therapeutic Index
8		0·96	2	391	1·05	150	1·1
9		0·35	3	422	0·41	—	—
10	6-MeO.Q.CHOH.CH_2.N(C_2H_5)$_2$ 2HCl	0·46	2	347	0·46	—	—
11	6-MeO.Q.CHOH.CH_2.N(C_4H_9)$_2$ 2HCl	1·9	3	403	2·14	175	2·5
12	6-MeO.Q.CHOH.CH_2.N(C_5H_{11})$_2$ 2HCl	0·49	2	431	0·59	—	—
13	Q.CHOH.CH_2.N(C_2H_5)$_2$ 2HCl	0·43	1	317	0·39	—	—
14		0·55	3	322	0·50	260	1·0
15		3·4	4	321	3·0	(175)	4·4
16		2·8±0·11	5	291	2·3	200 (250)	4·1
17		2·4	4	317	2·1	170 (175)	3·0
18		1·4	2	241	0·94	(175)	1·8

TABLE I (*continued*)

Q = 4-quinolyl N = α-naphthyl Ph = phenyl

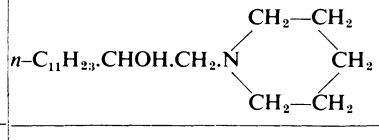
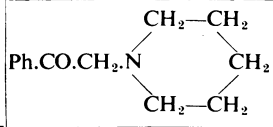
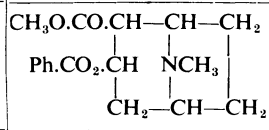
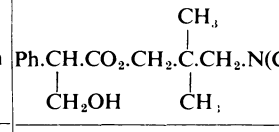
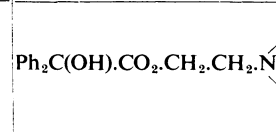
Name or Number	Formula	Activity (Quinidine = 1)	Number of Auricles used	Molar Weight	Activity per mol.	LD50 mg./kg. mice IP	Therapeutic Index
19		0·18	3	319	0·15	(400)	0·53
20	$Ph.CO.CH_2.N(C_2H_5)_2$ HBr	0·32	2	272	0·24	—	—
21		0·90	2	284	0·71	65	0·43
Cocaine		6·2	5	325	5·6	—	—
Procaine	$p\text{-}NH_2.Ph.CO_2.CH_2.CH_2.N(C_2H_5)_2$. HCl	0·8	3	272	0·6	180 (250)	1·1
Butethanol	$p\text{-}C_4H_9NH.Ph.CO_2.CH_2.CH_2.N(CH_3)_2$. HCl	13·8 ± 4·6	10	300	11·5	70	6·4
Butyn	$p\text{-}NH_2.Ph.CO_2.CH_2.CH_2.CH_2.N(C_4H_9)_2\frac{1}{2}H_2SO_4$	5·5	4	355	5·3	80	3·3
Syntropan		1·3	2	405	1·5	—	—
Trasentin	$Ph_2CH.CO_2.CH_2.CH_2.N(C_2H_5)_2$ HCl	0·63	2	347	0·59	—	—
22	$Ph_2C(OH).CO_2.CH_2.CH_2.N(CH_3)_2$. HCl	2·1	2	335	2·0	—	—
23	$Ph_2C(OH).CO_2.CH_2.CH_2.N(C_2H_5)_2$ HCl	3·0	4	363	3·0	160	3·5
24	$Ph_2C(OH).CO_2.CH_2.N(CHMe_2)_2$ HCl	6·8 ± 1·1	6	391	7·4	155	7·8
25		5·4 ± 1·9	10	375	5·6	150	6·0

TABLE I (*continued*)

Q=4-quinolyl N=α-naphthyl Ph=phenyl

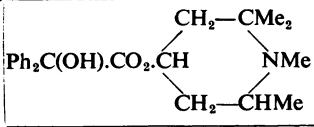
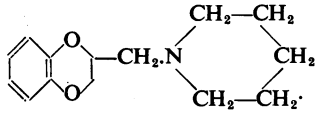
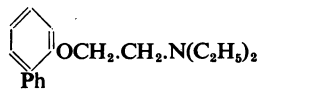
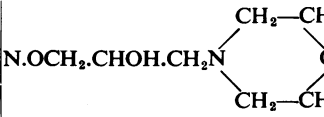
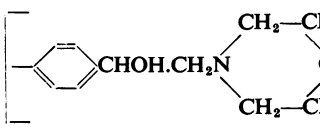
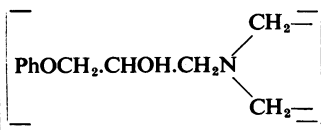
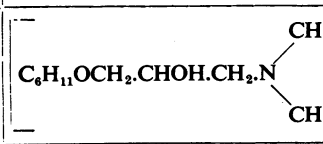
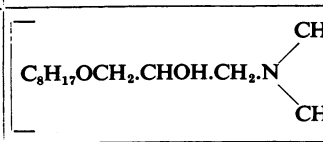
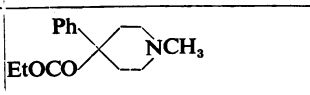
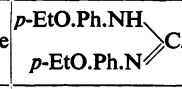
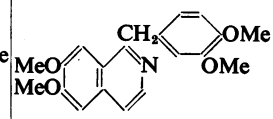
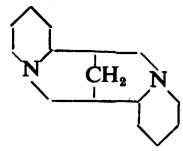
Name or Number	Formula	Activity (Quinidine=1)	Number of Auricles used	Molar Weight	Activity per mol.	LD50 mg./kg. mice IP	Therapeutic Index
26		4·6	4	403	5·1	75	2·6
F933		3·2	4	269	2·4	180	4·3
F1262		4·7±0·67	5	305	3·9	125	4·4
27		4·4±1·4	5	321	4·0	150	4·8
28		0·17	1	481	0·23	—	—
lower m.p.		3·2	4	459	4·4	140	4·1
29		2·1	2	459	2·9	—	—
higher m.p.							
30		0·63	2	471	0·82	—	—
31		<0·10	1	531	—	—	—
Pethidine		0·83	2	283	0·65	—	—

TABLE I (*continued*)

Q=4-quinolyl N=α-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	 HCl	4·5	4	334	4·2	80	2·7
Papaverine	 HCl	0·50	3	375	0·52	—	—
Sparteine	 H_2SO_4	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: (α) melts to an opaque liquid at 89° which becomes clear at 102° C.; hydrochloride, m.p. 238° efferv.; (β) 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 benzilic 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 benzilic 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 LD50 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 (β-phenyl β-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; β-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

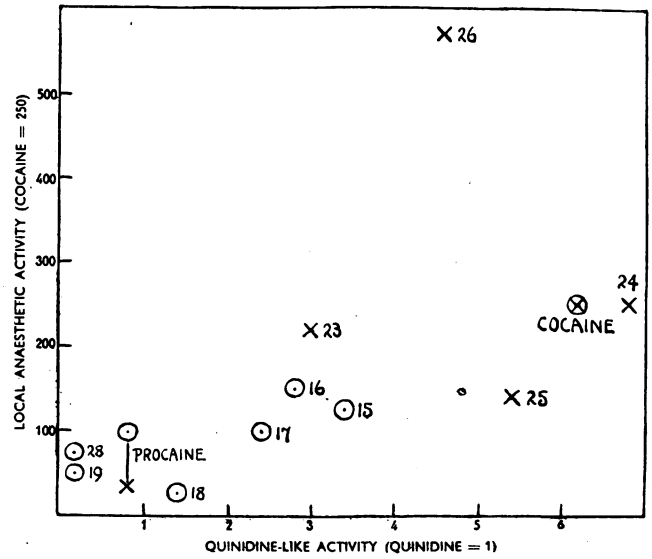


FIG. 5.—Ordinates: Local anaesthetic activity (cocaine=250). Abscissae: Quinidine-like activity (quinidine=1). Numbers refer to compounds in Table I. The data for local anaesthetic activity are taken from MacIntosh and Work (circles) and from Wajda (crosses).

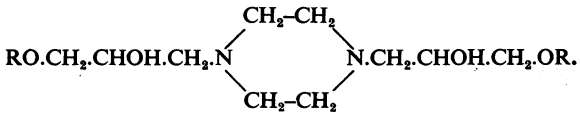
local anaesthetic activity. In addition there is nupercaine; the latter is difficult to assay upon the auricle since it causes a profound and prolonged depression of amplitude, as well as a reduction in the maximal rate at which the auricle will respond. Papaverine (Pal, 1914; Macht, Johnson and Bollinger, 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 benzilic 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 C_6H_{13} or C_7H_{15} they had respectively 8 and 22 times the activity of cocaine upon the rabbit's cornea. In compound 31 R is C_8H_{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

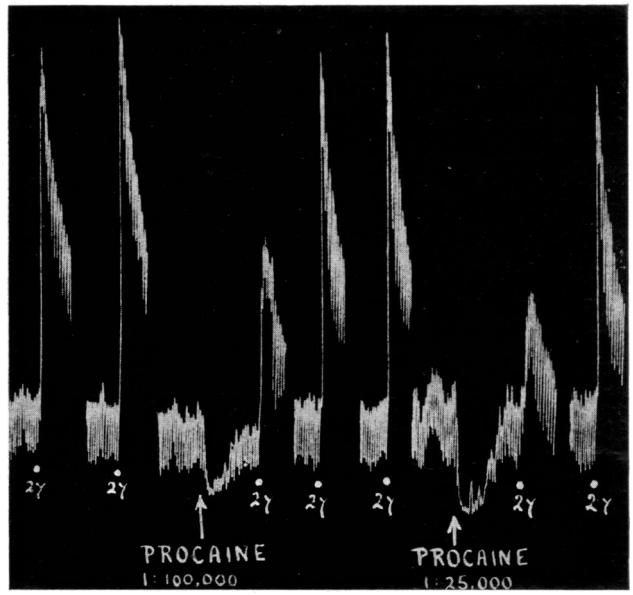


FIG. 6.—Isolated rabbit duodenum suspended in oxygenated Ringer-Locke at 37° C. Procaine hydrochloride in a concentration of 1 : 25–100,000 reduces the contraction caused by 2 μg. acetylcholine (50 ml. bath).

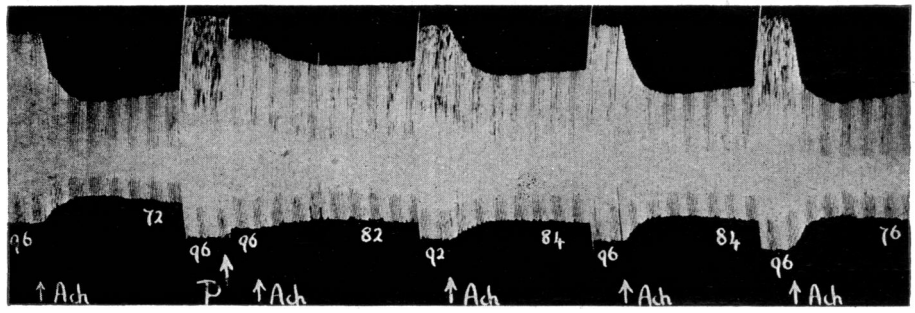


FIG. 7.—Isolated rabbit auricles suspended in oxygenated Ringer-Locke at 29° C. Acetylcholine 10^{-6} (Ach) was added at 8-minute intervals; the initial rate and the subsequent slowest rate per minute are recorded on the tracing. Procaine (1 : 25,000) was added to the bath at P and the drum stopped for 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-β-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-β-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 benzilic ester series (22–26) is also particularly active, but trasentin, 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 benzilic 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 *a*-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 β-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 anaethetics 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 *p*H 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 $R_3\overset{+}{N}H$ and the base R_3N in equilibrium:

$$R_3\overset{+}{N}H + \overset{-}{O}H \quad \rightleftharpoons \quad R_3N + H_2O$$

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. Bülbring (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.

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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. Pharmak.* **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.

Brit. J. Pharmacol. (1946), **1**, 113,

THE ASSESSMENT OF ANALGESIC ACTIVITY IN NEW SYNTHETIC DRUGS

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Many methods for the measurement of analgesic effect have been described recently. They may be classified into mechanical pressure methods as described by Eddy (1928, 1932), electrical stimulation methods used by Koll and Reffert (1938), Macht and Macht (1940) among others, and lastly heat stimulus methods, which have been employed extensively in recent years by Hardy, Wolff and Goodell (1940).

The method of electrical pain threshold determination of Macht and Macht may have some quantitative value. Electrical stimuli from an induction coil are applied to the scrotal sacs of a rat, and when the stimulus is sufficiently strong to produce a sensation of pain the animal squeaks. If, however, the animal is heavily narcotized the stimulus may be high enough to contract the cremaster muscle and compress the testes without causing signs of pain. An attempt was made to use this method with an electronic stimulator, measuring the intensity of the stimulus by means of a peak volt-meter, but the contact resistance at the electrodes was too variable for the method to be reliable and it was not pursued further.

The method described by Hardy, Wolff and Goodell was next examined, but with human subjects our results did not accord with theirs. The apparatus employed was exactly as described by these workers with the single exception that absolute values of the radiant heat stimulus were not measured. A volt-meter and ammeter were connected to the lamp so that the power being dissipated as heat could be calculated. This presented no disadvantage, as we were only interested in the change of pain threshold in the course of several hours. Day-to-day changes such as blackening of the lamp bulb and deposit on the lens system are therefore of no importance in this application. The ammeter was

omitted after a calibration curve had been prepared correlating the voltage across the lamp with the power dissipated in the lamp in watts.

Wolff, Hardy and Goodell (1940), in a second paper, described some findings with morphine and codeine and their effects upon the pain threshold of man. They give time-action curves for morphine sulphate with doses of 0.1 mg. to 30 mg. for man. We have been unable to reproduce these curves, and even when 10 mg. of morphine was injected into human subjects, with the actual drug unnamed, the rise in pain threshold was not statistically significant. A dose of pethidine of 100 mg. by mouth in several subjects caused a slight elevation of the pain threshold, but the recognition of the actual threshold intensity became a very difficult matter. Similar difficulties have been experienced by Dodds, Lawson, Simpson and Williams (1945) in testing diphenylethylamine compounds for analgesic action in the human subject. For ourselves we cannot say that therapeutic doses of morphine or pethidine raise the pain threshold as determined by the apparatus of Hardy et al., but we did find it increasingly difficult to discern a critical end-point after the doses of the drugs mentioned above. It may be that we were ignoring the point of obvious pain sensation and concentrating too much on the detection of a minimal sensation, but it was usually a most uncertain method.

The method of Hardy, Wolff and Goodell, however, gives clear-cut values for changes in the pain threshold which result from ischaemia, as shown in Fig. 1. The pain threshold of a male subject, who had previously shown a high degree of discrimination for small changes in the intensity of the radiant heat stimulus, was determined three times at ten-minute intervals and found to be constant. A sphygmomanometer cuff was then put on the right arm and inflated to a pressure of 200 mm. Hg, and the pain threshold was determined at intervals of

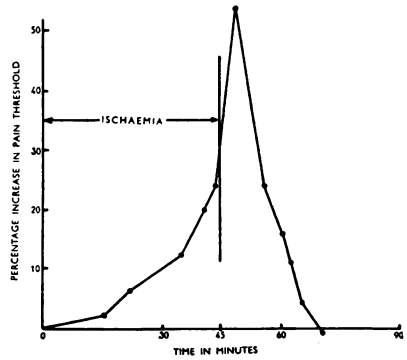

FIG. 1.—The effect of local ischaemia on the pain threshold in man.

approximately ten minutes. After forty-five minutes the pressure in the cuff was released and the pain threshold determinations continued until the values obtained were similar to those at the commencement of the experiment.

It will be seen that there is a real rise in the level of threshold stimulus, and this rise became much more rapid immediately after the sphygmomanometer cuff had been deflated; at the same time the subject complained that the pain was much more intense in the arm as a result of the resumption of blood flow than it was at any time during arterial occlusion. In this case the end-point of the pain threshold determination was quite well defined. In the experiments described by Hardy, the pain threshold started to fall as soon as the circulation was restored.

In view of the difficulty of detecting a precise end-point when drugs have been administered to man, and more especially since the toxicities of many of the substances we had to examine were not well established, it was decided to try the apparatus on various small laboratory animals.

The first animal used was the guinea-pig, and a shaved and blackened area of the flank was used for the radiant heat stimulus. Since the aperture employed by Hardy was too large in this case, and in view of their evidence that the greater the area of skin stimulated the greater is the heating sensation, whereas there is no similar area summation of pain sensitivity, the area of the aperture was altered to 1 sq. cm. Since guinea-pigs are excitable and did not give a precise end-point their use was abandoned and attention was turned to the use of rats.

Rats were first used in the same way as guinea-pigs; the pain threshold was indicated by a flinching reaction of the rat in which there is a general quivering of the body just before the shutter stops the radiant heat stimulus. At this point our attention was drawn to the work of D'Amour and Smith (1941), in which rats were used in a similar apparatus, but the tip of the tail was used as the sensory area. The method differs somewhat from that of Hardy, as the time of exposure to a set intensity of radiation is the adjustable factor and is timed with a stop-watch. The differences observed are small and need some automatic device to eliminate the reaction time of the observer. We endeavoured to do this by allowing the rat tail to lie in a groove in a board and across the tail was placed a lightly spring-loaded contact strip dipping in a mercury cup arranged to stop an electric chronometer if the tail was withdrawn.

The method did not give such consistent results as the use of rats with the Hardy apparatus mentioned above and was therefore abandoned. In the course of these investigations, however, we found that the rat tail was a very sensitive area and more suitable for stimulation than the flank. The Hardy apparatus was therefore modified by fitting a platform at right angles to the radiant heat aperture so that the rat could be placed upon it with its tail in front of the 1 sq. cm. aperture. The duration of stimulus was decreased to 2 secs. so that the heating effect would be less and the shutter was adjusted to operate silently once in 15 seconds. This apparatus is drawn diagrammatically in Fig. 2.

The rat, its tail blackened with Indian ink, is placed upon the platform with the tip of the tail across the aperture and the operator restrains it gently with

his right hand. The stimulus intensity is adjusted by the variable resistance controlling the lamp current and a sufficient number of trials are made, usually 4 to 6, to enable the operator to ascertain the pain threshold. The detection of the pain threshold stimulus is not a simple observation, and it is necessary to

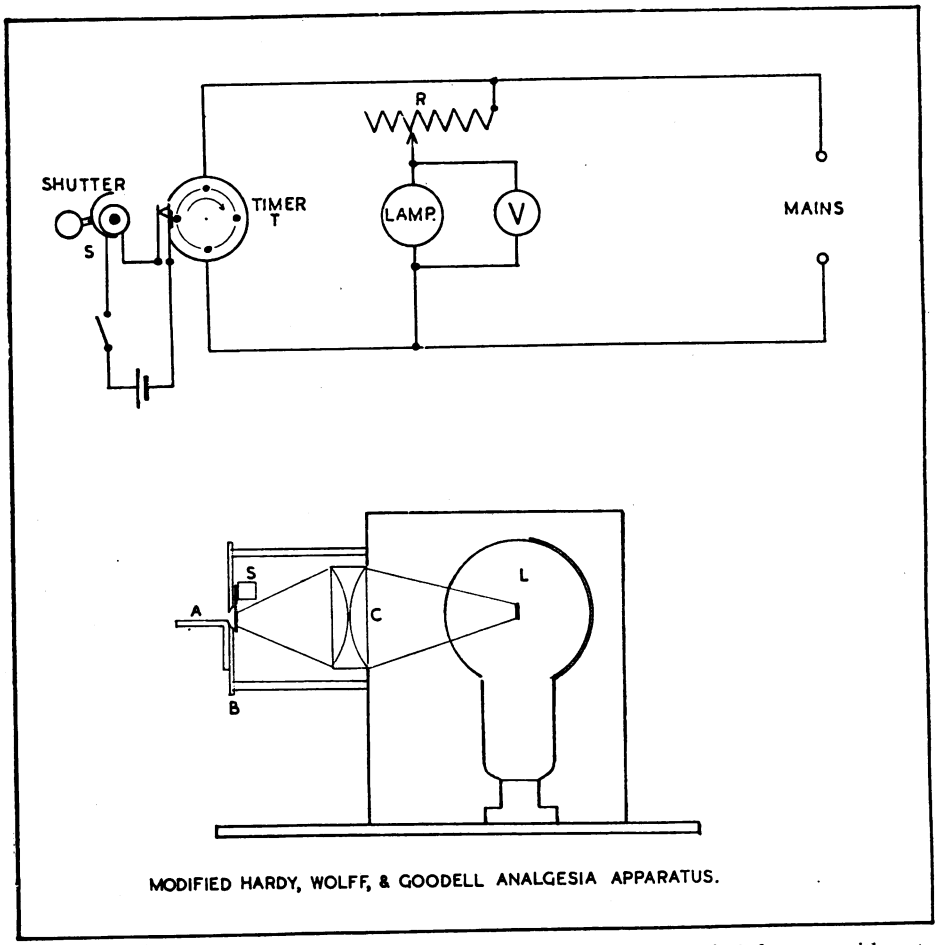

FIG. 2.—The apparatus of Hardy, Wolff and Goodell (1940) modified for use with rats.

Upper diagram: Electrical circuit. R, resistance to reduce the voltage across the lamp to 1/3 of its rated value when fully in circuit (50 ohms in this apparatus using a 200 volt lamp). T, timer for magnetic shutter ; this consists of a synchronous clock motor with a four-point cam so that the shutter is lifted for 2 secs. at intervals of 15 secs. S, shutter, made from an old domestic electric bell indicator and operated on a 1.5v. dry cell.

Lower diagram: A, small shelf to support the rat with the tip of the tail across the radiant heat aperture. B, paxolin front panel now fitted with water cooling as described in the text and carrying the shutter, S. C is a lantern condenser of 4.5 in. diameter and about 10 cm. focal length. L, projection lamp with grid filament, 1,000 watts, mirror-backed.

allow the operator to become sufficiently experienced before tests are conducted. Furthermore, it has been found that it is necessary to train new rats to discriminate between stimuli of different intensity before using them for the test. This is done by allowing several successive stimuli of high intensity to fall upon the tail until the rat removes its tail in a positive manner after each stimuli. The stimuli are repeated at a slightly lower intensity and the rats are then able to discriminate sufficiently accurately. This training is only necessary with rats which have not been used before for these experiments on the same day. If the necessity of training the rats is not appreciated the determination of the initial pain threshold becomes very time-consuming. No single response is used as a criterion, but a general assessment is made from slight movements of the tail, quivering of the body of the rat, or in cases of excessive stimulus removal of the tail. If the stimulus is below the threshold, no change in the rat's activity will be observed.

A recent paper by Slaughter and Wright (1944) draws attention to the need for stabilization of the electricity supply to the apparatus and the use of a thermocouple to measure the temperature of the surface being stimulated. Stabilization is not necessary in our case, because the stimulus is measured as the product of the instantaneous readings of the lamp current and voltage. We were not able to show that the temperature of the radiant heat upon a thermocouple had any advantage over our purely electrical measurements when each experiment was self-contained and completed in one day.

The method described above has been adopted in these laboratories and has proved very satisfactory for assessing the presence or absence of analgesic activity in new drugs and giving a quantitative comparison between them.

The method of pain threshold determination having been established it was necessary to investigate a suitable experimental design to enable the maximum information to be obtained. Rats are convenient in this respect, as all our animals are a pure Wistar strain and it is easy to use litter-mates in such an experiment. A series of preliminary experiments was made to find out whether there is a greater variation in the response of rats to analgesic drugs when they are not taken from the same litter than when litter-mates are used. It was also hoped to discover whether the " cross-over " construction of a test would result in a greater consistency of response.

The experiments were arranged as follows:

Experiment I.

Three sets of three rats, not litter-mates, were used and the experiment was arranged as a three-way cross-over test on three days so that each set of three rats received different doses of morphine on each day and had each received all three doses at the conclusion of the experiments.

TABLE I

EXPERIMENT I. THREE-WAY CROSS-OVER TEST ON RATS WHICH WERE NOT FROM THE SAME LITTERS.

DAY 1.

Rat No.	1	2	3	4	5	6	7	8	9
Initial pain threshold W. ..	268	259	230	222	248	222	213	213	222
Morphine dose	2·5 mg./kg.			3·0 mg./kg.			3·5 mg./kg.		
Pain threshold after 30 min. W.	279	277	307	286	268	320	279	286	320
Response %	+20	+8	+33	+30	+8	+44	+31	+34	+44

DAY 2.

Initial pain threshold W. ..	230	279	237	222	230	237	259	213	279
Morphine dose	3·5 mg./kg.			2·5 mg./kg.			3·0 mg./kg.		
Pain threshold after 30 min. W.	351	286	259	342	259	230	259	230	268
Response %	+53	+3	+3	+54	+12	−4	0	+8	−4

DAY 3.

Initial pain threshold W. ..	237	268	230	222	237	213	237	222	230
Morphine dose	3·0 mg./kg.			3·5 mg./kg.			2·5 mg./kg.		
Pain threshold after 30 min. W.	307	307	286	351	320	334	230	279	259
Response %	+25	+15	+25	+58	+35	+57	−4	+25	+13

Response % is a term used for the percentage change in the initial pain threshold after treatment with analgesic agents.

MEAN RESPONSES OF ALL RATS.

Dose mg./kg.	Response %
2·5	+17·6
3·0	+16·7
3·5	+35·3

The actual results obtained are reproduced *in extenso* in Table I to show the general type of record obtained. We had previously found that the peak of analgesic action following the injection of morphine into rats occurred at a quarter to half an hour after injection, and consequently the reading was taken for each rat half an hour after injection.

Table II shows an analysis of these results, and it will be seen that there was a significant difference in response between the doses of morphine of 0.3 and 0.35 mg./100 g. but not between 0.25 and 0.3 mg./100 g. This is probably due to all three dose levels not lying on the steepest part of the dose response curve.

TABLE II

ANALYSIS OF RESULTS RECORDED IN TABLE I

	Dose, mg./kg. 2·5	3·0	3·5
Mean Response	17·6	16·7	35·3
Variance of mean response	37·5	23·0	48·4
Standard deviation	6·1	4·8	6·9
Response difference		0·9%	18·6%
Variance of mean difference		60·5	71·4
Standard deviation		7·8	8·4
"t"		1·17	2·21
Degrees of freedom		16	16
"P"		0·9	0·04

Experiment II.

A similar experiment, using litter-mates in such a manner that each set of rats had the same litter history as the other two sets, was next performed. It will be seen from the results in Table III that a difference in dose of .05 mg./100 g.

TABLE III

ANALYSIS OF RESULTS OBTAINED IN EXPERIMENT II IN WHICH LITTER-MATE RATS WERE USED

	Dose, mg./kg. 2·5	3·0	3·5
Mean response	3·8	23·2	33·6
Variance of mean response	20·8	8·7	75·0
Standard deviation	4·6	2·9	8·7
Response difference		19·4%	10·4%
Variance of mean difference		29·5	83·7
Standard deviation		5·4	9·1
"t"		3·6	1·14
Degrees of freedom		16	16
"P"		0·005	0·25

was distinguished at the lower end of the dose scale but not at the higher level. It can be concluded that these rats were probably more sensitive than those of Experiment I.

The mean variance is, however, very much the same as that of the previous experiment, so that it does not seem that litter-mate rats are superior to mixed rats from the same stock.

Experiment III.

In this experiment three sets of nine rats with litter-mates in each of the three sets were used on the same day. The results given in Table IV show that there was discrimination between each pair of doses, but the dose difference of 0.05 mg./100 g. was too close for the difference in response to be statistically significant with such a small number of animals. The mean variance was rather less than in the other two experiments.

TABLE IV

ANALYSIS OF RESULTS OBTAINED IN EXPERIMENT III IN WHICH LITTER-MATE RATS WERE USED ON THE SAME DAY.

	Dose, mg./kg.		
	2·5	3·0	3·5
Mean response	14·0	18·0	25·0
Variance of mean response	4·4	13·4	32·0
Standard deviation	2·1	3·7	5·6
Response difference	4%	7%	
Variance of mean difference	17·8	45·4	
Standard deviation	4·2	6·73	
"t"	0·953	1·04	
Degrees of freedom	15	16	
"P"	0·3	0·3	

In the absence of any outstanding differences in the results, it was concluded from these experiments that the use of litter-mates did not give any great increase in accuracy to the test, and the advantage of using a cross-over arrangement was not particularly apparent. Although there is apparently no outstandingly desirable form for the experiment, we find it more convenient to use some form of cross-over test where any high degree of accuracy is required, as the work is thus divided over several days. If only approximate indications of analgesic potency are required a comparison is usually made between two litter-mate groups on the same day.

THE DURATION OF ACTION OF MORPHINE ON RATS

Although some previous experiments had indicated that the peak of analgesic action of morphine in rats occurred about half an hour after injection an experiment was performed in which the pain threshold was determined at frequent intervals after injection of morphine.

Two sets of six rats were used, the rats of one set being litter-mates of the corresponding rats in the other set. All rats were given 0.35 mg./100 g. of morphine by subcutaneous injection after the initial pain threshold readings had been taken. One set of rats was used for pain threshold measurement after a quarter, half, three-quarters and one hour from the time of injection and the second set for readings at 10 minutes, 20 minutes, and 35 minutes.

It was not possible to use the same rats at all these time intervals as the actual readings take about 10 minutes for six rats. The following results were obtained:

Set I

Time after injection, minutes	15	30	45	60
Actual percentage rise of threshold	23.4	13.8	12.4	–3.5

Set II

Time after injection, minutes	10	20	35
Actual percentage rise of threshold	4.6	17.9	2.8

This experiment shows that the peak of morphine analgesic action occurs after about 15–20 minutes in rats, and this time interval is now employed for drug comparisons with morphine together with a time-response determination for the drug under examination.

We had noticed on several occasions that the front panel of the apparatus in which the light aperture was cut became increasingly hot when readings were taken at such frequent intervals that the lamp was left on for long periods. This produced an apparent lessening of the analgesic effect after the initial reading because less radiant heat was needed to elicit a response. This was observed especially when control rats were incorporated in a test. The mean pain threshold of a group of such rats usually appeared to fall by some 10 per cent after the initial reading.

Since the above experiments were done a new front panel has been made for the apparatus. This was constructed from paxolin sheet 1/4 in. thick. An annular recess was milled out in the side facing the lamp and surrounding the stimulus aperture. The recess was 1 in. wide and 3/16 in. deep, and the rear of the panel was faced with a piece of 1/16 in. paxolin, cemented and screwed over the annular recess and having an aperture in its centre coinciding with the stimulus aperture. Two thin tubes were fitted in the edge of the panel so that a stream of water could be passed through the annular space. With this device the temperature of the panel remained very constant even after the lamp had been on for a period of some hours.

In addition to this we have found that experience of the method enables the end-points of the threshold determination to be found more accurately, and consequently smaller differences in response assume more significant proportions.

Dose Response Curve for Analgesic Action of Morphine in Rats

Six litters of rats were used with five or more rats in each group, and so grouped that one rat from each litter was placed into each of the five groups. The rats were then given doses of morphine of 0.27, 0.30, 0.33, 0.36, and 0.40 mg./100 g. to each group respectively and the pain threshold change determined as a percentage of the initial value after 20 minutes.

TABLE V

RESULTS OBTAINED IN AN EXPERIMENT TO ESTABLISH A DOSE/RESPONSE RELATIONSHIP FOR THE ANALGESIC ACTION OF MORPHINE

Group	Dose of Morphine, mg./kg.	Percentage increase in pain threshold for individual rats						Mean Response
		1	2	3	4	5	6	
1	2·7	25	32	20	16	21	17	21·8
2	3·0	16	18	8	38	39	20	23·1
3	3·3	54	36	66	43	23	38	43·0
4	3·6	57	89	74	82	66	66	72·3
5	4·0	97	91	85	77	83	72	84·1

The results obtained are given in Table V. When plotted on a linear scale the mean response values gave the curve of Fig. 3, and when plotted in a

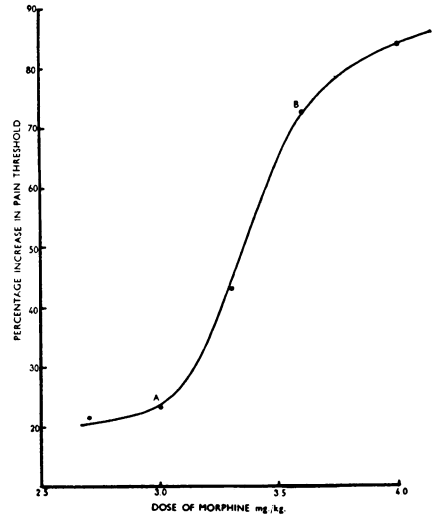

FIG. 3.—Curve relating the dose of morphine injected and the mean elevation of the pain threshold 20 minutes after injection. Each point is a mean value for six rats.

semi-logarithmic manner the usual formula to a regression-line of this type was applied to section AB and upon calculation the slope of this response was found to be 576.

Formula to log-dose/response curve:

$$y = 48.9 + 576\ (x + 0.483)$$

(by substitution in the formula $y = \bar{y} + b\ (x - \bar{x})$)

therefore $b = 576$.

Several days later two of the groups of rats were taken at random and injected with two solutions of morphine, both made up to a strength such that

a dose of 1.0 c.c./100 g. would cause a response within the measurable limits of the method. The percentage increase in pain threshold was determined for each rat and the mean value calculated for each group.

Group	*Dose*	*Mean percentage increase in threshold*
1	Soln.A 1.0 c.c./100 g.	20.6
2	Soln.B 1.0 c.c./100 g.	63.1

When these values were substituted in the equation and using the slope value of 576 the following results were obtained:

	Soln. A	*Soln. B*
Log. of estimated dose =	$\bar{1}.465$	$\bar{1}.543$
Therefore estimated dose =	0.292 mg.	0.349 mg.
Estimated Concentration of solutions =	0.292 mg./c.c.	0.349 mg./c.c.
Limits of error P = 0.95 =	0.265–0.312 mg.	0.323–0.375 mg.
Actual strengths of solutions prepared =	0.30 mg./c.c.	0.36 mg./c.c.

It is very rarely that we have any need to perform an accurate assay of analgesic drugs, but it would seem that it is quite a practical procedure. Normally with new compounds it is sufficient to aim at obtaining approximately a 50 per cent increase in pain threshold in a comparison with morphine and then the relative activity of the two drugs can be roughly represented by the ratio of the doses employed.

A comparison was made between morphine and heroin to illustrate this method of approximate comparisons and results are given in Table VI. Since heroin is known to be a more effective analgesic than morphine a dose of 0.1 mg./100 g. was given to nine rats and a dose of 0.35 mg./100 g. of morphine to a set of nine rats of similar litter constitution.

It was found that the effect of the dose of heroin was such that the pain threshold level of most of the rats rose above the upper limit of the test stimulus range. The results are shown graphically in Fig. 4.

The experiment thus showed that heroin was more than 3.5 times as effective as morphine as an analgesic and a second experiment arranged as a cross-over test with two groups of nine rats was performed with doses of 0.05 mg./100 g. and 0.35 mg./100 g. of heroin and morphine respectively.

The mean results for eighteen rats are given in Table VI and graphically in Fig. 4. The graph indicates that the maximum analgesic action of morphine occurs one hour after injection. This is most probably not so and previous experiments have indicated that it does, in fact, occur between fifteen and forty-five minutes after injection. The readings at one quarter and one hour for the animals receiving morphine probably lie on either side of the time for maximal analgesic

action, so that the true point of maximal action is not seen in these curves. It is, of course, unsound to compare the analgesic response of a group of rats given a certain dose of a drug with another response from a different group of rats at a different time given the same treatment, especially in view of the very steep slope

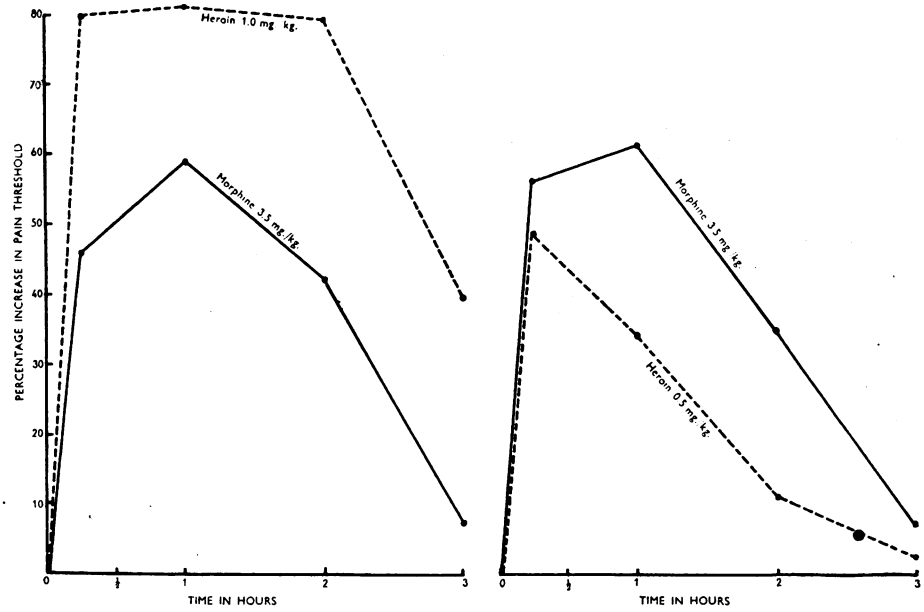

FIG. 4.—A comparison between the analgesic effects of morphine and heroin.

Left-hand curves: mean values obtained from groups of nine rats in each case.

Right-hand curves: mean values obtained from groups of eighteen observations at each point. Eighteen rats were used in the form of a cross-over test.

of the log. dose/response relationship usually given by analgesic drugs. It does seem, however, that these later experiments, performed after water-cooling had been fitted to the apparatus, show a bigger analgesic response for a given dose of morphine than do the earlier ones, a result rather to be expected.

It will be seen that heroin is rather less than seven times as potent as morphine as an analgesic, and that the duration of action is in both cases similar, which is supported by clinical experience.

From this experiment we may draw some general conclusions. If a test is arranged as a cross-over test using nine animals in each group and deriving eighteen observations on each treatment it is usually found that differences in the mean response percentages to each treatment exceeding ten are significant to $P > 0.95$ limits.

The variance of the response is naturally higher at times when the analgesic action is near the centre of the dose/response curve, but it will be seen in the

TABLE VI

THE RESULTS OBTAINED IN AN EXPERIMENT TO COMPARE THE ANALGESIC ACTIONS OF MORPHINE AND HEROIN

Dose	Percentage increase in pain threshold (Mean value for 18 rats)			
	½ hr.	1 hr.	2 hr.	3 hr.
Morphine 3·5 mg./kg. S.C.	56	61	35	9
Heroin 0·5 mg./kg. S.C.	48·5	34	11	2
Difference in response %	7·5	17	24	7
Variance of mean difference in response	66·2	48·0	38·0	19·4
Standard deviation	8·14	6·9	6·16	4·4
"t"	0·922	2·46	3·9	1·592
Degrees of freedom	34	34	34	34
"P"	0·4	0·02	$< 0·001$	0·1

experiment that a difference of seventeen between the two treatments at the first hour was highly significant.

It has been shown that the method is suitable for the comparison of the analgesic action of new drugs and is at the same time suitable as the basis of a relatively exact biological assay. A dose of morphine should always be included as a standard of comparison for the examination of new drugs, and we also include a group of animals receiving both morphine and the new compound as separate injections at the same time, in order that potentiation phenomena may not be overlooked.

SUMMARY

1. A brief review of the methods for the measurement of analgesic action is given together with an account of experiments with the apparatus described by Hardy, Wolff and Goodell on human subjects. The results which these workers obtained with morphine could not be repeated, but their observation that local ischaemia causes an elevation of the general pain threshold was confirmed.

2. In order to test drugs of unknown toxicities for analgesic action the method has been modified for use with rats. The blackened tip of the tail is used as the sensitive area and the pain threshold is represented by the power of the lamp in watts.

3. After an initial training period the rat is able to distinguish small changes in the intensity of the applied stimuli, and using several sets of rats at the same time comparisons between analgesic drugs can be made. The slope of the log. dose/response curve for analgesic drugs is very high and can be determined within the experiments when an accurate assay is required. Usually, however, it is sufficient to adjust the dose of the drugs so that approximately a 50 per

cent increase in the pain threshold results. The doses then represent the ratio of the analgesic actions of those drugs in inverse proportion.

4. Little advantage could be shown in the use of litter-mates or in the adoption of a cross-over arrangement. The experiments on these points did not show any outstandingly preferable arrangement and were therefore not continued to a point of statistical finality. It was found that dividing a group of animals equally among the drugs to be tested and repeating the experiment on a sufficient number of days for all the animals to have received all the treatments was a practical arrangement, since it is not possible to test a large number of rats at short time intervals.

5. As an example of the method a comparison was made between the analgesic effects of morphine and heroin, and heroin was found to be rather less than seven times as potent as morphine.

REFERENCES

D'Amour, F. E., and Smith, D. L. (1941). *J. Pharmacol.*, **72**, 74.
Dodds, E. C., Lawson, W., Simpson, S. A., and Williams, P. C. (1945). *J. Physiol.*, **104**, 47.
Eddy, N. B. (1928). *J. Pharmacol.*, **33**, 43.
Eddy, N. B. (1932). Ibid., **45**, 339.
Hardy, J. D., Wolff, H. G., and Goodell, H. (1940). *J. clin. Invest.*, **19**, 649.
Koll, W., and Reffert, H. (1938). *Arch. exp. Path. Pharmak.*, **190**, 176.
Macht, D. I., and Macht, M. B. (1940). *J. Amer. Pharm. Ass.*, Sci. Ed., **29**, 193.
Slaughter, D., and Wright, F. T. (1944). *Anesth. & Analg.*, **23**, 115.
Wolff, H. G., Hardy, J. D., and Goodell, H. (1940). *J. clin. Invest.*, **19**, 659.

Brit. J. Pharmacol. (1946), **1**, 127.

METHYL-THIOURACIL AND THIOURACIL AS ANTITHYROID DRUGS

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4-Methyl-2-thiouracil is beginning to replace 2-thiouracil in the treatment of thyrotoxicosis. It is cheaper and easier to prepare than thiouracil. Leys (1945) has reported the treatment of sixteen patients without any serious adverse effects and O'Donavon (1944) states that it is less toxic than thiouracil. Favourable preliminary results have also been obtained on a large number of patients in Denmark by Freiesleben, Kjerulf-Jensen, Meulengracht and Schmith (1944) and Jersild and Nissen (1944).

The present experiments were undertaken to compare the antithyroid activities of methyl-thiouracil and thiouracil fed to rats.

METHODS

Seventy-five young male albino rats, 3 to 4 weeks old, were placed on Coward's diet and allowed food and drinking water *ad libitum*. Ten controls received Coward's diet only, 36 received the diet supplemented with 0.11 per cent methyl-thiouracil, and 29 the diet supplemented with 0.1 per cent thiouracil. (The molecular weights of methyl-thiouracil and thiouracil are in the proportion of 11 to 10). The rats were weighed twice weekly, and at fortnightly intervals 1 control rat, 3 methyl-thiouracil rats, and 3 thiouracil rats were killed and the thyroids removed, weighed, and kept for histological examination. After 100 days on diet, most of the remaining rats were killed and the thyroids, adrenals, pituitaries, liver, kidneys, spleen, testes, and pancreas removed for histological study.

Metabolism experiments were carried out on 4 rats treated with methyl-thiouracil and on 4 treated with thiouracil after 23 weeks on diet. The water intake, food intake, and urine output were measured for each group of 4 rats over four consecutive 24-hour periods. The methyl-thiouracil and thiouracil contents of the urines were determined colorimetrically, using Grote's reagent. This reagent was prepared according to Williams, Jandorf, and Kay (1944). It was diluted 1 in 20 with 0.05 M phosphate buffer at *p*H 6.0 before use as advocated by Chesley (1944). Better colour development was obtained at this *p*H than at *p*H 8.5 to 9.0 used by Williams *et al.* (1944). For the urine estimations, an equal volume of the dilute Grote reagent was added to a 1 in 20 dilution of the urine. The green colour developed was read in a photo-electric colorimeter, using a Wratten filter No. 29. The time required to attain full colour development varies considerably not only in different urine samples but also in standard methyl-thiouracil and thiouracil solutions set up on different days. For this reason, it was found necessary to construct a calibration curve for methyl-thiouracil and thiouracil each day and to take readings of both standards and urine samples at frequent intervals until constant values were obtained.

Metabolic rate determinations were carried out in a constant temperature room at 25° C. after a fasting period of 24 to 30 hours using, in principle, the closed circuit method of Regnault and Reiset (1943). Both O_2 consumption and CO_2 output were measured over a period of 45 minutes.

Respiration rate determinations were carried out on thyroids and diaphragm. The rats were killed by a blow on the head, the thyroids removed as quickly as possible, weighed,

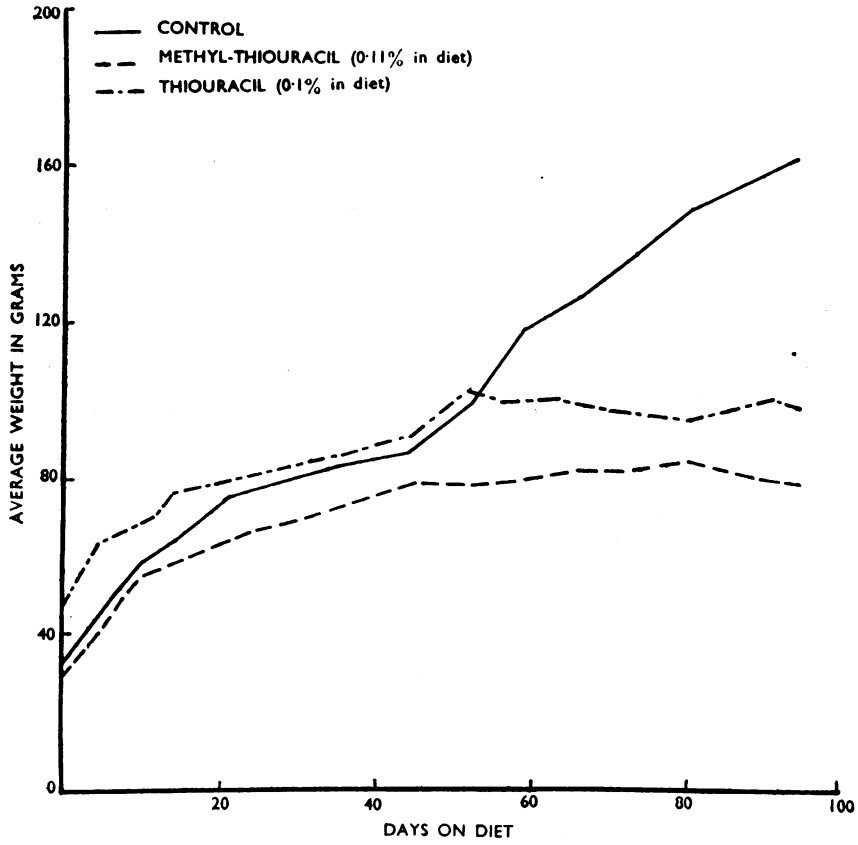

FIG. 1. Growth curves of rats

cut into thin slices with scissors, suspended in 2 cc. Ringer phosphate at *p*H 7.4, and the O_2 uptake measured in O_2 at 37.5° C. in Warburg manometers over a period of 60 minutes. Since it was found necessary to use at least 50 mg. wet weight tissue for each estimation, this necessitated using the thyroids from five or six control rats, one or two thiouracil rats, and three methyl-thiouracil rats. The diaphragms were cut in halves and one estimation carried out on each half.

The results were expressed on a wet weight basis.

RESULTS

The growth curves of the three groups of rats are shown in Fig. 1.

Both methyl-thiouracil and thiouracil produce approximately the same inhibition of growth.

The thyroid weights are given in Fig. 2.

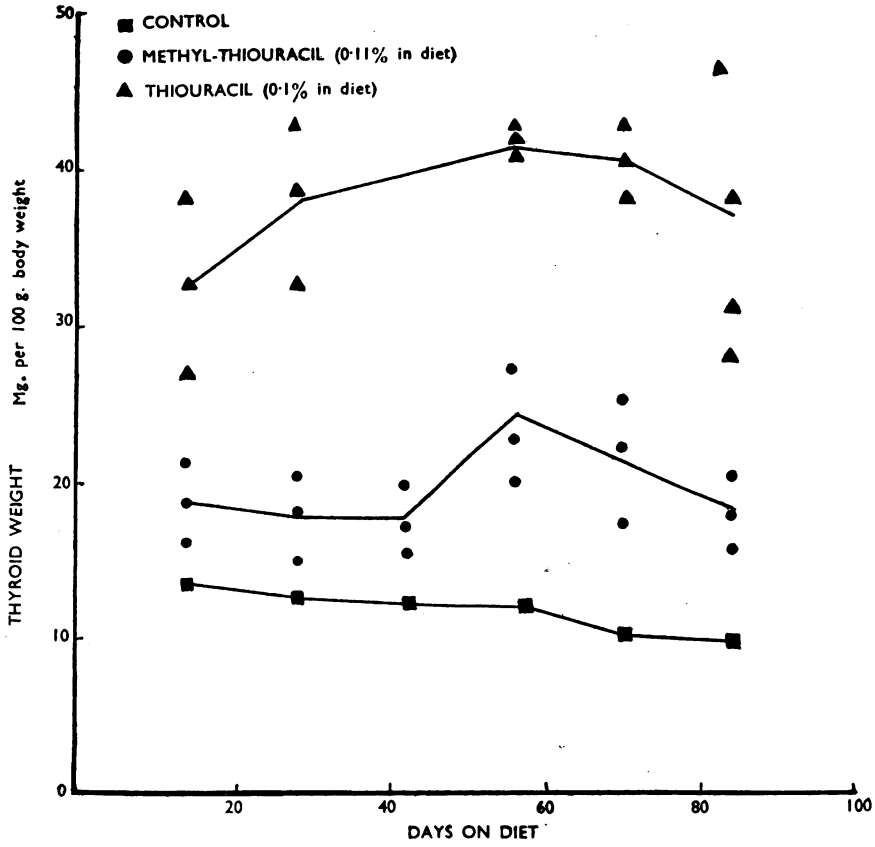

FIG. 2. Thyroid weights of rats

All the thyroids of the rats treated with methyl-thiouracil were larger than those of the controls but smaller than any of the rats treated with thiouracil. The thyroids of the rats treated with methyl-thiouracil were of the same pinkish-buff colour as the controls, whereas those of the rats treated with thiouracil were extremely red and vascular. This greater goitrogenic effect of thiouracil is reflected in the microscopic appearance of the thyroids. The thyroids of the control rats (Fig. 3) have regular-shaped, colloid filled alveoli lined by flattened epithelium. In contrast to this, the thyroids of the rats treated with thiouracil (Fig. 5) have enlarged irregular-shaped alveoli containing no colloid and are lined by high cubical epithelium. Many of the epithelial cells are vacuolated. The

Sections of rat thyroids stained with haematoxylin and eosin after 100 days on diet. (Magnification 105.)

FIG. 3.—Thyroid of control rat showing regular-shaped alveoli filled with colloid and lined with flattened epithelium.

FIG. 4.—Thyroid of methyl-thiouracil treated rat showing alveoli lined with high cubical epithelium. Many alveoli contain vacuolated colloid.

FIG. 5.—Thyroid of thiouracil treated rat showing irregular-shaped alveoli lined with high cubical vacuolated epithelium. Very little colloid remains.

thyroids of the rats treated with methyl-thiouracil (Fig. 4) are intermediate in structure. Although the alveoli are not so regular as the controls and the epithelium is high cubical, most alveoli still contain some colloid.

The greater cellular activity of the thyroids of the rats treated with thiouracil is also shown in the respiration rate of thyroid slices. Table I gives the results after the rats had been on diet for 24 weeks. The values for the respiration rate

TABLE I

RESPIRATION RATE OF THYROID SLICES

(Q_{O_2} = cu. mm. O_2 at N.T.P. per mg. wet weight tissue per hour.)

Control (C)	Methyl Thiouracil (MT)	Thiouracil (T)
		−1·57
−0·87		−1·50
−0·69		−1·37
−0·83		−1·18
−0·79	−1·02	−1·50
−0·80	−1·09	−1·29
Mean −0·79	Mean −1·055	Mean −1·40

Mean difference MT–C t=4·8, n=5, p=0·0048
T–MT t=3·0, n=6, p=0·025

of the thyroids of the rats treated with methyl-thiouracil are intermediate between those of the controls and of the thiouracil-treated rats. The Q_{O_2} appears to be proportional to the degree of thyroid hyperplasia.

Neither thiouracil nor methyl-thiouracil has any effect on the respiration rate of diaphragm removed from rats fed these drugs. Values for the Q_{O_2} of diaphragm are given in Table II. This is very difficult to reconcile with the fact

TABLE II

RESPIRATION RATE OF DIAPHRAGM

(Q_{O_2} = cu. mm. O_2 at N.T.P. per mg. wet weight tissue per hour)

Control	Methyl Thiouracil	Thiouracil
−1·35	−1·24	−1·34
−1·51	−1·34	−1·38
−1·41	−1·48	−1·52
−1·27	−1·27	−1·28
−1·42	−1·37	−1·38
−1·30	−1·27	−1·35
−1·29		−1·29
Mean and Standard Deviation 1·36 ± 0·0045	1·33 ± 0·0065	1·36 ± 0·0041

that both drugs produce a considerable decrease in the metabolic rate and that in thyroidectomized rats the Q_{O_2} of skeletal muscle is reported to be greatly reduced (Dye and Maugham, 1929). Jandorf and Williams (1944) have also found that

feeding thiouracil to rats produces no alteration in the values for the Q_{O_2} of liver and diaphragm.

Although thiouracil is only sparingly soluble in water (approximately 0.2 per cent at 20° C.), it is nearly five times as soluble as methyl-thiouracil. It was thought that the smaller goitrogenic activity of methyl-thiouracil might be due to incomplete absorption of the drug, and consequently some metabolism experiments were carried out. The results (Table III) clearly indicate that the major part of the methyl-thiouracil is absorbed, since approximately 62 per cent is excreted in the urine.

TABLE III

METABOLISM EXPERIMENT ON FOUR METHYL-THIOURACIL AND FOUR THIOURACIL-TREATED RATS AFTER 23 WEEKS ON DIET

24-hr. Period	Water Intake (cc.)	Urine Output (cc.)	Drug ingested (mg.)	Drug excreted (mg.)	% Drug excreted*
METHYL-THIOURACIL GROUP (MT)					
1	42	14·5	27·5	16·0	58
2	40	10·5	27·5	16·4	60
3	35	17·5	33·0	23·7	72
4	37	9·5	22·0	12·3	56
THIOURACIL GROUP (T)					
1	40	22·5	25·0	17·4	69
2	57	18·0	25·0	19·7	79
3	40	17·0	30·0	20·0	67
4	44	14·0	25·0	18·0	72

*Mean difference T–MT $t=2{\cdot}298$, $n=6$, $p=0{\cdot}058$.

The metabolic rates of a few rats have also been determined. These results are given in Table IV. Both methyl-thiouracil and thiouracil produce approximately the same depression in metabolic rate.

TABLE IV

HEAT PRODUCTION OF FASTING RATS AT 25° C.

Calories per sq. m. body surface per 24 hr.

Control (C)	Methyl Thiouracil (MT)	Thiouracil (T)
	642	
	533	
	520	
	633	632
832	687	650
890	557	656
838	635	585
930	591	600
Mean 872	Mean 600	Mean 625

Mean difference C–MT $t=8{\cdot}23$, $n=10$, $p=\ll 0{\cdot}001$
T–MT $t=0{\cdot}835$, $n=11$, $p=0{\cdot}4$

Both thiouracil and methyl-thiouracil produce changes in organs other than the thyroid. After rats had been on diet for 100 days, the anterior pituitaries of both groups showed changes identical with or closely similar to those seen after thyroidectomy, that is, a considerable reduction in the number of acidophil cells and an increase in the number, size, and degree of vacuolation of the basophil cells. No differential counts of basophils and acidophils were undertaken, but there was no obvious difference between the anterior pituitaries of the two groups. Slight changes were also observed in the adrenals, but nothing approaching the gross changes in the terminal stages with thiourea and thiouracil, previously reported (Glock, 1944). In both groups the reticularis was congested, there was swelling of the superficial cells of the fasciculata, and a marked reduction in the sudanophil staining material, particularly in the fasciculata. The most marked difference between the appearance of rats treated with methyl-thiouracil and thiouracil was that the rats treated with methyl-thiouracil were sexually very immature. With three exceptions, the testes of this group of rats had not descended after six months on diet. The descended testes were normal in structure, whereas sections of the undescended testes showed that spermatogenesis was inhibited and the tubules considerably reduced in size and containing relatively few cells ; this is similar to the appearance of undescended testes in man. In the thiouracil-treated rats the testes had invariably descended.

Discussion

The antithyroid activity of new compounds is generally tested by ascertaining the increase in thyroid weight of rats administered these drugs for a fixed period. Although antithyroid activity is associated with some degree of thyroid hyperplasia, the present results show that these two effects are not quantitatively related, and that it is essential to determine the metabolic rate in order to be able to assess antithyroid activity. Thus, although both methyl-thiouracil and thiouracil produce approximately the same depression in metabolic rate, the degree of thyroid hyperplasia and vascularity is very much less with methyl-thiouracil than with thiouracil. In this connection, it is interesting to note that Ciereszko (1945), testing purified thyrotropic hormone on chicks, found that thyroid size gave no indication of thyrotropic potency. Recently, McGinty and Bywater (1945) have taken the total iodine content of the thyroid as an index of antithyroid activity.

Since the completion of these experiments, the results of Danish workers have become available. Freiesleben, Kjerulf-Jensen and Schmith (1945) and Jensen and Kjerulf-Jensen (1945) report methyl-thiouracil to be the most active goitrogenic compound they have tested. No data, however, are given for thiouracil. Their results differ in some respects from those obtained in this country and America, notably in the action of 2-thiobarbituric acid. Christensen (1945) found it to be inactive, whereas Astwood (1943) found it to be almost as active as thiouracil.

Summary

1. The antithyroid activities of methyl-thiouracil and thiouracil have been compared on rats.

2. Both methyl-thiouracil and thiouracil produce approximately the same inhibition of growth and the same depression of metabolic rate.

3. Methyl-thiouracil produces considerably less thyroid enlargement and vascularity than thiouracil. The greater cellular hyperplasia of the thyroids of the thiouracil-treated rats is reflected in the higher values of the respiration rate of thyroid slices.

4. Thyroid size should not be taken as a criterion of antithyroid activity. Metabolic rate determinations are essential.

5. The use of methyl-thiouracil in the treatment of human thyrotoxicosis is advocated, since, although it produces the same depression of metabolic rate as thiouracil, the degree of thyroid hyperplasia and vascularity is considerably less.

Acknowledgements

I am indebted to Dr. C. L. Oakley and Dr. D. Trevan, who carried out all the histological examinations, and to Mr. O. F. Hutter for valuable assistance.

References

Astwood, E. B. (1943). *J. Pharmacol.*, **78**, 79.
Chesley, C. (1944). *J. biol. Chem.*, **152**, 571.
Christensen, B. G. (1945). *Acta pharmacol.*, **1**, 98.
Ciereszko, L. S. (1945). *J. biol. Chem.*, **160**, 585.
Dye, J. A., and Maughan, G. H. (1929). *Proc. Soc. exp. Biol., N.Y.*, **26**, 439.
Freiesleben, E., Kjerulf-Jensen, K., Meulengracht, and Schmith, K. (1944). *Ugeskr. f. Laeger (Copenhagen)*, **106**, 867. Cited by Freiesleben, E., Kjerulf-Jensen, K., and Schmith, K. in *Acta pharmacol.* (1945), **1**, 82.
Freiesleben, E., Kjerulf-Jensen, K., and Schmith, K. (1945). *Acta pharmacol.*, **1**, 82.
Glock, G. E. (1944). *Nature*, **156**, 508.
Jandorf, B. J., and Williams, R. H. (1944). *Amer. J. Physiol.*, **141**, 91.
Jensen, K. Ar., and Kjerulf-Jensen, K. (1945). *Acta pharmacol.*, **1**, 280.
Jersild, M., and Nissen, N. J. (1944). *Ugeskr. f. Laeger (Copenhagen)*, **106**, 918. Cited by Freiesleben, E., Kjerulf-Jensen, K., and Schmith, K. in *Acta pharmacol.* (1945), **1**, 82.
Leys, D. (1945). *Lancet*, **1**, 461.
McGinty, D. A., and Bywater, W. G. (1945). *J. Pharmacol.*, **84**, 342.
O'Donavon, D. K. (1944). *Brit. med. J.*, **2**, 91.
Regnault and Reiset. Cited by von Muralt, A. (1943). *Einführung in die Praktische Physiologie*, p. 133 ; Berlin: Springer.
Williams, R. H., Jandorf, B. J., and Kay, G. A. (1944). *J. Lab. clin. Med.*, **29**, 329.

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AUTOMATIC APPARATUS FOR PHARMACOLOGICAL ASSAYS ON ISOLATED PREPARATIONS

BY

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In a pharmacological assay on an isolated preparation the experimenter has to repeat at regular time intervals various operations requiring his continued concentrated attention. These include control of the drum, adjustment of the fluid level of the isolated organ bath, addition of a drug to the bath, and changing of the bath fluid. If any of these operations be delayed the timing of injections of the drug is likely to be upset and the accuracy of the assay impaired. The present apparatus, which consists of standard component parts of automatic telephone exchanges, has been designed to perform automatically all the operations needed in an assay except the injection of drugs into the bath. Regularity of timing and constancy of fluid volume is thus assured and the operator is left free to concentrate his attention on the solutions to be injected. The method, which has been in use in this laboratory for several years, is particularly useful for carrying out two or more assays simultaneously.

Principle of the Method

Telephone relays are converted to compress rubber tubing as shown in Fig. 1. When the relays are activated the rubber tubing is decompressed and fluid is allowed to flow. These relays control the emptying and filling and the adjustment of fluid level of an isolated organ bath. They are activated at regular time intervals through a telephone uniselector which makes 12 successive contacts in a cycle. The duration of each contact is usually of 15 seconds, thus producing a cycle of 3 minutes. The selector also controls the movements of the drum and a light signal to time the injection of drugs.

The driving magnets of the uniselector are energized through a clockwork mechanism producing electrical pulses at regular time intervals. Since the wipers of the uniselector move on to the next contact every time it is energized, the duration of each step, and consequently of the whole cycle, may be adjusted at will.

If it is desired to alter the *relative* duration of individual operations, for instance the duration of the wash-out period in relation to the whole cycle, the connections of the selector must be rearranged so as to increase or decrease, as the case may be, the number of steps in a cycle. Since the standard P.O. uniselector has eight rows of 25 contacts, whilst only two rows of 12 contacts are needed for the present apparatus, it is possible not only to increase the number of steps per cycle up to 25, but also to have several alternative arrangements on the same selector by utilizing additional rows of contacts which may be switched into the circuit as required.

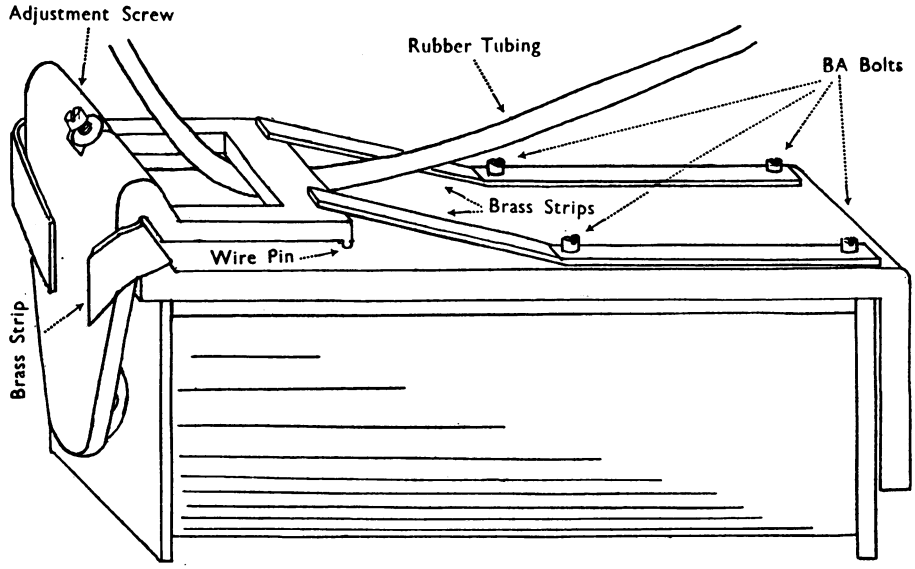

FIG. 1.—Telephone relay with adjustments enabling it to be used for compressing rubber tubing. Two brass strips screwed in place of the relay contacts act as springs tending to compress the rubber tubing. The strip inserted between relay and armature has the effect of increasing the movement of the armature when the relays are energized.

DETAILS OF OPERATIONS

The following operations are performed during a 12 × 15 seconds cycle:

Emptying and Filling of the Bath.—The bath fluid is changed twice in succession, the bath being emptied during periods 1 and 3 and filled during periods 2 and 4 by means of the relays shown in Fig. 2. A stock bottle containing Tyrode's solution is placed several feet above the table and the rate of inflow is adjusted by means of a screw clip.

Overflow.—The level of fluid in the isolated organ bath is kept constant by means of a bent glass tube fused into its side, which acts as an overflow. Shortly before addition of the drug the bath is allowed to empty to the overflow level through suction from a water pump. During the rest of the cycle the rubber tubing leading to the overflow is compressed by a relay, thus preventing the overflow from acting during the time in which drugs are added to the bath.

Alternative Arrangement for Changing the Bath Fluid.—This would consist in energizing the inflow and overflow relays simultaneously and eliminating the normal outflow altogether. The bath would thus be emptied through the overflow and the tissue would remain continuously immersed in fluid.

Drum and Signal.—The motor driving the drum is started 15–30 seconds before addition of the drug and is stopped when the bath begins to empty. The

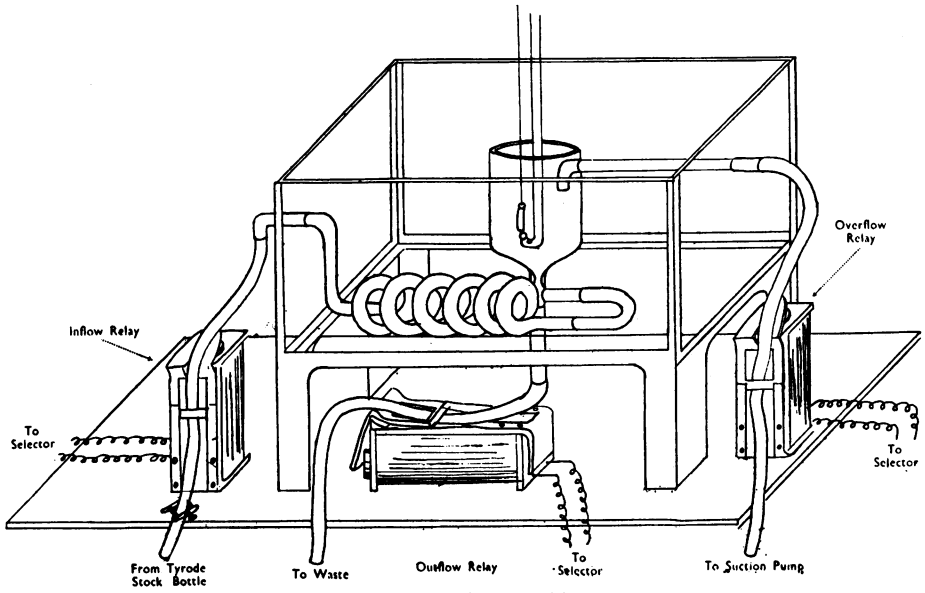

FIG. 2.—Bath assembly.

light signal for injection of the drug may conveniently be given at the beginning of period 10 or 11, depending on the length of time during which the effect of the drug is to be observed.

APPARATUS—DIAGRAM OF CONNECTIONS

The following main pieces of apparatus are required:

Interval Timer.—A "Londex" timer has been used, providing in addition to an interval of 15 seconds, intervals of 6, 10, 15, 20 and 30 seconds.

Uniselector.—A standard P.O. uniselector (M.A.I. No. 1) has been used. It is operated from D.C. mains (230 volts) in series with a suitable lamp resistance. Since the selector has 25 contacts the first and last twelve contacts are wired in parallel whilst the thirteenth contact is jumped by connecting it to the interrupter spring as shown in Fig. 3.

Relays.—Standard P.O. relays of 3000 ohms resistance are connected directly to D.C. mains without any resistance in series. They thus develop considerable power, whilst owing to the short periods of activation they do not become unduly

hot. By means of a few simple adjustments as shown in Fig. 1 the gap may be increased sufficiently to take rubber tubing of 3 × 5 mm. diameter.

Drum Motor.—If a D.C. motor is available it may be connected directly to the selector without the mediation of a relay as shown in Fig. 3.

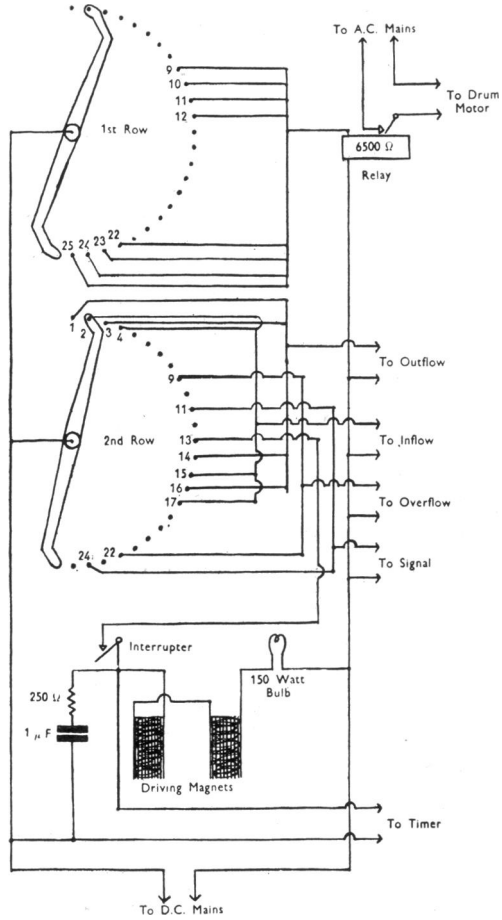

FIG. 3.—Diagram of connections of uniselector.

SUMMARY

Apparatus is described which performs automatically and at regular time intervals all the operations needed in an assay on isolated preparations, save the injection of drugs.

REFERENCE

P.O. Engineering Dept., *Engineering Instructions, M.A.I. No.* 1, *Uniselector.*

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SULPHONAMIDES IN THE TREATMENT OF CAECAL COCCIDIOSIS OF CHICKENS

BY

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(Received April 3, 1946)

Beneficial results obtained with sulphamezathine (sulphadimethylpyrimidine) and sulphadiazine (sulphapyrimidine) in the treatment of caecal coccidiosis in chickens have been reported by Horton-Smith and Taylor (1942, 1943, 1945), who found the mortality among treated chicks to be reduced by 50 to 73 per cent of that among untreated controls in induced epidemics. Hawkins (1943) also obtained satisfactory results when a saturated sulphamezathine solution was substituted for drinking-water 98 hours after infection of chicks. Ripsom and Herrick (1945) found sulphadiazine to be effective when administered in the food, and Swales (1944) found that both sulphamezathine and sulphamerazine (sulphamethylpyrimidine) had a definite curative effect upon established infections even up to the time when intestinal haemorrhage appeared.

Although good results were obtained in the treatment of caecal coccidiosis in chickens by dosing with sulphamezathine incorporated in the food and as a saturated solution in drinking-water, neither of these methods of dosing was perfect. It is difficult in practice to obtain a completely uniform mixture of a small amount of drug with dry food. The low solubility of the drug makes the preparation of saturated solutions of sulphamezathine from the powder troublesome, and such solutions are apt to vary in strength according to the hardness or softness of the water used and the method of preparing the solution. Sulphapyrazine and all the sulphapyrimidine derivatives are more soluble in hard water than in distilled or soft water because the calcium salts are more soluble than the free drugs. Thus a saturated solution in distilled water contained 0.06 per cent sulphamezathine, while a similar solution made in Weybridge tap water at the same time contained 0.13 per cent.

EXPERIMENTAL

I. *The Sodium Salt of Sulphamezathine as a Convenient and Effective Means of Administering the Drug*

1. *Method of Preparation.*—The readily soluble sodium salts of sulphonamides can be prepared by dissolving the drugs in a little over the theoretical amount of sodium hydroxide solution or sodium carbonate solution. If sodium carbonate is used the solution must be boiled. A concentrated stock solution of sodium sulphamezathine was prepared

by dissolving 160 g. of sulphamezathine in 200 ml. of 3N.NaOH (12 per cent) and diluting to one litre. Such a solution is marketed by Imperial Chemical (Pharmaceuticals) Ltd. The solution was diluted, just before use, to the concentration required. With hard waters the diluted material slowly produces a deposit of calcium carbonate on exposure to air. An additional advantage of the sodium salts is that one equivalent of alkali is given with the drug, so that the sulphonamides and their acetyl derivatives are unlikely to be deposited in the kidney.

2. *Therapeutic Experiments with Sodium Sulphamezathine after Artificial Infection.*—The satisfactory action of sodium sulphamezathine was demonstrated in the following experiments. In each of four experiments one-week-old Light Sussex × Rhode Island Red cockerels were infected by one administration into the crops by pipette of equal heavy infective doses of sporulated oocysts of *Eimeria tenella.* Groups of chicks were given access to different concentrations of sodium sulphamezathine at intervals of 24, 48, 72 and 96 hours after infection. Treatment, which consisted of substituting the solutions to be tested for the ordinary drinking-water, was carried on for approximately seven days after the death of all the controls. The percentage mortality from acute caecal coccidiosis is shown in Table I.

TABLE I

PERCENTAGE MORTALITY FROM CAECAL COCCIDIOSIS AMONG GROUPS OF 50 CHICKS AFTER DELAYED TREATMENT WITH VARIOUS STRENGTHS OF SODIUM SULPHAMEZATHINE SOLUTION

Time that treatment was delayed after infection (in hours)	Percentage mortality at various strengths of sodium sulphamezathine solution, and in control groups.					
	Water control	0·025 per cent	0·05 per cent	0·1 per cent	0·15 per cent	0·2 per cent
24	82	40	14	0	not tested	0
48	100	not tested	14	0	0	0
72	90	not tested	46	26	10	0
96	100	not tested	76	not tested	not tested	50

The surviving chicks were in good condition when they were killed 16 days after the deaths of the last controls. Post-mortem examination revealed scattered lesions of coccidiosis in the caeca of chicks receiving the 0.025, 0.05, 0.1 and 0.15 per cent solutions at all times after infection and in all groups treated from the ninety-sixth hour after infection. The experiments point to the 0.2 per cent solution as being the most effective and reliable in controlling the disease. (Percentage strength of all solutions is in grams per 100 ml.)

3. *Sodium Sulphamezathine in the Control of an Induced Epidemic.*—A single experiment was carried out with a view to testing the efficacy of three strengths of sodium sulphamezathine solution in controlling such an epidemic as might occur in the field.

Sixty-eight three-week-old chicks were placed on sawdust litter in each of four pens. The litter of each pen was infected with equal quantities of a heavy suspension of sporulated oocysts of *Eimeria tenella.* Blood appeared in the faeces of the chicks of each pen five days after the infection of the litter; the chicks were then randomized by transferring 17 chicks (i.e., a quarter of the number in each pen) to each of the other three pens. Each pen then contained equal numbers of chicks made up of 17 chicks originally present plus 17 from each of the other three pens. This procedure was adopted in an attempt

to correct differences in the original distribution of the oocysts and therefore in infections of the chicks. The chicks in three pens were then given 0.05, 0.1 and 0.2 per cent solutions of sodium sulphamezathine respectively in the place of drinking-water. The chicks in the fourth pen served as controls and continued to receive ordinary drinking-water. The results of this experiment are summarized in Table II.

TABLE II

CONTROL OF AN INDUCED EPIDEMIC OF CAECAL COCCIDIOSIS PRODUCED BY *Eimeria tenella* IN GROUPS OF 68 CHICKS BY THE SUBSTITUTION OF SODIUM SULPHAMEZATHINE SOLUTION OF DIFFERENT STRENGTHS FOR THE DRINKING-WATER

No. of days after commencement of treatment.	Deaths from caecal coccidiosis among chicks on various percentages of sodium sulphamezathine solution and in a control group.			
	0·05 per cent	0·1 per cent	0·2 per cent	Water
0	5	2	3	3
1	3	3	–	7
2	2	–	–	5
3	–	–	–	3
4	–	–	2	2
5	–	–	1	1
6	4	–	–	2
7	3	3	–	11
8	7	2	–	14
9	4	2	–	3
10	–	–	–	3
11	–	–	–	1
12	–	–	–	1
13	–	–	–	–
14	–	–	–	–
Total number of deaths	28	12	6	56

The results compare favourably with those previously reported by Horton-Smith and Taylor (1945) for sulphamezathine, and again show the superiority of the 0.2 per cent solution.

4. *Duration of Treatment with Sodium Sulphamezathine.*—An experiment was carried out to determine the minimum time of treatment necessary for effective results. Four groups of 13 chicks each were heavily infected with sporulated oocysts of *Eimeria tenella.* Three groups were given 0.2 per cent sodium sulphamezathine 48 hours after being infected. Ordinary drinking-water was substituted for the solution 24, 48 and 72 hours respectively after the deaths of the fourth group of untreated controls which succumbed to acute caecal coccidiosis on the fifth day. Two deaths from coccidiosis occurred in the 24-hour group and one in the 48-hour group. No deaths occurred in the group that was returned to water three days after the deaths of the controls. The surviving chicks were killed 16 days after the deaths of the controls, and post-mortem findings showed them to be normal apart from some minor lesions in a few of the caeca. From these results it would appear that a treatment carried on for three days after the last deaths from coccidiosis is probably sufficient to control an outbreak of the disease.

Recent work (Asplin, Boyland, and Horton-Smith, 1946) has shown that there are dangers in using sulphamezathine over long periods. If young chicks receive sulphamezathine for two or more weeks the blood-clotting time is lengthened, possibly owing to the decreased synthesis of vitamin K in the gut. In a few cases multiple petechial

haemorrhages of the intestines have been found post mortem after prolonged dosing. Dosing with sulphapyrimidines, particularly sulphamezathine, causes hyperplasia of the seminiferous tubules of the testes of cockerels. The testicular enlargement is accompanied by the precocious development of the comb and wattles. It is recommended that the duration of treatment with sulphamezathine should not exceed one week.

II. *Comparison of Various Sulphonamides with Sodium Sulphamezathine in the Treatment of Caecal Coccidiosis*

1. *Method Used in Tests.*—The therapeutic effects of solutions of sulphaguanidine and of the sodium salts of sulphadiazine, sulphamethylpyrimidine, sulphapyrazine, and sulphathiazole were compared with those obtained with solutions of sodium sulphamezathine as substitutes for drinking-water. Equal heavy infective doses of sporulated oocysts of *Eimeria tenella* were administered to groups of chicks which later were given the different drugs at different concentrations. One group, the control, remained on drinking-water. In each case one group was treated with sodium sulphamezathine and served as a standard for comparison. The results of these trials are shown in Table III.

2. *Tests made with Sulphapyridine and Sulphaguanidine.*—A test was carried out with a ration to which 1 per cent sulphapyridine was added. Groups of chicks had access to the medicated ration 48 hours before and 48 hours after being heavily infected with sporulated oocysts of *Eimeria tenella.* The treatment did not prevent deaths from coccidiosis ; 9 of the 10 chicks in each of the treated groups and in the untreated control group succumbed.

Levine (1941), Farr and Allen (1942), and Horton-Smith (1942) have shown that chicks receiving a ration containing 1 to 2 per cent of sulphaguanidine are protected against infection with caecal coccidiosis provided treatment is instituted before the ingestion of the infective dose of oocysts. In view of these findings an experiment was carried out with sulphaguanidine incorporated in the ration on lines similar to those described in Section I for sodium sulphamezathine. Fifty-four chickens were placed in a single pen and the litter was infected. Thirteen days later 10 chickens died of acute caecal coccidiosis. The remaining 44 chickens were then distributed in two groups of 22 chickens each. One group was treated with 2 per cent sulphaguanidine in the food and the other continued to receive a normal ration. Treatment exerted little or no effect on the course of the infection, as 20 of the treated and 21 of the untreated chickens succumbed to the disease.

3. *Resistance of Chickens to Caecal Coccidiosis after Recovery due to Treatment with the Sodium Salts of Sulphamezathine and Sulphapyrazine.*—Previous work (Horton-Smith and Taylor, 1945) showed that a strong immunity to coccidiosis developed in chicks which had survived a previous epidemic as a result of treatment. A single experiment was carried out to find whether similar results were obtained when solutions of sodium sulphamezathine and sodium sulphapyrazine were used in treatment.

Three heavily infected groups of 10 chicks each were treated with 0.05, 0.1, and 0.2 per cent sodium sulphamezathine respectively. Another group of 10 chicks was treated with 0.1 per cent sodium sulphapyrazine solution. Treatment was commenced 24 hours after the chicks had been infected. A fifth group received a similar infection, but was maintained on water and served as a control group for the first part of the experiment. A sixth group was not infected, maintained on water, and served as a control for the second part of the experiment. Only one of the chicks from the 0.05 per cent sodium sulphamezathine group died from acute caecal coccidiosis as compared with 10 chicks from the control group. All surviving chicks were restored to water five days after the deaths of the controls. Five days later all the chicks, together with the second control group,

TABLE III

COMPARISON OF SODIUM SULPHAMEZATHINE AND OTHER SULPHONAMIDES INTRODUCED INTO THE DRINKING-WATER IN THE TREATMENT OF CAECAL COCCIDIOSIS.

Test substance (as Na-Salt, except Sulphaguanidine)	Time Treatment was delayed after Administration of Oocysts (in hrs.)	Percentage Strength of solution of Test Substance	Mortality after Treatment with Test Substance	Control Groups				
				Mortality after Treatment with Sodium Sulphamezathine of Various Strengths				Mortality among Untreated Control Chicks
				·025	·05	0·1	0·2	
Sulphadiazine (a)	24 24	0·025 0·05 0·10	13/16 13/16 0/10	11/16	3/16	–	–	16/16
(b)	24	0·05 0·10	1/8 2/7	2/7	0/7	–	–	8/8
Sulphathiazole	24	0·10 0·30	10/10 7/10	-	–	0/10	0/10	10/10
Sulphamethyl-pyrimidine (Sulphamerazine)	24	0·05 0·1 0·2	9/13 8/13 0/13	2/13	0/13	0/13	–	13/13
Sulphapyrazine	24 (1)	0·025 0·05 0·1	1/7 0/7 0/7	2/7	1/7	0/7	–	9/10
	24 (2)	0·1 0·2	0/10 0/10	4/10	1/10	0/10	–	10/10
	48	0·025 0·05 0·1	1/6 1/6 0/6	6/6	0/6	0/6	–	5/6
	72 (1)	0·2	0/10	4/10	1/10	0/10	–	9/10
	72 (2)	0·025 0·05 0·1	2/7 2/7 0/7	6/7	3/7	3/7	–	6/7
Sulphaguanidine	24	0·1	4/8	–	–	–	–	6/8
	48	0·1	10/16	–	–	–	–	–
	72	0·1	14/16	–	–	6/16	4/16	–

received heavy doses of sporulated oocysts. The results of this experiment are set out in Table IV.

TABLE IV

RESISTANCE OF CHICKS, WHICH HAD SURVIVED INFECTION AS A RESULT OF TREATMENT WITH SODIUM SULPHAMEZATHINE AND SODIUM SULPHAPYRAZINE, TO A SECOND HEAVY DOSE OF OOCYSTS ADMINISTERED FIVE DAYS AFTER THEIR RETURN TO WATER

Solution or Water	No. of Chicks in Group	Deaths during Treatment	Deaths from Infection after 5 days on Water
Sulphamezathine 0·05%	10	1	1
„ „ 0·1%	10	0	1
„ „ 0·2%	10	0	1
Water (1st controls)	10	10	–
Water (2nd controls)*	8	–	7
Sulphapyrazine 0·1%	10	0	3
Water (1st controls)	10	10	–
Water (2nd controls)*	10	–	9

*The second controls remained uninfected until the chicks which survived the first infection as a result of treatment had received their second heavy infection of oocysts.

4. *Discussion of Results of the Comparisons Made.*—Sulphapyridine, sulphathiazole, and sulphaguanidine were all ineffective in treatment of established infections. Sulphadiazine and sulphamethylpyrimidine are both less effective than sulphamezathine. Thus in Table III it will be seen that the mortality was similar in groups treated with 0.1 per cent sulphadiazine and 0.025 per cent sulphamezathine. Similarly, the concentration of sulphamethylpyrimidine required for complete protection (0.2 per cent) was much higher than that of sulphamezathine (0.05 per cent) in groups of chicks dosed at the same time. These results suggest that sulphadiazine and sulphamethylpyrimidine have only about one-quarter of the therapeutic effect of sulphamezathine. The results obtained with sulphadiazine were rather erratic.

The results were interesting in that they showed one sulphonamide, sulphapyrazine, to be more effective than sulphamezathine. In comparative experiments 0.1 per cent sulphapyrazine and 0.2 per cent sulphamezathine have prevented symptoms in almost all chicks even when treatment has been delayed to 72 hours after infection. In practice it is recommended that infections should be treated by the substitution of 0.2 per cent sodium sulphamezathine or of 0.1 per cent sodium sulphapyrazine for the drinking-water as soon as coccidiosis is diagnosed. The work of Asplin, Boyland, and Horton-Smith (1946) has shown that sulphapyrazine and sulphathiazole have no ill effect on the clotting power of the blood or on the testes comparable with that of sulphamezathine.

III. *Concentration of the Drugs in the Blood*

In an endeavour to gather some information on the mode of action of sulphonamides on the parasite within the epithelial cells we made a study of the concentrations of the various drugs in the blood of chickens of different ages and at various times of day (Tables V and VI).

The concentrations of the sulphonamides in the blood of chickens were estimated by a modification of the method described by Bratten and Marshall (1939). Blood was taken

from the wing vein of adult birds and from the hearts of recently killed young chicks. The blood (0.5 ml.) was allowed to haemolyse in 6.5 ml. of distilled water for fifteen minutes, after which 1.0 ml. of 30-per-cent trichloracetic acid was added and the precipitated proteins were removed by filtration. The amount of free drug was estimated by comparison of the colour produced in 2 ml. of the filtrate after the addition of 0.2 ml. of 0.1 per cent sodium nitrite, 0.2 ml. of 0.5 per cent ammonium sulphamate, and finally 0.2 ml. of 0.1 per cent N-(1-naphthyl)-ethylene diamine hydrochloride, with the colour developed by similar treatment of standard solutions. The amount of total drug was estimated by the same procedure after heating 2 ml. of the blood filtrate with 0.2 ml. of 2N hydrochloric acid at 100° C. for twenty minutes. The "free drug" refers to that which is estimated directly and is probably present as such in the blood. The "total" figures refer to the amount estimated after hydrolysis, and include the drug which is acetylated or in other combined forms.

The individual variations in concentration of sulphamezathine in the blood of groups of chickens (Table V) are considerable, but the differences between

TABLE V

CONCENTRATIONS OF SULPHAMEZATHINE AND SULPHADIAZINE IN MG. PER 100 ML. OF BLOOD OF CHICKENS RECEIVING DIFFERENT STRENGTHS OF THESE DRUGS IN WINTER

Drug	Times Samples were Taken	Concentration of Drug in the Drinking-water								
		0·05 per cent			0·1 per cent			0·2 per cent		
		Free Drug	Total Drug	Percentage of Total Present as Free Drug	Free Drug	Total Drug	Percentage of Total Present as Free Drug	Free Drug	Total Drug	Percentage of Total Present as Free Drug
Sulphamezathine	7.30 a.m.	1·4	2·0	70	4·9	6·3	78	8·5	9·9	86
		2·5	3·2	78	3·3	4·3	77	5·7	5·8	98
		1·9	2·4	79	3·9	5·2	75	6·3	8·0	79
		1·6	2·5	64	2·3	2·5	92	5·5	7·1	78
Mean		1·8	2·5	73	3·6	4·6	80	6·5	7·7	85
	3.30 p.m.	3·6	6·1	59	9·1	9·9	93	17·9	19·6	91
		2·0	2·2	91	5·7	5·8	98	10·0	12·0	83
		2·7	4·4	62	4·3	6·6	65	15·8	17·3	91
		2·5	2·9	86	4·9	5·1	96	12·2	14·6	83
Mean		2·7	3·9	75	6·0	6·8	88	14·0	15·9	87
Sulphadiazine	Morning	1·5	2·7	56	1·6	1·7	94	10·3	11·1	93
		1·4	2·5	56	3·8	5·0	76	3·0	3·1	97
		2·6	2·7	96	1·5	3·2	47	0·5	0·6	83
		3·1	3·4	91	7·3	8·1	90	0·4	0·7	57
Mean		2·1	2·8	75	3·5	4·5	76	3·5	3·8	82
	Evening	4·9	5·9	83	1·4	2·4	58	1·3	2·4	54
		5·4	6·7	81	1·3	2·6	50	12·6	15·9	79
		3·7	3·9	95	9·5	9·8	97	14·1	4·7	96
		4·1	4·6	89	6·7	8·5	79	6·1	6·3	97
Mean		4·5	5·3	87	4·7	5·9	71	8·3	9·8	82

different levels of dosing and between morning and evening are quite clear and greatly exceed individual variation. The concentrations of sulphadiazine showed much individual variation except when a low level (0.05 per cent in drinking-water) was administered. There was also considerable variation in curative effect with sulphadiazine ; the two effects may be related and be due to some individual chickens not drinking or not absorbing the more concentrated sulphadiazine solutions. The blood concentrations given in subsequent tables and Figure 1 are

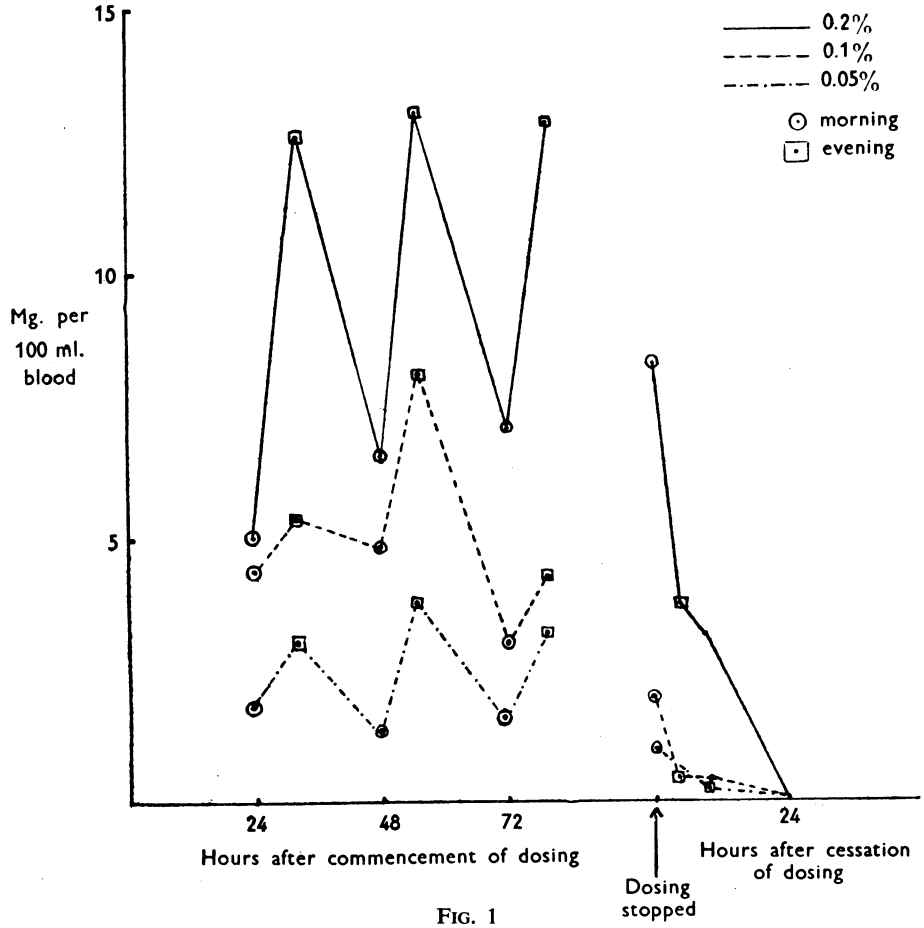

FIG. 1

all mean values for four chickens sampled at the same time. The individual variations in blood concentrations in the case of the other drugs examined were much less than with sulphadiazine. The variation in concentration of sulphamezathine with time of day and the fall in concentration after cessation of dosing are shown in Table V (one-week old chicks) and Figure 1 (twelve-week old chickens).

TABLE VI

CONCENTRATIONS OF SULPHAMEZATHINE, SULPHAPYRAZINE AND SULPHATHIAZOLE (IN MG. PER 100 ML.) IN THE BLOOD OF CHICKS IN WINTER AND SUMMER. CONCENTRATIONS WERE DETERMINED AT LEAST 48 HOURS AFTER COMMENCEMENT OF DOSING AND ARE MEAN VALUES FOR FOUR CHICKS SAMPLED AT THE SAME TIME.

Drug and Season When Dosed		Age of Chickens and Time of Day Samples were Taken	Concentration of Drug in Drinking Water								
			0·05%			0·10%			0·20%		
			Free Drug	Total Drug	Percentage of Total Present as Free Drug	Free Drug	Total Drug	Percentage of Total Present as Free Drug	Free Drug	Total Drug	Percentage of Total Present as Free Drug
Sulphamezathine	Winter	One week old:									
		Morning	1·8	2·4	75	3·7	5·2	71	7·1	9·7	73
		Evening	3·0	3·8	79	5·8	9·4	62	10·1	13·0	78
		Two weeks old:									
		Morning	2·0	2·8	72	3·6	5·0	72	9·3	13·9	67
		Evening	3·1	4·2	72	5·2	7·9	66	9·3	13·1	71
		Twelve weeks old:									
		Morning	1·4	2·3	61	3·9	5·6	70	6·6	7·8	85
		Evening	3·4	5·2	65	5·7	8·4	68	10·4	14·4	72
	Summer	Two weeks old:									
		Morning	3·9	4·2	93	8·0	10·2	78	10·9	11·9	91
		Evening	3·3	4·0	83	8·6	11·0	78	10·5	12·6	83
		Twelve weeks old:									
		Morning	1·9	2·8	68	5·1	5·5	93	9·2	10·2	90
		Evening	2·0	3·0	66	6·1	6·4	95	9·2	10·2	90
Sulphapyrazine	Summer	Two weeks old:									
		Morning	5·7	6·4	89	8·7	9·4	92	16·8	17·9	94
		Evening	3·9	4·5	87	6·0	6·6	91	14·4	15·6	92
		Twelve weeks old:									
		Morning	1·7	2·0	85	3·7	4·3	86	9·9	11·3	88
		Evening	2·7	3·6	75	4·2	4·8	87	10·3	12·0	86
Sulphathiazole	Summer	Two weeks old:									
		Morning	1·0	1·1	91	0·9	0·9	100	2·4	2·6	92
		Evening	1·1	1·5	73	0·9	1·4	65	0·9	1·5	60

When the drug was administered as a 0.2 per cent solution the blood concentration was very rarely below 5 mg. per 100 ml. As the most satisfactory therapeutic results were obtained by dosing with the 0.2 per cent solution it appears that a blood concentration of 5 to 10 mg. per 100 ml. is necessary for optimal results. There was a tendency for the blood concentrations to be higher in younger chicks when dosed in summer. The blood concentrations are of the order of one-twentieth of the concentration of drug in the drinking-water. Table VI indicates the mean values of concentrations obtained in chicks of different ages in winter and summer. The blood concentrations varied with the concentrations of drug in the drinking-water and to some extent with the time of day. Samples of blood taken at 7.30 a.m. (G.M.T.) in the winter had consistently lower concentrations than similar samples taken at 3.30 p.m. (G.M.T.). This is presumably due to the chicks drinking less during the hours of darkness, so that the blood concentrations fall during the night. Similar determinations carried out in the summer (June) showed no regular variations between morning (6.30 a.m., G.M.T.) and afternoon (2.30 p.m., G.M.T.) values.

The concentrations of sulphapyrazine in young chicks (two weeks old) were considerably higher than in older (twelve weeks old) chickens treated at the same time. The difference with age is similar but much greater than that noticed with sulphamezathine. The concentrations of sulphapyrazine were higher than those for comparable dosage levels of sulphamezathine and the higher therapeutic action is probably due to the higher blood concentrations obtained. It is remarkable that sulphapyrazine should be so well absorbed when it is poorly absorbed from the alimentary tract of mammals (cf. Robinson *et al.*, 1943)

The differences in blood concentrations obtained in chickens and canaries after oral administration of sulphonamides have been described by Marshall (1945). In his experiments sulphapyridine derivatives appeared to be more easily absorbed and more slowly excreted than was sulphathiazole, and the antimalarial activity was correlated with the attainable blood concentration. In our experiments the values for sulphathiazole, which are given in Table VI, show that this drug is poorly absorbed or quickly excreted, and possibly has no therapeutic action for this reason.

Sulphapyridine, given as 1 per cent of the chickens' ration, was fairly well absorbed. The blood concentrations were as follows: morning values were, free 6.2 and total 6.6 mg. per 100 ml., while evening values were, free 6.5 and total 7.6 mg. per 100 ml. Sulphapyridine is ineffective in curing the disease presumably because it does not inhibit the growth of coccidia.

Sulphaguanidine is relatively poorly absorbed from the alimentary tract of mammals and is therefore used in the treatment of intestinal diseases. When a solution (0.1 per cent) of this drug was given to chicks the blood concentrations appeared to rise very slowly (Table VII). Even so, the concentrations obtained after five days were insufficient to have a curative or prophylactic effect as experiments have shown. When 1 to 2 per cent of sulphaguanidine was administered in

the food it was found to have a prophylactic effect, and under these conditions the blood concentrations were quite high and appeared to be rapidly attained. The method of determination would not differentiate between sulphaguanidine and any derivative (with an intact aminophenyl group) which might be formed from sulphaguanidine either in the gut or in the body.

TABLE VII

CONCENTRATION OF SULPHAGUANIDINE IN THE BLOOD OF CHICKS AT DIFFERENT TIMES FOLLOWING THE COMMENCEMENT OF DOSING

Concentration of Drug and the Means of Dosing	Time after Commencement of Dosing in Hours	Sulphaguanidine mgs. per 100 ml. Blood	
		Free	Total
0·1% in drinking-water	16	1·8	2·1
	26	1·9	2·6
	42	2·2	2·5
	66	2·6	3·2
	84	3·3	4·5
	108	3·2	3·7
	192	4·0	4·4
1% in food	24	7·6	8·1
	96	5·7	7·5
	120	5·3	6·1
2% in food	24	20·0	21·8
	96	20·9	24·6
	120	19·5	27·3

IV. *Antagonism of Sulphonamide Action by* p-*Aminobenzoic Acid*

The bacteriostatic action of sulphonamides on streptococci has been shown to be inhibited *in vitro* by *p*-aminobenzoic acid (Woods, 1940), and in view of experiments on the neutralization of the therapeutic action of sulphonamides in small animals (Selbie, 1940) it would seem probable that a similar mechanism would operate *in vivo*. An experiment was carried out to test this assumption in the case of avian coccidiosis. Groups of chicks, heavily infected with coccidia, were given (1) sulphamezathine and sulphapyrazine solutions, (2) these solutions with the addition of different concentrations of *p*-aminobenzoic acid (PAB), and (3) PAB only. As *Eimeria tenella* cannot be grown *in vitro* the effect can only be shown in infected chicks.

The results (Table VIII) show that the therapeutic effect of 0.2 per cent sulphamezathine was largely neutralized by the presence of 0.01 per cent PAB and that of 0.1 per cent sulphamezathine by 0.005 per cent PAB. This means that the therapeutic action of ten molecules is neutralized by one molecule of PAB. The action of 0.05 per cent sulphapyrazine was neutralized by 0.002 per cent PAB. Compared with the effect of sulphanilamide on streptococci *in vitro*, where the amount of PAB required to neutralize the effect of the drug is small (i.e., one molecule of PAB nullifying the effect of several thousand molecules of the drug), the amount of PAB required to neutralize sulphamezathine or sulphapyrazine is large (i.e., ten molecules are neutralized by one molecule), the ratio

being of the same order as that found in the treatment of streptococcal infections of mice (Selbie, 1940). The action of sulphamezathine in the animal appears to be dependent on the use of PAB by the coccidia.

TABLE VIII

THE EFFECT OF DIFFERENT CONCENTRATIONS OF PAB ON THE THERAPEUTIC EFFECT OF SULPHAMEZATHINE AND SULPHAPYRAZINE

Percentage Concentrations of Drugs and of PAB			Mortality
Sulphamezathine	Sulphapyrazine	PAB	
Exp. A.			
–	–	0·1	6/6
0·2	–	–	0/7
0·2	–	0·1	8/8
0·2	–	0·01	5/8
0·2	–	0·001	1/7
Exp. B.			
Controls –	–	–	10/10
0·1	–	–	0/10
–	–	0·02	10/10
0·1	–	0·02	10/10
0·1	–	0·01	10/10
0·1	–	0·005	6/10
0·1	–	0·002	6/10
–	0·05	–	0/10
–	0·05	0·005	8/10
–	0·05	0·002	9/10

DISCUSSION

The results show clearly that a protozoal infection can be cured by certain heterocyclic sulphonamides and that the therapeutic action resembles the antibacterial action of sulphonamides in being neutralized by *p*-aminobenzoic acid. The two most effective drugs, sulphamezathine and sulphapyrazine, differ from each other in some properties to a greater extent than does sulphamezathine from the less effective compounds like sulphadiazine and sulphamethyldiazine. Thus sulphapyrazine is much less soluble in neutral solution than the three sulphapyrimidine compounds. The effects of the different drugs are summarized in Table IX.

The difference in the therapeutic efficiencies of the drugs is no doubt partly due to differences in absorption or excretion ; thus, with the effective compounds, the therapeutic value increases with the concentration of the drug in the blood for a given dosage level. But absorption (or delay in excretion) is not the only factor, because sulphapyridine is absorbed but has no therapeutic action ; it may be assumed that this drug is not toxic to the parasite. Consideration of the similarity of the effects of sulphathiazole and sulphadiazine on the growth of bacteria *in vitro* would suggest that the variation in, or lack of, effect on coccidia

is due to the poor absorption of the drug by the host. In our present state of knowledge of sulphonamide metabolism the unpredictable variations in absorption between different drugs in different species, and in chickens of different ages, can only be found by trial. From the fact that only drugs which are absorbed appear to be effective in the treatment of chickens already infected, and consider-

TABLE IX

EFFECT OF DIFFERENT SULPHONAMIDES ON INFECTIONS OF *Eimeria tenella* IN CHICKENS

Drug	Effect on *Eimeria tenella* Infection	Number of Hours after Infection that Treatment was Delayed.
Sulphanilamide	No prophylactic effect (Levine, 1939)	–
Sulphapyridine	No prophylactic effect although absorbed	–
Sulphathiazole	1-2% was effective when administered in food before or at time of infection (Ripsom and Herrick, 1945). Dosing gives low blood concentrations.	–
Sulphaguanidine	2% in food had prophylactic effect if fed to chickens before infection.	–
Sodium Sulphadiazine	0·1% in drinking-water had some therapeutic effect.	24*
Sodium Sulphamerazine	0·2% in drinking-water had some therapeutic effect.	24*
Sodium Sulphamezathine	0·2% in drinking-water had excellent therapeutic effect.	24–72
Sodium Sulphapyrazine	0·1% in drinking-water had excellent therapeutic effect.	24–72

*Longer delays in treatment were not carried out with these sulphonamides.

ing that the life-cycle of the protozoon is principally intracellular, it would seem that effective treatment for caecal coccidiosis consists in attacking the parasite in the tissues.

SUMMARY

1. Caecal coccidiosis in chickens caused by the protozoon *Eimeria tenella* can be effectively treated by substituting solutions of 0.2 per cent sodium sulphadimethylpyrimidine (sodium sulphamezathine) or 0.1 per cent sodium sulphapyrazine for drinking-water. Sulphadiazine and sulphamethylpyrimidine (sulphamerazine) will also cure the infection but are not so reliable as the other drugs. Sulphathiazole and sulphapyridine are completely ineffective in preventing symptoms of caecal coccidiosis in infected chicks.

2. The relative therapeutic effectiveness of sulphapyrazine and the sulphapyrimidines depends upon the blood concentrations obtained. Effective doses result in blood concentrations of 5 to 10 mg. per 100 ml.

3. Chickens which survive an infection of caecal coccidiosis as a result of treatment with sulphamezathine or sulphapyrazine are resistant to subsequent infections with *Eimeria tenella* within the period tested.

4. The action of sulphamezathine and sulphapyrazine on coccidia in the chicken is antagonized by *p*-aminobenzoic acid.

REFERENCES

Asplin, F. D., Boyland, E., and Horton-Smith, C. (1946). *Biochem. J.*, **40**, ii.
Bratton, A. C., and Marshall, E. K. (1939). *J. biol. Chem.*, **128**, 537.
Farr, M. M., and Allen, R. W. (1942). *J. Amer. vet med. Ass.*, **100**, 47.
Hawkins, P. A. (1943). *Poult. Sci.*, **22**, 459.
Horton-Smith, C. (1942). *Vet. Record*, **54**, 259.
Horton-Smith, C., and Taylor, E. L. (1942). *Vet. Record*, **54**, 516.
Horton-Smith, C., and Taylor, E. L. (1943). *Vet. Record*, **55**, 109.
Horton-Smith, C., and Taylor, E. L. (1945). *Vet. Record*, **57**, 35.
Levine, P. P. (1939). *Cornell Vet.*, **29**, 309.
Levine, P. P. (1941). *Cornell Vet.*, **31**, 107.
Marshall, P. B. (1945). *J. Pharmacol.*, **84**, 1.
Ripsom, C. A., and Herrick, C. A. (1945). *Jour. Parasit.*, **31**, 98.
Robinson, H. J., Siegel, H., and Graessie, O. B. (1943). *J. Pharmacol.*, **79**, 354.
Selbie, F. R. (1940). *Brit. J. exp. Path.*, **21**, 90.
Swales, W. E. (1944). *Canad. J. Res. D.*, **22**, 131.
Woods, D. D. (1940). *Brit. J. exp. Path.*, **21**, 74.

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