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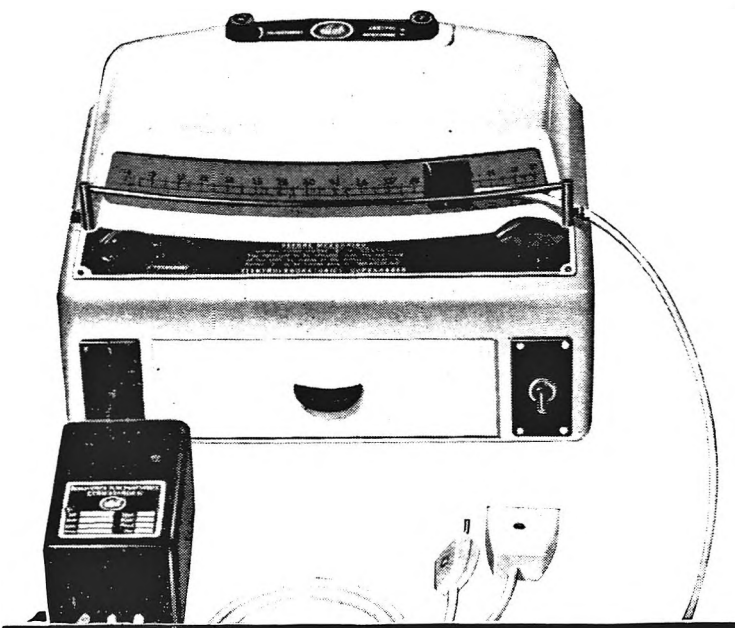

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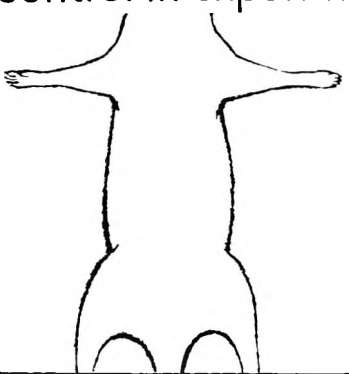

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The electrically stimulated ileum of the guinea-pig for measuring acetylcholine antagonism at different sites

G. D. H. LEACH

The transmurally stimulated guinea-pig ileum preparation was used to determine quantitatively the antagonism developed by hexamethonium and atropine against the emptying reaction and the longitudinal muscle response. Hexamethonium in concentrations of 1·5–6·0 μg/ml blocked the emptying reaction but larger doses, 8–10 μg/ml, failed to depress the longitudinal response to less than 50% of its original height. Atropine, on the other hand, in concentrations of 0·001–0·02 μg/ml, reduced the longitudinal response without affecting the emptying reaction. Thus, the preparation discriminates between acetylcholine antagonists acting at either the nicotinic or muscarinic site.

SINGLE or repeated electrical shocks applied across the wall of the guinea-pig ileum (Paton, 1955) produce a characteristic "twitch" or contraction of the longitudinal muscle in the undistended preparation which can be abolished by atropine in concentrations of 0·02 μg/ml. When the intraluminal pressure is raised to 1·5–3·0 cm of water, transmural stimulation has the effect of producing a co-ordinated movement of the circular and longitudinal muscles, termed an emptying reaction, which can be antagonised by ganglion blocking drugs. If the antagonism exhibited by atropine against the twitch response, and that of ganglion blocking drugs against the emptying reaction can be shown to be reasonably specific for their respective sites of action, the preparation would seem to be a convenient one for determining the relative intensity of action of acetylcholine antagonists at the muscarinic and nicotinic cholinergic sites. The quantitative effects are reported for atropine and hexamethonium in antagonising the responses of the transmurally stimulated guinea-pig ileum.

Methods

Adult guinea-pigs of either sex, 300–500 g, were killed and 3–5 cm of ileum removed from the small intestine 20 cm proximal to the ileo-caecal junction. This was set up in 10 ml of Krebs solution containing neostigmine 0·025 μg/ml at 34°, in the manner of a modified Trendelenburg preparation (Fig. 1).

Longitudinal muscle movements were isometrically recorded on smoked paper using a photoelectric method (Bell & Robson, 1936–37) with a mirror attached at the fulcrum of the lever; intraluminal pressure changes associated with either the emptying reaction or peristaltic reflex were measured with a polythene float recorder (Leach, 1958). The preparation was transmurally stimulated with platinum electrodes (Paton, 1956).

Transmurally elicited longitudinal contractions of the undistended preparation were obtained at 2 min intervals with a frequency of 1 shock/sec for 10 sec and a pulse duration of 0·1 msec. To ensure that

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the intraluminal pressures required to produce the emptying reaction (E) and the peristaltic reflex (P) remained constant throughout the experiment, two constant level fluid reservoirs, arranged at heights of 1·5–3·0 cm (E) and 6·0–8·0 cm (P) respectively (Fig. 1), above the fluid level of the organ bath, were used to distend the preparation. Transmural stimulation to elicit the emptying reaction was at a frequency of 1 shock/sec for 5 sec at a pulse duration of 0·03 msec.

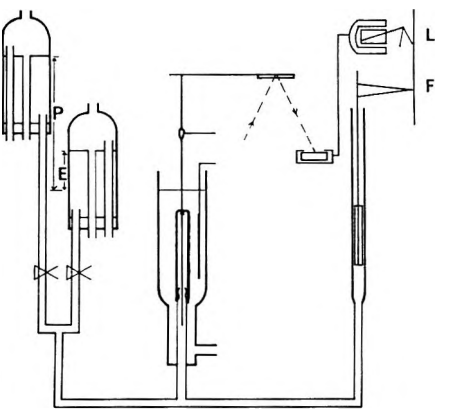

FIG. 1. Diagram of experimental arrangement. The ileum preparation suspended in a 10 ml bath is distended with Krebs solution from one of the two constant level reservoirs (E) and (P). Longitudinal muscle movements (L) are photo-electrically recorded on a smoked drum, and volume changes measured by the float recorder (F).

Longitudinal responses to transmural stimulation were obtained 1, 3, and 5 min after antagonist administration and emptying reactions at 7 and 9 min.

Estimates of antagonist potency against longitudinal responses were obtained by plotting response height, 5 min after the addition of the antagonist, as a percentage of the control height against log concentration of antagonist. The amount of antagonist required to reduce the response height by 50% could then be calculated. The difficulty in obtaining graded inhibition against the emptying reaction was overcome by expressing the antagonism to this response as the minimal concentration required to suppress two consecutive responses.

Results

The suitability of the preparation to discriminate between acetylcholine antagonists at the muscarinic and nicotinic site depended on tests with atropine and hexamethonium.

ATROPINE

Longitudinal response. Atropine, 0·001–0·02 μg/ml, reduced the longitudinal "twitch" muscle response (Fig. 2), the extent of the decrease depending upon the concentration of antagonist (Fig. 4).

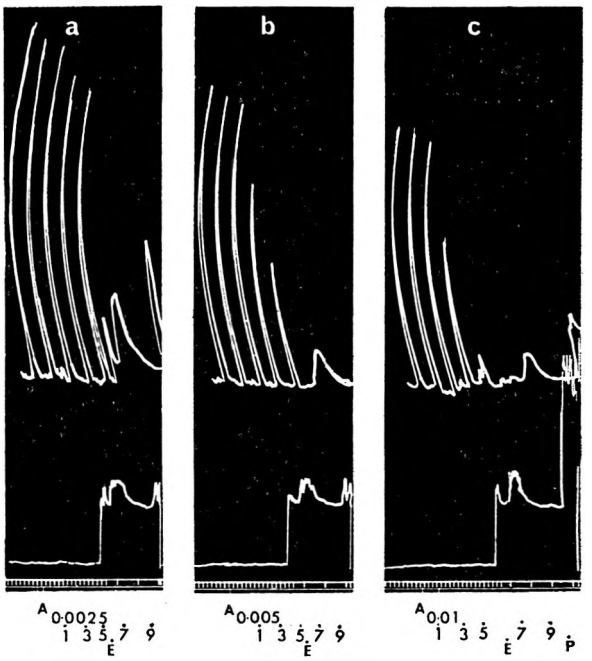

FIG. 2. Effect of atropine (A) on transmurally stimulated guinea-pig ileum. Upper record longitudinal responses; lower record intraluminal pressure. Atropine (a) 0·0025 μg/ml; (b) 0·005 μg/ml and (c) 0·01 μg/ml. 1, 3 and 5 refer to time in min after drug addition at which longitudinal responses were obtained; emptying reaction (E) elicited at 7 and 9 min after raising intraluminal pressure. Peristaltic reflex (P), Time = 30 sec.

TABLE 1. THE CONCENTRATION OF ANTAGONISTS REQUIRED TO INHIBIT THE LONGITUDINAL RESPONSE, THE EMPTYING REACTION AND THE PERISTALTIC REFLEX OF THE TRANSMURALLY STIMULATED GUINEA-PIG ILEUM

Exp. No.	Longitudinal response (calc. conc., μg/ml, producing 50% inhibition after 5 min)	Emptying reaction (conc., μg/ml, inhibiting two consecutive responses)	Peristaltic reflex (inhibitory conc., μg/ml)
(A) Atropine			
45	0·0046	>0·01	
46	0·0025	>0·01	
47	0·001	>0·01	
77	0·0047	0·06	<0·08
78	0·0046	0·04	0·04
79	0·0038	0·04	0·08
80	0·0024	0·04	0·06
Mean	0·0032	0·045 (Expts 77–80)	0·06 (Expts 78–80)
s.e.	0·0017 (n = 7)	0·016 (n = 4)	0·01 (n = 3)
(B) Hexamethonium			
18		1·5	
19		6·0	
20		2·0	
21		2·0	>8·0
22		4·0	
25		2·0	
26		2·0	>100·0
Mean		2·8	
s.e.		0·75 (n = 7)	
23	Contracture	130·0	
24	Contracture	320·0	

Emptying reaction. Atropine in concentrations needed to produce a 50% inhibition of the longitudinal response failed to suppress the emptying reaction; larger doses, 0·01–0·04 μg/ml, abolished the emptying reaction (Fig. 2).

Peristaltic reflex. An impairment of the peristaltic reflex was seen with doses of atropine, 0·04–0·08 μg/ml.

The mean concentrations of atropine needed to inhibit the longitudinal response and emptying reaction were 0·0032 μg/ml and 0·045 μg/ml respectively (Table 1).

HEXAMETHONIUM

Longitudinal response. Hexamethonium, 0·1–0·5 μg/ml, had no effect

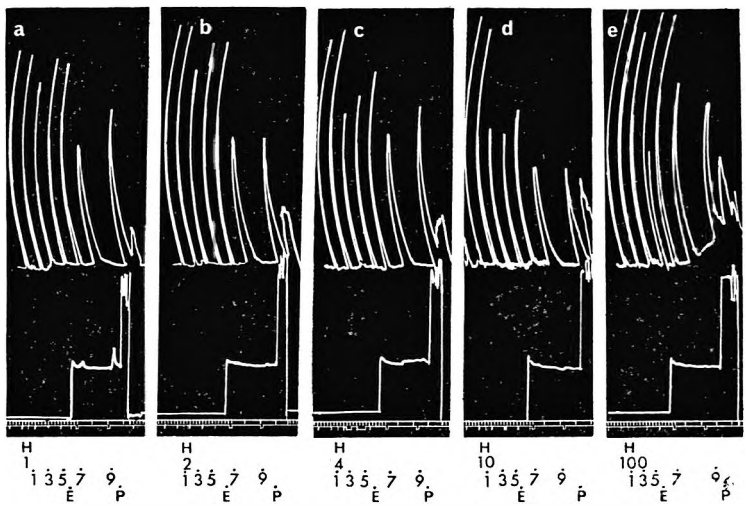

FIG. 3. Effect of hexamethonium (H) on transmurally stimulated guinea-pig ileum. Upper record longitudinal responses; lower record intraluminal pressure. Hexamethonium (a) 1·0 μg/ml; (b) 2·0 μg/ml; (c) 4·0 μg/ml; (d) 10 μg/ml and (e) 100 μg/ml. 1, 3 and 5 refer to time in minutes after drug addition at which longitudinal responses were obtained; emptying reaction (E) elicited 7 and 9 min after raising intraluminal pressure. Peristaltic reflex (P), Time = 30 sec.

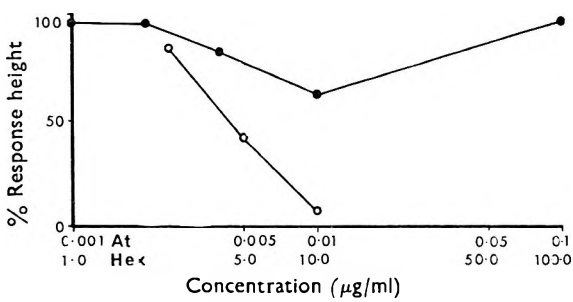

FIG. 4. Effect of hexamethonium and atropine concentrations on longitudinal response. Ordinate response height 5 min after drug addition as percentage of control. ●——● Hexamethonium (Hex), ○——○ Atropine (At). Calculated 50% inhibitory concentration of atropine in this experiment 0·0046 μg/ml.

on the longitudinal response. 0·5–3·0 μg/ml produced an initial decrease in the longitudinal response which was not sustained over the 5 min period of drug contact, and with the larger doses the 3 and 5 min stimulations were often seen to be greater than the initial control response (Figs 3 and 4).

The failure of hexamethonium to block the longitudinal response even in concentrations of 8 μg/ml or more, was not due to an inability of the preparation to respond to atropine-like compounds. In one experiment hexamethonium, 8 μg/ml, blocked the emptying reaction but the preceding longitudinal responses were unaffected; after washing, 0·01 μg/ml of propantheline reduced the longitudinal response to 35% of its initial height without affecting the emptying reaction.

Emptying reaction. Small doses of hexamethonium, 1·5–6·0 μg/ml, were able to suppress the emptying reaction (Fig. 3).

In two out of nine experiments, large doses of hexamethonium, 130–320 μg/ml, were required to inhibit the emptying reaction; the longitudinal response was not reduced, but was usually augmented; with the larger doses a contracture of the preparation was also seen. This unusual variation in sensitivity was not seen with other antagonists.

Peristaltic reflex. Although hexamethonium, 1·5–6·0 μg/ml, prevented the occurrence of an emptying reaction, increasing the intraluminal pressure to 7·5 cm water still produced a peristaltic reflex. In the preparations tested, hexamethonium, 100 μg/ml, did not impair the peristaltic reflex.

The mean concentration of hexamethonium needed to inhibit the emptying reaction was found to be 2·8 μg/ml; no value for longitudinal response inhibition could be determined for hexamethonium using this preparation (Table 1).

Discussion

In introducing the transmurally stimulated guinea-pig ileum preparation, Paton (1955) suggested that because of the sensitivity of the longitudinal twitch responses to atropine and the emptying reaction to ganglion blocking drugs, it is possible to "distinguish in a single preparation excitation of preganglionic, postganglionic . . . cell structures".

If these two responses of the preparation are indeed specific, then the preparation should prove useful not only in determining the type of cholinergic receptor involved in the antagonism, but also in providing quantitative information about the relative antagonistic potency at the muscarinic and nicotinic sites.

The results obtained in these experiments compare well with those quoted by Paton (1955, 1956) for this preparation. The mean concentration of atropine needed to produce a 50% inhibition of the longitudinal response was found to be 0·0032 μg/ml compared with 0·01–0·02 μg/ml quoted by Paton to abolish the longitudinal response; higher concentrations of 0·045 μg/ml affected the emptying reaction.

It may be seen from the results that although hexamethonium produces

an initial decrease in the longitudinal response, this is not sustained and with larger doses may even increase beyond the initial response height after 5 min contact. The appearance of stimulatory properties with respect to hexamethonium was reported by Feldberg (1951) and by Paton & Zaimis (1951), the latter authors ascribing the increased tone and rhythmic contractions to a release of vagal and sympathetic tone. Mantegazza, Tyler & Zaimis (1958), after summarising reported response enhancements to administered hexamethonium, considered the effect to be due to sensitisation of peripheral receptors. Increased vascular responses to 5-hydroxytryptamine and sympathomimetics after hexamethonium were also investigated by Laverty (1962).

However, the emptying reaction can be abolished with concentrations of 1·5–10·0 μg/ml of hexamethonium, even though the longitudinal muscle can still respond to stimulation. On the other hand, atropine in doses of 0·0032 μg/ml produced a 50% decrease in the longitudinal muscle responses to transmural stimulation, a concentration having no observable effect on the emptying reaction which was abolished only by larger doses of 0·01–0·04 μg/ml. The preparation would therefore appear to discriminate satisfactorily between those drugs which antagonise acetylcholine at its nicotinic and muscarinic sites of action.

One further point of interest is the extreme sensitivity of the emptying reaction to ganglion blocking drugs, as seen in Fig. 3, the emptying reaction being abolished with 8 μg/ml of hexamethonium, whilst increasing the intraluminal pressure still initiated a peristaltic reflex. Similar differences in the sensitivity of atropine were not seen, 0·04–0·08 μg/ml of atropine depressed both the emptying reaction and the peristaltic reflex.

Acknowledgement. I should like to express my grateful thanks to Professor G. Brownlee for his encouragement and advice during the course of this work.

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Viscosity of phosphatidylcholine (lecithin)

J. H. PERRIN* AND L. SAUNDERS

The viscosities of egg lecithin dispersed in water have been measured by means of a Couette viscometer. The more concentrated dispersions are thixotropic, but below 1·8% w/w of lecithin the sols become Newtonian. From the extrapolated ratio of specific viscosity to volume fraction, the dispersed particles are considered to be of an elongated rod-like shape. When the coarse dispersions of lecithin are irradiated ultrasonically, an irreversible clearing of the sols occurs, accompanied by a big decrease in viscosity. None of the irradiated sols examined was thixotropic and the extrapolated viscosity ratio indicated a low asymmetry and degree of hydration for the dispersed particles. The effect of ultrasonic irradiation is not only to reduce size and asymmetry of the aggregates but also to expel water from them.

PREVIOUSLY, Saunders (1957) and Thomas & Saunders (1958), using capillary viscometers and a Ferranti-Shirley cone and plate viscometer, showed that sols containing lecithin alone and mixtures of lecithin and lysolecithin exhibit anomalous flow behaviour. They also showed that, in the mixed sols, the intrinsic viscosity depends on the ratio of the two phospholipids. A Couette viscometer has been constructed to investigate these effects in more detail at known low shear rates. This paper gives the results obtained for lecithin sols.

Experimental

THE COUETTE VISCOMETER

The viscometer was based on published designs (Lawrence, Needham & Shen, 1944; Ogston & Stanier, 1953; Perrin, 1962).

An electronic speed control was used, with a 0·08 h.p. electric motor designed to give infinitely variable control over speeds from 0 to 86 rpm. The unit was modified by replacing the 50,000 ohm variable resistance with a helical potentiometer of 50,000 ohm resistance and fitted with a ten turns counting dial. This modification gave a much closer control over the speed of the motor, but a variation of up to 2% in speed was noticed during the day with each potentiometer setting. All revolutions were therefore timed against a stop-watch for Newtonian systems; a previous calibration of potentiometer setting against rev min^{-1} was used only with thixotropic sols.

The outer cylinder of the viscometer was made of stainless steel and had a length of 13 cm and a bore of 2·1 cm. A perspex window was cemented into the bottom end of the cylinder and was scribed with a concentric cylinder of 8 mm diameter. The inner surface of the outer rotating cylinder was highly polished. The inner cylinder or bob was made of titanium, chosen because of its lightness, high corrosion resistance and its ability to take a high polish. Two bobs of 1·9 cm diameter were made. The larger bob was 9 cm long and weighed approximately 125 g with fittings, whilst the small bob was 7 cm in length and weighed approximately

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100 g. To minimise end effects, the lower ends of the bob were made concave to trap an air bubble.

A beryllium-copper alloy wire, hardened at 300° for 4 hr, was used to suspend the bobs; using 0·13 cm diameter wire in lengths of approximately 30 cm to suspend the small bob, deflections of 14–17 scale divisions were obtained with water at 10 rev min^{-1}. The heavier bob was suspended by 0·14 cm diameter wire of approximately 30 cm length, giving deflections of 9–11 scale divisions at 10 rev min^{-1} with water. The suspension wire was attached to the bob by means of a brass rod. Vanes attached to the bob suspension rod rotated freely in an annular trough containing silicone oil. This damping served to minimise the effects of draughts without appreciably affecting the sensitivity of the apparatus.

To measure the deflections of the bob, a 0·6 cm diameter concave aluminised mirror was attached to the bob suspension rod. This mirror projected an image of a vertical hair line onto a circular scale at its focal distance (1 metre), the 200 cm long perspex scale being graduated in 1 mm divisions. Water was pumped through the thermostat jacket surrounding the housing for the rotating cylinder by the external pumping circuit of a thermostat water-bath maintaining a temperature of 25·0° ± 0·1° within the rotating cylinder.

Method of operation. The instrument was supported on a balance table, and the axis of rotation was made accurately vertical by using two spirit levels at right angles to one another, placed on the base plates containing the cylinder housing. 10 ml of water was placed in the outer cylinder and the bob was lowered into the cylinder until it just touched the bottom; it was then raised about 0·5 cm from the bottom of the inner cylinder so permitting free movement. The bob was manipulated to trap an air bubble of similar diameter to the circle scribed on the perspex base of the outer cylinder and was then centred, its position being observed by an inclined mirror placed on the balance table underneath the perspex base of the outer cylinder. Fifteen min were allowed for temperature equilibrium and then the zero deflection on the scale was noted. Deflection readings were noted at various speeds of the outer cylinder (timed against a stop watch) If the material under study was thixotropic, previous calibration of the speed control setting against a stop watch was used. Using this procedure a 20% w/w solution of sucrose gave a relative viscosity of 1·81 ± 0·01 compared with a value of 1·79 computed from published figures (National Bureau of Standards, Circular 440). Errors with phospholipid sols were probably greater than with the sucrose solution due to increased difficulty in centering the bob and filtering the solutions, and at higher relative viscosities (above 6) the deflection became increasingly difficult to read, possibly because of slight variation in rotational speed.

PREPARATION OF LECITHIN

Egg yolks were extracted with ethanol-ether (4 : 1) and the extract was taken up in ether and precipitated with acetone four times. The precipitate was dissolved in absolute ethanol and freed of ninhydrin reacting

material by passing through a Dowex 1 × 2 50–100 mesh ion-exchange column in the bicarbonate form (Perrin & Saunders, 1960). The choline-containing phospholipids were then freed from lysolecithin by silicic acid chromatography. The product was recrystallised from methyl ethyl ketone-acetone (1 : 3) and then stored at 0° in ethanol under nitrogen. A typical analysis was as follows (%): phosphorus 3·82; quaternary nitrogen 1·71; total nitrogen 1·71; plasmologen 0·3; sugar (galactose) 0·1; iodine number 72; ester bond/phosphorus = 2·00 (theoretical = 2·00); quaternary nitrogen/phosphorus = 0·993 (theoretical = 1·00).

PREPARATION OF LECITHIN SOLS

A measured quantity of the ethanolic stock solution of lecithin in a weighed flask was evaporated to dryness in a vacuum oven overnight, and the flask was then reweighed. The lecithin was dissolved in as small a quantity of ether as possible, the requisite amount of de-ionised water was then added and the ether removed by bubbling nitrogen through the dispersion, warmed to 30°. This bubbling was continued for 30 min and the flask was then gently shaken for 1 hr to remove any last traces of ether. The flask was reweighed and the dispersion aged for 24 hr. All sol concentrations reported are percentage weight in weight.

MEASUREMENT OF VISCOSITY

Measurements were extremely difficult with the turbid lecithin sols since the dispersions above 2% could not be spun on the laboratory centrifuge because they sedimented giving clear and turbid liquid layers, and the air bubble and the inner cylinder could not be seen.

To overcome the first difficulty, filtered water was used and care taken to keep the sol dust free, and the solution was de-gassed by means of a vacuum pump. Using water, it was found that if the inner cylinder was put into position by the same technique every time then the size of the air bubble was reproducible; a similar technique was therefore used with the lecithin sols. A circuit containing a milliammeter was used to ensure that the bob did not touch the outer cylinder. In this circuit the bob was insulated from the rest of the viscometer and so with a solution in the outer cylinder and the bob accurately centered no reading was observed on the ammeter. On the other hand if the bob touched the wall of the outer cylinder a large deflection was observed. Before any experiment with lecithin sols was attempted, the bob was accurately centred using water, and then the water was replaced by the sol. Preliminary experiments showed the lecithin sols to be thixotropic, and to check whether or not the flow curves passed through the origin the apparatus was geared to give speeds as low as 0·2 rev min^{-1} (shear rate of 0·2 sec^{-1}).

Results

A plot of deflection against rev min^{-1} for a Newtonian liquid is a straight line passing through the origin. Water and sols containing less than 1·83% lecithin behaved in a Newtonian manner at the shear rates

used. In such instances relative viscosity = η_{rel} = viscosity of sol/viscosity of solvent, η_{rel} = slope of line with sol/slope of line with water; specific viscosity = $\eta_{sp} = \eta_{rel} - 1$.

Simha's equations (below) assume ellipsoidal particles in dilute Newtonian systems, correlating a viscosity factor ν with the axial ratio of the ellipsoids; ν is the ratio of specific viscosity to volume fraction of solute ϕ, extrapolated to zero volume fraction, and f is the axial ratio.

$$(a)\quad \nu = \frac{f^2}{15(\log 2f - 1{\cdot}5)} + \frac{f^2}{5(\log 2f - 0{\cdot}5)} + \frac{14}{15}, \text{ for rods}$$

$$(b)\quad \nu = \frac{16f}{15 \tan^{-1} f} \text{ for discs}$$

Where f is the axial ratio.

$$\nu = [\eta_{sp}/\phi]_{\phi \to 0}$$

$\phi = \dfrac{\text{volume of particles}}{\text{volume of solution}} = \dfrac{c}{100\rho}$ to a close approximation where c = concentration expressed as percentage weight in weight and ρ = density of lecithin = 1·016 g/ml (Elworthy, 1959).

Values of axial ratios for various viscosity factors have been computed using the above equations (Scheraga, 1955).

VISCOSITY AT VARIOUS CONCENTRATIONS

Less concentrated sols were prepared by weight in weight dilution with de-ionised water. If the sols exhibited thixotropy, the rate of shear was found from the previous calibration of the potentiometer setting and a period of 1½ hr was allowed between dilutions for the sols to recover. The degree of thixotropy was diminished on dilution until at 1·83% no thixotropy was exhibited.

The values of η_{rel} shown were obtained from the usual plots of deflection against rev min^{-1}. The η_{rel} at a given shear rate (rev min^{-1}) being taken

TABLE 1. DEFLECTION READINGS FROM THE COUETTE VISCOMETER AND VALUES OF η_{rel} AT ZERO SHEAR FOR NON-NEWTONIAN SOLS

Deflection readings 3·2% sol			Relative viscosities of lecithin dispersions at zero shear	
Revs min^{-1}	A	B	Sol. conc. w/w %	η_{rel} at zero shear
0·22	26·2	5·5	3·81	190
0·35	32·8	8·5	3·55	177
0·43	35·8	9·5	3·33	149
0·62	44·8	12·3	2·95	118
0·84	53·5	14·7	2·78	88
1·08	61·6	16·8	2·62	60
1·26	67·5	19·1	2·29	49
1·43	73·4	21·8	2·12	9·7
1·72	78·0	23·8	1·95	2·2
1·97	80·5	26·5		
2·53	88·8	31·7		
3·11	99·0	37·3		
3·78	106·5	42·2		
4·25	112·2	46·5		
4·88	118·2	53·0		
5·45	126·8	57·3		

as deflection with sol at a given shear rate/deflection with water at same shear rate.

Column A in Table 1 gives the deflection readings at the rates of rotation shown, for a 3·2% lecithin sol, aged after each reading; column B shows results obtained when the sol had been sheared at 5·45 rev min^{-1} to a constant deflection and then the speed had been dropped to the figure shown. For B, Table 1, all the readings were taken within 10 sec of reducing speed.

Some values of η_{rel} at zero shear for non-Newtonian sols are also shown in Table 1; in Table 2 the viscosities for more dilute dispersions which showed Newtonian behaviour, are given.

TABLE 2. VISCOSITIES OF DILUTE DISPERSIONS OF LECITHIN SHOWING NEWTONIAN BEHAVIOUR

Sol conc. w/w %	Volume fraction, $\phi \times 10^3$	η_{rel}	η_{sp}	η_{sp}/ϕ
1·833	18·04	1·562	0·562	31·2
1·727	16·99	1·486	0·486	28·6
1·621	15·96	1·421	0·421	26·4
1·531	15·06	1·394	0·394	26·2
1·430	14·07	1·378	0·378	26·9
1·339	13·18	1·336	0·336	25·5
1·255	12·35	1·300	0·300	24·3
1·165	11·46	1·286	0·286	25·0
0·938	9·23	1·258	0·258	28·0
0·849	8·36	1·218	0·218	26·1
0·783	7·71	1·194	0·194	25·2
0·687	6·77	1·181	0·181	26·8
0·628	6·19	1·176	0·176	28·5
0·535	5·27	1·118	0·118	22·4

The values of η_{sp}/ϕ in Table 2 appear to fluctuate about a mean value of 26·5. Taking this value as the viscosity factor gives an axial ratio of 15·5 for a prolate ellipsoid (rod) or 37 for an oblate ellipsoid (disc).

SOLS DISPERSED BY ULTRASONICS

Lecithin was dispersed in ion-free water using a 60 W Mullard ultrasonic generator, to drive a titanium ultrasonic drill head. The generator was

TABLE 3. VISCOSITIES OF LECITHIN SOLS DISPERSED BY ULTRASONICS

Sol conc.	ϕ	η_{rel}	η_{sp}	η_{sp}/ϕ
(*a*) Couette viscometer				
4·993	0·0492	1·206	0·206	4·1
4·653	0·0458	1·184	0·184	4·0
4·321	0·0425	1·162	0·162	3·8
3·941	0·0383	1·136	0·136	3·5
3·517	0·346	1·113	0·113	3·3
3·083	0·0303	1·089	0·089	2·9
(*b*) Cannon-Fenske viscometer No. 25 viscometer				
1·302	0·0128	1·057	0·057	4·5
1·205	0·0119	1·049	0·049	4·1
1·143	0·0113	1·045	0·045	3·9
1·091	0·0107	1·040	0·040	3·7
1·044	0·0103	1·037	0·037	3·6
0·995	0·0098	1·036	0·036	3·7
0·948	0·0093	1·034	0·034	3·6
0·906	0·0089	1·033	0·033	3·7

tuned to give maximum cavitation in the liquid. All irradiations were carried out in an atmosphere of nitrogen and the vessels containing the sols were surrounded by ice cold water. The clear sols were irradiated until there was no change in opacity, they were then mixed with Amberlite Monobed MBI ion-exchange resin and spun on the centrifuge at 6,000 rpm. Measurements in the Couette viscometer showed that even a 5% ultrasonically irradiated sol behaved in a Newtonian manner and had a low relative viscosity. The results are given in Table 3.

Averaging the last five of the capillary viscometer results in Table 3 gives a viscosity factor of 3·7. When substituted in Simha's equation this gives an axial ratio of 3·0 for a prolate ellipsoid and 3·5 for an oblate ellipsoid.

Discussion

In water, phosphatidylcholine or lecithin prepared from egg yolk or brain, exists as large molecular aggregates with the polar phosphorylcholine groups orientated outwards; X-ray diffraction studies of concentrated sols indicate that bimolecular leaflets are present. In the more concentrated sols these particles are probably associated to form larger structures which are easily broken on shearing. The structure present in the more concentrated sols is shown by the large values of η_{rel} at zero shear; however, this association is weak enough to be broken down at low shear rates. During the recovery time the smaller particles reassociate by Brownian motion. In more dilute sols the tendency to association is less, and the thixotropy is lost on dilution.

Simha's equations are derived assuming low shear rates, low concentrations and Newtonian behaviour, the viscosity factor of 26·5 compares with a value of 27·9 calculated from previous investigations (Thomas & Saunders, 1958) using a Cannon-Fenske viscometer.

Coarse lecithin dispersions by light-scattering (Robinson, 1960) gave an axial ratio of 13·2 for disc shaped particles and a molecular weight of $27{\cdot}2 \times 10^6$ using data obtained in the concentration range of 1 to 20×10^{-5} g ml.$^{-1}$ Taking a rod as a model and using the above particle weight, Robinson suggested a triple-layer laminated structure. This unlikely structure would give an axial ratio of approximately 6 for a rod-shaped aggregate. These values compare with the axial ratio of 15·5 for a rod and 37 for a disc reported above (Table 3). The discrepancies between the viscosity and light-scattering results may be due to hydration, to the different concentration ranges used in the two investigations, and to the fact that the scattering of the sols that have not been dispersed by ultrasonics, is above the level at which reasonably accurate calculations of size and shape can be made.

At the extremely high shear rates obtained by ultrasonic irradiation, the sols become optically clear and stable. These ultrasonic irradiated sols are of much lower viscosity than the untreated sols and give a viscosity factor of 3·7 corresponding to an axial ratio of 3·5 for a rod and 3·0 for a disc. Diffusion and sedimentation coefficients (Gammack, Saunders, &

Perrin, 1964) have been obtained for these optically clear sols and correlation with the above viscosity data suggests a micellar weight in the region between 2 and 7 $\times$ 10^6. A more exact estimate, based on light scattering, shows that prolonged ultrasonic irradiation reduces the aggregate size to 2 $\times$ 10^6; the low axial ratio estimated from the viscosity results is confirmed by light scattering (Attwood & Saunders, 1965).

The absence of thixotropy and the low value of ν for the ultrasonic dispersions suggest that the amounts of water bound in these sols is comparatively small. In addition to the reduction in size of the dispersed particles, the effect of ultrasonics appears to be to expel water from the aggregates present in the coarse dispersions. It is this latter effect, which is irreversible, that causes a change of configuration of the aggregates.

Fuller accounts of the effect of ultrasonics on lecithin sols have been reported (Perrin, 1962; Saunders, Perrin & Gammack, 1962; Attwood & Saunders, 1965).

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Further effects of imipramine and its desmethyl derivative on the hypothermia induced by reserpine

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Imipramine and desipramine injected intracerebrally increase the temperature of fully reserpinised rats. Desipramine is more effective than imipramine in this. The effect of imipramine seems to be independent of the formation of desipramine in the brain. That imipramine, injected intraperitoneally, leads to an accumulation of brain desipramine has been confirmed.

IT is well known that imipramine-like antidepressant drugs prevent or counteract some of the biochemical, pharmacological and behavioural effects induced by reserpine or tetrabenazine. These include prolongation of barbiturate narcosis (Domenjoz & Theobald, 1959), bradycardia (Costa, Garattini & Valzelli, 1960), blepharospasm (Garattini, 1959; Costa & others, 1960; Sulser, Bickel & Brodie, 1961), gastric erosions (Garattini, Giachetti, Jori, Pieri & Valzelli, 1962; Metysova, Metys & Votava, 1964), and depletion of adrenal (Zbinden, 1962) but not brain catecholamines (Garattini & Valzelli, 1962; Pletscher & Gey, 1962; Sulser, Watts & Brodie, 1962). We have paid particular attention to the interaction between imipramine-like antidepressant drugs and reserpine, using as an end-point the change of body temperature. Thus it has been shown that imipramine potentiates the initial hyperthermia induced by reserpine (Jori, Paglialunga & Garattini, unpublished) and antagonizes the subsequent hypothermia (Garattini & others, 1962) at dose levels and times which do not modify the behavioural syndrome and the depletion of amines induced by reserpine (Garattini & others, 1962). However, the prolongation of the reserpine hyperthermia occurring in desipramine-treated rats was recently correlated with a decrease of the rate of noradrenaline release (Manara, Sestini, Algeri & Garattini, 1966). When imipramine-like drugs are given to fully reserpinised animals there is a reproducible and gradual increase of body temperature (Askew, 1963; Jori, Carrara, Paglialunga & Garattini, 1965; Morpurgo & Theobald, 1965). This effect could be considered specific because imipramine-like agents show, if anything, a small decrease of body temperature in normal animals and do not affect hypothermia induced by other agents such as chlorpromazine, meprobamate, α-methyldopa or 5-hydroxytryptamine (Garattini & others, 1962; Garattini & Valzelli, 1962).

A possible explanation may be found in the work of Axelrod and of Brodie and their colleagues (Axelrod, Whitby & Hertting, 1961; Brodie, Dick, Kielholz, Pöldinger & Theobald, 1961; Glowinski & Axelrod, 1964); and also of Iversen (1965). These authors have shown that imipramine-like drugs prevent the uptake of catecholamines, thereby enhancing their pharmacological effects (Sigg, 1959; Kaumann, Cousso & Izquierdo, 1962; Thoenen, Hürlimann & Haefely, 1964).

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We have also shown that imipramine-like drugs potentiate the hyperthermia induced by catecholamines either exogenously supplied by intravenous infusion (Jori, Paglialunga & Garattini, unpublished; Jori & Garattini, 1965) or endogenously released from storage sites by reserpine. Furthermore, unpublished results (P. Morselli) show that noradrenaline given intracerebrally acts like imipramine in increasing body temperature of fully reserpinised animals.

Gillette, Dingell, Sulser, Kuntzman & Brodie (1961) concluded that imipramine was not active by itself in stimulating tetrabenazine sedated animals, but that the effect was due to the formation of a metabolite, desipramine (DMI), which accumulates in the brain (Gillette & others, 1961; Dingell, Sulser & Gillette, 1964). Desipramine is more active than imipramine in some experimental conditions including the antagonism to reserpine-induced hypothermia (Garattini & others, 1962).

We now show that imipramine and more markedly desipramine counteract the hypothermic effect of reserpine, and that this effect is probably centrally mediated. Other data indicate that in this respect imipramine may act independently from desipramine, and therefore that formation of this metabolite is no prerequisite for this activity.

Experimental

MATERIALS AND METHODS

Female Sprague-Dawley rats, of mean weight 150 g, were kept in Makrolon cages at 22° and a relative humidity of 60%. Groups of at least 6 animals each were used.

Imipramine and desipramine were suspended as the hydrochloride in liquid paraffin and injected intracerebrally in a volume of 10 μl according to the technique described by Valzelli (1964). Reserpine (commercial ampoules) was given intraperitoneally at a dose of 2·5 or 5 mg/kg. Corresponding controls received only reserpine solvent and liquid paraffin respectively. Rectal temperature was measured with an electrical thermometer. Imipramine and desipramine in whole brain were determined according to the method of Dingell & others (1964).

Results

Fig. 1 illustrates the decrease of body temperature induced by reserpine as a function of time. When desipramine was given intracerebrally either together with or 4 or 16 hr after reserpine at a dose of 100 μg, there was an elevation of body temperature lasting for several hours, whereas no effect on the behavioural syndrome induced by reserpine was seen. The controls showed that an intracerebral injection of liquid paraffin does not affect the lowering of body temperature induced by reserpine. Furthermore, desipramine given intracerebrally to rats treated intraperitoneally with the reserpine solvent did not increase body temperature.

In Table 1 a comparison of the action of imipramine and desipramine on reserpine hypothermia is made. A clear effect is already present with

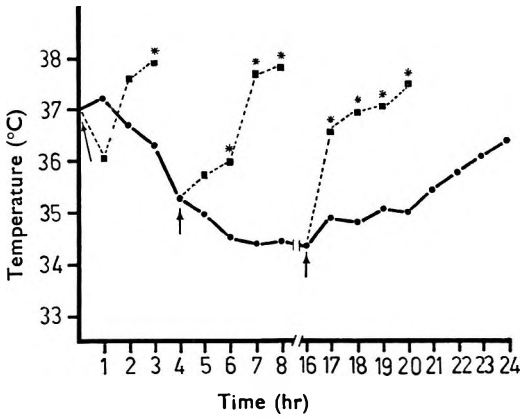

FIG. 1. Body temperature of rats treated with reserpine (2·5 mg/kg i.p. (—●—). At the arrows desipramine (100 μg) was injected intracerebrally (- - ■ - -). ($P < 0{\cdot}01$ *).

25 μg of desipramine whereas it is necessary to give 100 μg of imipramine to obtain a well defined effect. Furthermore, with imipramine there was always an initial hypothermia, and antagonism toward reserpine hypothermia was only observed after a latent period of about 2–3 hr. Five hr after the administration of desipramine or imipramine their levels in brain were measured. The results are similar for the two drugs. On the average only about 10% of the quantity injected was recovered. There was no formation of desipramine in brain after administration of imipramine even at the largest dose. (In separate experiments not reported here in detail it was demonstrated that no desipramine could be detected 15, 30 or 60 min after imipramine was given intracerebrally to normal or reserpinised animals.)

Other experiments were made to verify if the concentrations of desipramine or imipramine detected in brain after intracerebral injection were similar to those found after an intraperitoneal administration of doses

TABLE 1. IMIPRAMINE AND DESIPRAMINE BRAIN CONCENTRATION AND EFFECT OF THESE DRUGS INJECTED INTRACEREBRALLY ON RESERPINE INDUCED HYPOTHERMIA IN GROUPS OF 4 RATS

Drug μg/rat		Difference in temperature (° C) between control and treated rats — Hr after treatment					Total increase °C	Brain conc. after 5 hr (μg)
		1	2	3	4	5		
Desipramine (intracerebrally)	15	0·1	0·6	0·9	0·4	0·8	2·8 ± 0·1	1·2 ± 0·1
	25	0·7	1·6	1·8	1·6	1·8	7·5 ± 0·4	3·9 ± 0·2
	50	1·0	1·9	2·3	1·9	2·3	9·5 ± 0·3	5·7 ± 0·5
	100	1·4	1·8	1·9	2·0	2·0	9·1 ± 0·4	8·5 ± 0·2
	150	1·2	1·3	2·7	2·2	2·6	10·0 ± 0·5	11·3 ± 1·5
Imipramine (intracerebrally)	15	0·2	0·8	0·4	0·5	0·3	2·2 ± 0·5	—
	25	−0·3	−0·1	0·2	0·9	0·6	1·3 ± 0·2	3·7 ± 0·6
	50	−0·6	−0·1	0·1	0·9	0·5	0·8 ± 0·1	8·4 ± 3·5
	100	−0·5	0·2	0·8	2·5	2·6	5·6 ± 0·3	7·2 ± 2·1
	150	−0·7	−0·2	0·3	3·0	3·4	5·8 ± 0·4	12·4 ± 1·7

All animals reserpinised (5 mg/kg i.p.) 16 hr before start of experiment.

known to antagonise reserpine hypothermia. The results, summarised in Table 2, show a good agreement with those in Table 1. Comparing the brain concentration of imipramine or desipramine in normal and reserpinised animals no striking differences were observed. When imipramine was injected intraperitoneally, desipramine accumulated in brain so that at the 5th hr there was at least twice as much desipramine as imipramine.

TABLE 2. THE EFFECTS OF IMIPRAMINE AND DESIPRAMINE ON RESERPINE-INDUCED HYPOTHERMIA WHEN INJECTED INTRAPERITONEALLY AND THEIR CONCENTRATIONS IN THE RAT BRAIN

No. rats	Treatment mg/kg i.p.	Reserpine 5 mg/kg (16 hr before)	Time between saline or drug and death (hr)	Body temp. (° C)	Brain conc. (μg)	
					Imipramine	Desipramine
5	Saline	+	1	28·9 ± 0·2	—	—
5		+	2	29·0 ± 0·1	—	—
5		+	5	29·8 ± 0·32	—	—
10	Imipramine 20	—	1	35·5 ± 0·35	14·7 ± 1·7	6·0 ± 0·6
5		—	2	35·4 ± 0·58	5·6 ± 1·3	6·4 ± 0·5
5		—	5	35·2 ± 3·4	4·0 ± 0·3	7·8 ± 1·2
5		+	1	32·5 ± 1·5	12·9 ± 1·3	3·5 ± 0·5
5		+	2	30·9 ± 1·4	9·6 ± 1·0	4·4 ± 0·9
5		+	5	36·4 ± 0·34	3·5 ± 0·68	8·0 ± 2·1
10	Desipramine 15	—	1	35·7 ± 0·35	—	14·2 ± 1·4
10		—	2	34·3 ± 0·59	—	14·0 ± 0·9
5		—	5	35·4 ± 0·14	—	8·9 ± 1·3
5		+	1	33·2 ± 1·30	—	7·8 ± 0·6
5		+	2	33·6 ± 1·32	—	9·9 ± 1·8
5		+	5	35·6 ± 0·71	—	8·2 ± 0·62

Discussion

An antagonism to the hypothermia induced by reserpine can be demonstrated when imipramine or desipramine is injected intracerebrally, suggesting that the drugs act centrally. This effect is not due to unspecific stimulation because the intracerebral injection of the solvent does not modify the hypothermia induced by reserpine. However, it must be stressed that with our experimental conditions imipramine or desipramine were effective only on body temperature without affecting the reserpine behavioural syndrome with the possible exception of a slight effect on blepharospasm.

When imipramine was given intraperitoneally there was an accumulation of desipramine in the brain. However the intracerebral administration of imipramine never gave concentrations of desipramine higher than 1 μg/brain (limit of sensitivity with the method used). These results are in agreement with previous data showing that liver, but not brain or other tissues, can demethylate imipramine (Dingell & others, 1964). Furthermore, the data obtained suggest that the effect on reserpine hypothermia exerted by imipramine does not seem to be mediated through the formation of desipramine. This direct action of imipramine has been reported for its anticonvulsant (Garattini & others, 1962) and the antioxytremorine (Lévy & Michel-Ber, 1965) effects.

It is possible that desipramine is formed from imipramine but rapidly metabolised or washed out from the brain, but this is unlikely because the half-life of desipramine in brain should be about 10 hr according to the data of Sulser, Bickel & Brodie (1964).

That a rather long latent period is necessary before the effect of imipramine on reserpine hypothermia is observed, suggests the possible formation of a metabolite.

Imipramine and desipramine are still effective in fully reserpinised animals when catecholamines are depleted, which does not detract from the hypothesis that they are acting by potentiating catecholamines. It has been shown that catecholamine synthesis is even increased after reserpine treatment (Brodie & Costa, 1962; Hillarp & Malmfors, 1964).

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The rigidity of gelatin-glycerin gels

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At 25° the presence of glycerin in gelatin gels increased the modulus of rigidity in all the samples used. An empirical relationship between rigidity and the concentrations of gelatin and glycerin was calculated which was valid within the range 4–15% gelatin and 0–40% glycerin. A critical concentration of gelatin was found at which neither Bloom number nor glycerin concentration affected the rigidity. No simple relationship was found between Bloom number and rigidity.

GELATIN is used in pharmaceutical preparations and gelatin-glycerin gels are still the basis for the preparation of many suppositories. The rigidity of gelatin gels has been extensively studied (Ferry, 1948a; Clamper & Alexander, 1952) but few equations have been derived relating rigidity to composition. Ferry (1948b) and Ferry & Eldridge (1949) have produced empirical equations relating gelatin concentration, weight molecular weight and temperature. The effect of certain nonelectrolytes on the modulus of elasticity has also been reported but not extensively studied (Sheppard & Sweet, 1921; Hatschek, 1932, 1933). Glycerin was found to increase the modulus of elasticity. No direct relationship has been reported between rigidity and molecular weight or any function of molecular weight such as Bloom number. In the present work the rigidity of gelatin-glycerin-water mixtures has been studied and an equation derived which relates the concentration of gelatin and glycerin to the rigidity of the gel.

Experimental

MATERIALS

Gelatins. Alkali processed hide gelatins with the characteristics given in Table 1 were used.

Glycerin. "Analar" grade material was used.

TABLE 1. CHARACTERISTICS OF GELATIN USED

Sample	Bloom No.	pH of solution	Isoelectric point	Viscosity of 6·67% solutions		Moisture content %
				40°	60°	
A	99	6·1	5·1	4·3 cps	2·9 mps	12·7
B	154	6·1	5·1	5·9 cps	3·9 mps	12·4
C	200	6·3	5·2	6·4 cps	4·3 mps	12·8
D	250	—	5·1	7·1 cps	4·9 mps	12·7

METHODS

Preparation of the gels. The gelatin and water mixture was allowed to swell at 5° for 1 hr before solution at 50°. The glycerin, also at 50°, was added and the mixture gently but thoroughly stirred. The gel was allowed to mature in a water-bath at 25 ± 0·1° for 16 hr before rigidity measurements were made.

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Measurement of rigidity. The rigidometer method used was originally devised by Kinkel & Sauer (1925) and was modified by Saunders & Ward (1954). The volume displacement of a gelatin column, maintained at 25° ± 0·1°, on the application of air pressure was measured by means of the movement of a mercury thread in a side arm (Fig. 1). This allowed

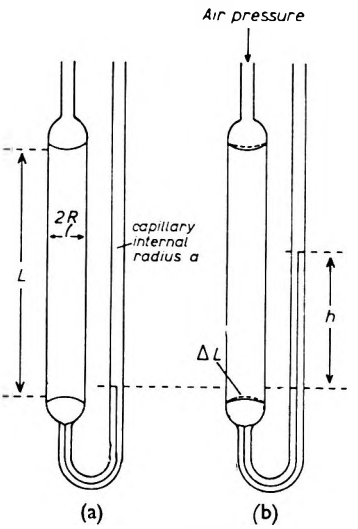

FIG. 1. Arrangement of apparatus (a) at rest; (b) under applied air pressure (after Saunders & Ward, 1954).

very small changes in volume to register as large movements of the mercury meniscus. Adhesion of the gel to the wall of the tube was sufficient to prevent slip. The rigidity is calculated using the formula

$$G = \frac{PR^4}{8La^2h} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where G = rigidity modulus in dynes/cm². P = net pressure corrected for the back pressure of displaced mercury. R = radius of tube, cm. L = length of gel column, cm. a = radius of mercury capillary, cm. h = displacement of mercury, cm. The formula was valid for the condition that $L \gg R$.

Results and discussion

Early reports asserted that the elasticity of gelatin gels was increased by a number of substances, one of which was glycerin. Hatschek (1932, 1933), who measured Young's modulus for 10% gels of "hard" gelatin with different percentages of glycerin at 10·8° and 21°, found that the modulus of elasticity increased continuously with time. These early results were incidental to the main purpose of our work and were not followed up. In the early reports, the gelatin was often not characterised and the different methods employed gave results which could not readily be correlated one with another. The method of measuring the rigidity we used gave an absolute value.

The effect of glycerin concentration on the rigidity of gelatin-glycerin-water gels is shown in Fig. 2. At all the gelatin concentrations studied, the curve was essentially linear up to 40% w/w glycerin concentration.

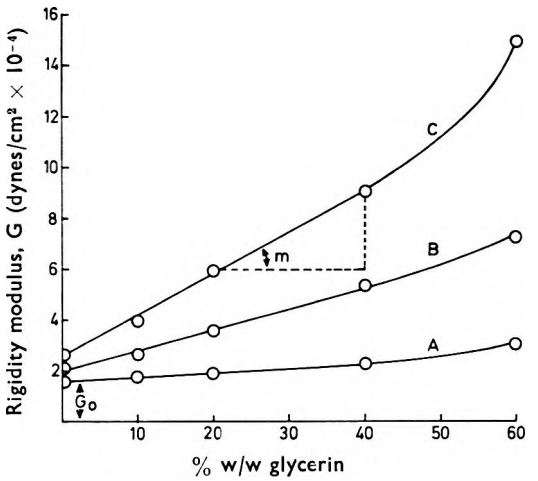

FIG. 2. The effect of glycerin concentration on the rigidity of gelatin/glycerin/water gels. Gelatin % w/w (99 Bloom): A, 5; B, 10; C, 15. Temperature, 25° ± 0·1°.

The non-linear increase in rigidity at higher glycerin concentrations could be correlated with the similar non-linear increase in the viscosity of the liquid phase (glycerin-water) at high glycerin concentrations.

For gels which contained up to 40% w/w glycerin it was possible to write the following equation

$$G = G_0 + mC \qquad (2)$$

where G = rigidity ($G \times 10^{-4}$ dynes/cm²), G_0 = rigidity at zero glycerin content, m = slope, C = concentration of glycerin (% w/w). If the value of G_0 is plotted against the square of the gelatin concentration (Fig. 3) then a second linear relationship is found

$$G_0 = \alpha + \phi Z^2 \qquad (3)$$

where α = intercept on rigidity axis at zero gelatin concentration, ϕ = slope. Z = gelatin concentration (% w/w).

Substituting for G_0 in equation (2)

$$G = \alpha + \phi Z^2 + mC \qquad (4)$$

The slope m depends on the concentration of gelatin, as is seen in Fig. 2, and is a linear function of the gelatin concentration (Fig. 4). The slope m could therefore be written as

$$m = \beta + \psi Z \qquad (5)$$

where β = the intercept with the m axis, ψ = slope.

Substituting for m in equation (4)

$$G = \alpha + \phi Z^2 + (\beta + \psi Z)C \qquad (6)$$

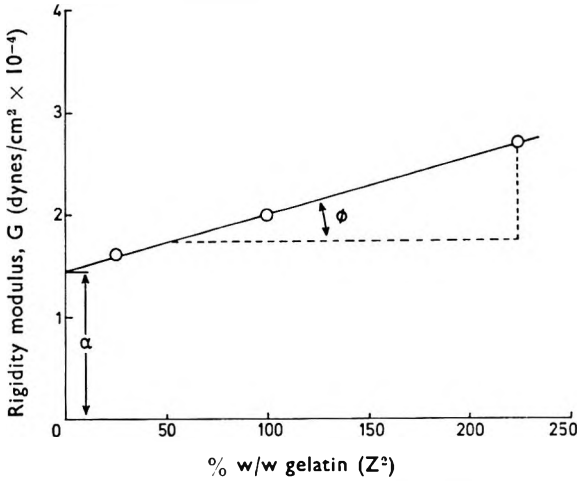

FIG. 3. The relationship between rigidity and gelatin concentration in the absence of glycerin. Gelatin (99 Bloom). Temperature, 25° ± 0·1°.

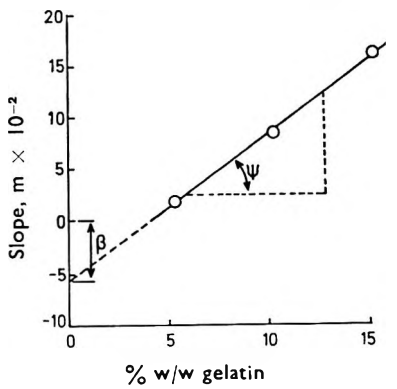

FIG. 4. The effect of gelatin concentration on the slope of the rigidity curve of gelatin/glycerin/water gels. Gelatin (99 Bloom). Temperature, 25° ± 0·1°.

Substituting the values for the constants calculated from Figs 2, 3 and 4

$$G \times 10^{-4} = 1{\cdot}48 + 5{\cdot}5 \times 10^{-3}Z^2 + (1{\cdot}45 \times 10^{-2}Z - 5{\cdot}45 \times 10^{-2})C \quad . . \quad (7)$$

This mathematical treatment was derived using a gelatin of 99 Bloom strength, but equivalent relationships were found with other gelatins. The constants for the other gelatins examined are given in Table 2 and a comparison of experimental and calculated rigidities is shown in Table 3. The general equation appeared to be valid for alkali treated hide gelatins at gelatin concentrations between 4 and 15% w/w and containing up to 40% w/w glycerin.

The equation was empirical and the criterion for distinguishing between the gelatins was Bloom number, which gives no indication of the molecular

TABLE 2. CONSTANTS FOR SAMPLES OF DIFFERENT GELATIN

Sample	Bloom No.	Constant			
		α	β	ϕ	ψ
A	99	1·48	$-5{\cdot}45 \times 10^{-9}$	$0{\cdot}55 \times 10^{-2}$	$1{\cdot}45 \times 10^{-2}$
B	154	1·46	$-4{\cdot}69 \times 10^{-9}$	$2{\cdot}2 \times 10^{-2}$	$1{\cdot}30 \times 10^{-2}$
C	200	1·52	$-11{\cdot}9 \times 10^{-9}$	$2{\cdot}68 \times 10^{-2}$	$2{\cdot}71 \times 10^{-2}$
D	250	1·57	$-7{\cdot}55 \times 10^{-9}$	$4{\cdot}38 \times 10^{-2}$	$2{\cdot}09 \times 10^{-2}$

TABLE 3. EXPERIMENTAL AND CALCULATED RIGIDITIES FOR GELATIN-GLYCERIN-WATER GELS

Bloom No.		Rigidity (dynes/cm²) × 10^{-4}								
	% Gelatin-% Glycerin	5/10	5/20	5/40	10/10	10/20	10/40	15/10	15/20	15/40
99	Experimental	1·76	1·94	2·34	2·66	3·6	5·47	3·97	5·97	9·1
	Calculated	1·79	1·98	2·34	2·93	3·8	5·65	4·3	5·97	9·23
	Difference %	+1·7	+2	0	+7·4	+5·5	+1·8	+7·7	0	+1·1
154	Experimental	2·15	2·41	2·76	4·3	5·0	6·91	7·6	9·5	13·1
	Calculated	2·18	2·37	2·73	4·49	5·32	6·98	7·89	9·37	12·37
	Difference %	−1·4	−1·7	−1·1	+4·4	+6	+1	+3·8	−1·4	−5·6
200	Experimental	2·3	2·7	3·3	5·29	6·88	9·9	9·96	12·2	16·8
	Calculated	2·35	2·52	2·85	5·7	7·24	10·28	10·42	13·29	18·6
	Difference %	+2·2	−6·6	−13·6	+7·5	+4·9	+3·8	+4·6	+8·9	+10·7
250	Experimental	3·02	3·3	4·0	7·5	9·5	12·2	12·51	16·49	24
	Calculated	2·98	3·27	3·85	7·32	8·65	11·32	13·83	16·21	20·97
	Difference %	−1·3	−0·9	−3·7	−2·41	−8·9	−7·2	+10·5	−1·6	−12·6

weight of the sample. In general a high Bloom number indicates a gelatin molecule of long chain length, but there is no simple relationship. None of the constants β, ϕ, ψ, showed any direct relationship to Bloom number. β and ψ depended on both the gelatin and glycerin concentration whilst ϕ depended on the gelatin concentration only. However, for each percentage w/w gelatin : glycerin ratio, it was possible to calculate a linear relationship between rigidity and Bloom number. As an example, when 5% w/w gelatin and 20% w/w glycerin were used,

$$\sqrt{G} = F + kN \qquad (8)$$

where F is the intercept on the rigidity axis, N is the Bloom number and k the slope.

The numerical values of the constants were $1{\cdot}11 \times 10^{-2}$ and $2{\cdot}84 \times 10^{-5}$ respectively and using the equation, a gelatin with a stated Bloom strength of 180 was found from rigidity measurements to be 179·5 Bloom.

Fig. 5 shows the effect of glycerin concentration on the rigidity modulus of gelatin gels. The rapid increase in rigidity at higher glycerin concentration is paralleled by the increased viscosity of the glycerin : water phase which occurred when the glycerin concentration exceeded 20%. The glycerin may therefore increase the rigidity either by providing weak extra linkages for the gel network or simply by increasing the resistance

to movement of the gelatin molecules. At low liquid phase viscosities the gelatin molecules may be easily moved on the application of a force, but if the viscosity of the interphase is very high then the ability of the gel network to distort under a given force will be severely restricted and be manifest as a high rigidity.

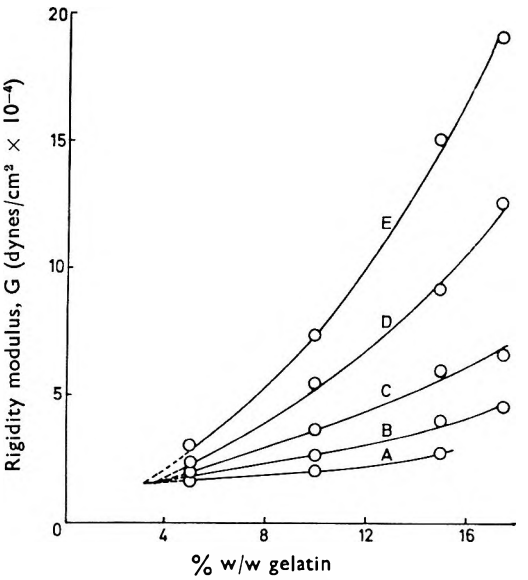

FIG. 5. The effect of gelatin and glycerin concentration on the rigidity of gelatin/glycerin/water gels. Gelatin (99 Bloom). Glycerin % w/w: A, 0; B, 10; C, 20; D, 40; E, 60. Temperature, 25° ± 0·1°.

If the curves in Fig. 5 were extrapolated, they intersected at a gelatin concentration of 3·2% w/w; below this the 'rigidity' was found to be constant and independent of glycerin concentration and Bloom number. The value of this critical rigidity was $1{\cdot}5 \times 10^{-4}$ dynes/cm² and corresponded to the apparent rigidity when the rigidometer was filled with water. Therefore no gel was detected under our experimental conditions at concentrations below 3 to 3·5% gelatin.

Acknowledgements. One of us (P.P.G.) would like to thank N.A.T.O. for financial assistance.

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Anticurare activity of tacrine (THA) *in vitro*

J. M. H. REES

Tacrine (THA) has been found to be a relatively weak anticurare agent having a significant anticurare activity on the rat isolated phrenic nerve diaphragm preparation in concentrations above 10^{-7} M. It is about 20 times less potent than neostigmine. Between 10^{-5} M and 10^{-4} M, anticurare activity of THA ceases to be reproducible, and in concentrations above 10^{-4} M the compound exerts a depressant action directly on the muscle. The qualitative and quantitative differences between the anticurare activities of THA and neostigmine are discussed.

ALTHOUGH the anticurare activity of tacrine (THA) has been demonstrated *in vivo* and *in vitro* (Gershon & Shaw, 1958), no quantitative estimation of its potency *in vitro* has been made.

A quantitative comparison has now been made between the anticurare activities of THA and neostigmine on the rat isolated phrenic nerve diaphragm preparation.

Bentley & Shaw (1949, 1953) showed THA to be a respiratory stimulant and they also reported "profound parasympathetic stimulation". They later showed THA to be an anticholinesterase of the same order of potency as neostigmine and eserine, and this has since been confirmed (Heilbronn, 1961; Ho & Freeman, 1965).

Experimental

METHODS

The preparation used was essentially that described by Bülbring (1946). Female rats, 200–250 g, were used. The tissue baths of 15 ml capacity were identical to those used by Starmer & Thomas (1961). The preparation was maintained in Krebs solution at 38° gassed with a 95% oxygen, 5% carbon dioxide mixture. The preparation was so arranged that it could be stimulated directly or by way of the phrenic nerve. Rectangular electrical pulses of 2·0 to 2·5 msec duration and of a strength just greater than that necessary to evoke maximal twitches were applied at a frequency of 6/min. The contractions were recorded by a Brodie Universal writing lever on a smoked drum.

The drugs used were (+)-tubocurarine chloride, neostigmine methyl sulphate, and 5-amino-1,2,3,4-tetrahydroacridine hydrochloride (tacrine, THA).

In all instances concentrations are expressed as final bath molar concentrations.

Results

Two series of experiments were made. In the first the anticurare agent was added to the bath fluid before or with the tubocurarine, and in the

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second the anticurare agent was added during partial block produced by tubocurarine.

The first series of experiments was designed according to the method of Bülbring & Chou (1947). A log dose response curve for tubocurarine was determined and this was then repeated in the presence of either THA or neostigmine. THA or neostigmine was added to the bath 5, 2, or 1 min before each dose of tubocurarine, or the tubocurarine and the antagonist were added together. The largest dose of each antagonist investigated was about 40 times bigger than the smallest. The antagonism was expressed as the molar concentration of anticurare agent that reduced the effect of a dose of tubocurarine to that of a dose half the size. These values are termed A_2 values (Schild, 1947) and are given in Table 1. The standard deviations of the A_2 values were usually small for experiments done on the same preparation but in different preparations the A_2 values for THA varied sometimes by as much as 100%. With neostigmine there was no more variation between preparations than there was in the same preparation.

TABLE 1. THE RELATIVE ACTIVITIES (± S.D.) OF NEOSTIGMINE AND THA AS ANTICURARE AGENTS

Pretreatment time with anticurare agent	A_2 value		Potency ratio, where activity of THA for 5 min pretreatment = 1·0	
	THA	Neostigmine	THA	Neostigmine
5 min	$1{\cdot}379 (\pm 0{\cdot}493) \times 10^{-6}$ (4)*	$2{\cdot}610 (\pm 0{\cdot}123) \times 10^{-8}$ (4)	1·0	54·0
2 min	$1{\cdot}568 (\pm 0{\cdot}092) \times 10^{-6}$ (5)	$6{\cdot}123 (\pm 1{\cdot}48) \times 10^{-8}$ (5)	0·9	22·5
1 min	$6{\cdot}264 (\pm 1{\cdot}32) \times 10^{-6}$ (4)	$3{\cdot}596 (\pm 1{\cdot}43) \times 10^{-7}$ (4)	0·2	3·8
Simultaneous addition	Could not be determined	$2{\cdot}437 (\pm 0{\cdot}946) \times 10^{-7}$ (5)	—	5·7

The A_2 value is the molar concentration of anticurare agent that would reduce the effect of a double dose of tubocurarine to that of a single dose.

* Figures in parenthesis indicate number of experiments.

In the second series of experiments, the bath concentration of tubocurarine produced each time was $1{\cdot}4 \times 10^{-7}$ M. Either 2 or 4 min after the addition of tubocurarine, the anticurare agent was added and the extent of the antagonism of the block was measured either 2 or 4 min later respectively. Two concentrations of each anticurare agent were used. For THA these were 4·2 and $2{\cdot}1 \times 10^{-6}$ M and for neostigmine, 9·2 and $4{\cdot}6 \times 10^{-7}$ M. The doses of each anticurare agent were added to the bath according to a Latin square design. When the 2 min contact time was used, the log dose response slopes for THA and neostigmine did not differ significantly from parallelism. The ratio of anticurare potencies was found to be neostigmine, 5·77 : THA, 1·00 ($P = 0{\cdot}05$, range of standard deviation 5·65–5·88).

When the 4 min anticurare contact time was used the partially blocked contractions were initially increased in strength by THA but the effect began to wane after about 2 min and the responses became erratic.

At concentrations near 10^{-4} M, THA depressed both directly and indirectly elicited contractions. A concentration of 10^{-4} M produced a

30% depression in 10 min and a concentration of $4{\cdot}3 \times 10^{-4}$ M abolished the twitches within 10 min. Neither potassium ion nor neostigmine reversed the effect.

The depression was rarely quantitatively reproducible after washing the preparation.

The mechanism of this action cannot be restricted to the neuromuscular junction.

Discussion

An A_2 value is indicative only of a small degree of antagonism, and Table 1 shows that an A_2 value could not be determined for simultaneous addition of THA; this could be because at concentrations above 10^{-5} M the anticurare activity of THA is antagonised by a direct inhibitory effect on the muscle. This effect has also been reported by Gershon & Shaw (1958).

Table 1 shows that at 5 min pretreatment neostigmine was over 50 times more potent than THA, and this ratio falls progressively so that at 1 min pretreatment neostigmine is only 17 times more potent. It has been established that the activity of anticurare agents decreases as the interval between their administration and that of tubocurarine decreases (Bülbring & Chou, 1947). From the ratio of potencies it will be seen that the activity of neostigmine falls by a greater fraction than the activity of THA.

On post-treatment, using a 2 min anticurare contact time, neostigmine was found to be only six times more potent than THA. However using the 4 min anticurare contact time, THA was less potent, for although its anticurare action appeared quickly, the effect was only transient. THA therefore appears to have a shorter duration of action than neostigmine.

It has been shown in man that THA is a relatively ineffective decurarising agent compared with neostigmine, particularly when there was profound curarisation (Hunter, 1965). The ratio of intravenous doses in man for decurarisation is neostigmine, 1 : THA, 15. It is of interest that these relative potencies are similar *in vivo* and *in vitro*.

THA is an inhibitor of acetylcholinesterase equipotent with neostigmine (Heilbronn, 1961), though it is a more potent inhibitor of pseudocholinesterase (Ho & Freeman, 1965). The ability of an anticholinesterase to reverse curarisation is believed to be due primarily to inhibition of acetylcholinesterase, though this has been the subject of some controversy (for references see Barlow, 1964).

With THA, its anticurare activity is less potent than its anticholinesterase activity would indicate. Porter (1965) has attributed the potentiation of acetylcholine on the toad rectus solely to inhibition of cholinesterase, and in this preparation THA is equipotent with neostigmine. There is therefore a strong indication that on the rat isolated phrenic nerve diaphragm preparation there is some essential difference between the anticurare activity of neostigmine and THA, for THA differs from neostigmine in its potency, time course of action, and the presence of an interfering action.

NOTE ON THE APPLICATION OF pA_x VALUES TO CURARE-ANTICURARE ANTAGONISM

In 1947 Schild introduced and discussed the pA_x value as a measurement of antagonist activity. The applicability of such a criterion to the present situation may be challenged on two counts. Firstly the degree of antagonism is not measured at equilibrium but at a fixed time known to be short of equilibrium. Secondly, curare-anticurare antagonism is probably not a simple antagonism at one receptor, but involves two distinct systems namely the cholinesterase site and the nicotinic receptor.

In an attempt to differentiate between the nature of neostigmine and THA curare antagonism, pA_2 values were calculated both after the method of Schild (1947), and from the graph of log dose ratio -1 plotted against the negative logarithm of the molar concentration of antagonist (Arunlakshana & Schild, 1959). From the slope of this line, the $pA_2 - pA_{10}$ value should equal approximately 0·95 for competitive antagonism. The results are given in Table 2.

TABLE 2. A COMPARISON OF THE pA_2 VALUES OF THA AND NEOSTIGMINE ANTAGONISM OF TUBOCURARINE CALCULATED AFTER SCHILD (1947) (i), AND ARUNLAKSHANA & SCHILD (1959) (ii)

Drug	Pretreatment min	pA_2 (i) (± s.d.)	pA_2 (ii)	$pA_2 - pA_{10}$
Neostigmine	simultaneous	6·64 (±0·18)	6·58	1·47
	1	6·47 (±0·19)	6·74	1·03
	2	7·22 (±0·10)	7·15	1·33
	5	7·58 (±0·22)	7·58	0·81
THA	simultaneous*		4·05	4·10
	1	5·22 (±0·08)	5·23†	2·60
	2	5·80 (±0·03)	5·56†	2·12
	5	5·88 (±0·17)	5·88	1·39

* pA_2 value (i) could not be determined practically. pA_2 (ii) and $pA_2 - pA_{10}$ calculated by extrapolation
† Denotes that the points do not lie on a straight line. These figures are therefore approximate.

For curare-anticurare antagonism to behave as true competitive antagonism, the concentration of anticholinesterase must be directly proportional to the additional acetylcholine available to compete with tubocurarine for the nicotinic receptors. However, the degree of antagonism is limited by the availability of acetylcholine.

It will be seen that neostigmine almost fulfils the requirements for competitive antagonism at all the time intervals investigated over a 40-fold increase in concentration. On the other hand, with THA this only occurs for 5 min pretreatment at a concentration of 10^{-6} M. Were THA merely a weaker anticholinesterase agent then it should still fulfil exactly the same requirements as does neostigmine.

The value of a pA_2 determination is that it is independent of agonist concentration, and is reproducible. It is of interest that if the results of Bülbring & Chou (1947) for neostigmine and tubocurarine are calculated in terms of pA_2 and $pA_2 - pA_{10}$, values close to the present ones emerge: 6·80 compared with the present study 6·74, and 0·95 compared with the present study 1·03 respectively. The neostigmine was added to the bath

1 min before tubocurarine and the reversal (%) of curarisation measured after 3 min.

Thus even taking into account theoretical criticisms of the application of pA_2 to curare-anticurare antagonism, it is evident that there is a large difference between THA and neostigmine as anticurare agents.

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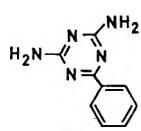
The pharmacology of 2-amino-4-methyl-6-phenylamino-1,3,5-triazine, a centrally acting muscle relaxant*

R. T. BRITTAIN

2-Amino-4-methyl-6-phenylamino-1,3,5-triazine (CB.2487) was found to resemble mephenesin both qualitatively and quantitatively in its actions in mice, and could be distinguished from meprobamate and pentobarbitone. In this species CB.2487 antagonised strychnine competitively but had no effect against leptazol or nicotine. In the anaesthetised cat, the most pronounced action of CB.2487 was to block multisynaptic crossed extensor and flexor reflexes in doses which did not suppress the monosynaptic patellar reflex. The compound also depressed selectively the crossed extensor reflex and not the patellar reflex in the spinal animal. CB.2487 had no effect on the myoneural junction. The results indicate that CB.2487 is a centrally acting muscle relaxant. Unfortunately the drug was poorly active by mouth and had a short duration of action. It is considered to have no advantage over mephenesin.

IT had been observed in these laboratories that 2,4-diamino-6-phenyl-1,3,5-triazine (I) caused paralysis in mice at levels well below the lethal dose (Brittain & Collier, unpublished observations). The precise mode of action was unknown but the properties of the drug were quickly shown to resemble those of mephenesin rather than those of curare. Previously, the synthesis of a series of related symmetrical triazines as potential antipyrimidines had been started by Dr. G. M. Timmis (Chester Beatty Research Institute). These structures were now investigated for their



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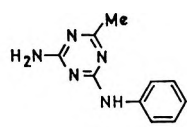

II

effects on the central nervous system since they might have specific depressant properties of value as centrally acting muscle relaxants or as mild tranquillisers. One compound of particular interest in the series, 2-amino-4-methyl-6-phenylamino-1,3,5-triazine (CB.2487) (II), was submitted to detailed pharmacological analysis, the results of which are now described.

Experimental

METHODS

Paralysing activity and acute toxicity in mice. Paralysing activity was determined by the rotating drum technique of Collier, Hall & Fieller (1949). Groups of 10 animals (18–22 g) were used for each dose level

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* This work formed part of a thesis accepted for the degree of Ph.D in the University of London.

and the drugs were given by oral, intraperitoneal and intravenous administration. The mean paralysing dose (ED50) for each compound was calculated by the method of Litchfield & Wilcoxon (1949). Acute toxicity (LD50) was determined after 7 days.

Anticonvulsant activity in mice. Compounds were investigated for their abilities to protect animals against the convulsive and lethal effects of leptazol and strychnine respectively and against tremor induced by nicotine. Groups of 10 animals received varying doses of drug intraperitoneally and were then challenged immediately with either leptazol, 100 mg/kg subcutaneously, or strychnine, 1·6 mg/kg intraperitoneally. Nicotine, 0·6 mg/kg intravenously, was injected 10 min after the administration of the test compound. The mean protective dose (PD50) was calculated for each drug. Antagonism to strychnine induced lethality was also used to find if a drug was active by mouth and, if so, to estimate the duration of activity. The nature of the antagonism between CB.2487 and strychnine in the mouse was investigated as follows. Using groups of 10 animals, LD50 values for strychnine were determined in the absence, or presence of, geometrically increasing doses of antagonist. The antagonist was injected intraperitoneally and immediately followed by strychnine injected by the same route.

Antagonism to maximal electric shock was determined using the method based on that described by Swinyard, Brown & Goodman (1952) and later modified by Cashin & Jackson (1962). A square wave stimulator (C. F. Palmer Model H.44) was connected through a time switch to ear electrodes of platinum wire. Rectangular pulses of 80 V, width 3 msec at a frequency of 100/sec for 0·3 sec, were found to induce a characteristic hind-leg tonic extensor seizure in all untreated mice. Drugs were administered orally in graded doses to groups of 10 animals 1 hr before the electric shock was applied. The median protective dose (PD50) was calculated for each drug. A similar test was used to estimate the duration of activity of the test compounds, by varying the interval between the administration of the compounds and the electric shock.

Antagonism of fighting behaviour in mice. The method used was based on that described by Tedeschi, Tedeschi, Mucha, Cook, Mattis & Fellows (1959). Drugs were administered orally to 5 pairs of animals at each of 3 dose levels of compound. Thirty min after the dose, each pair was subjected to 2 min continuous footshock and the number of fighting episodes were recorded. Three or more fighting episodes was taken as a positive response and 2 or less as negative so that each group could have a score of 0, 20, 40, 60, 80 or 100% response. Control groups of 5 pairs of non-drug-treated animals were also subjected to the footshock and had to achieve a 100% score before the test was considered to be valid. Drugs were compared on the basis of their ED50 values.

Antagonism of drug effects in mice by bemegride. It was of interest to determine whether the paralysis induced by CB.2487 was similar to that induced by barbiturates. Accordingly, varying doses of the compounds under test were injected intraperitoneally into groups of 10 mice, and the mean duration of paralysis or motor inactivation for each dosage group

was determined in the absence or in the presence of, 10 mg/kg intraperitoneally of bemegride. The bemegride was injected immediately after the onset of paralysis in each mouse. The duration of paralysis for each animal was determined using the rotating drum technique of Collier & others (1949) and was taken as the time interval from the bemegride injection to the time of recovery.

Action on spinal reflexes. Cats were lightly anaesthetised with chloralose (70–80 mg/kg intraperitoneally) or spinalised under ether anaesthesia and then maintained under artificial respiration. Reflex contractions of the quadriceps femoris or of the tibialis anterior muscles were recorded kymographically: in some experiments contractions of the same quadriceps were elicited by alternately tapping the patellar tendon (Schweitzer & Wright, 1937) and by stimulating the cut contralateral sciatic nerve with rectangular pulses of 5 msec duration and strength 2–5 V at a frequency of 20–40/sec for 0·2 sec. Flexor contractions of the tibialis anterior muscle were elicited by stimulating the ipsilateral superficial peroneal nerve with rectangular pulses of 10 msec duration and strength 2–8 V at a frequency of 20–30/sec for 0·2 sec, the nerve being ligated peripherally to the electrodes. Blood pressure was recorded from the carotid artery with a mercury manometer. Drugs were injected intravenously through a cannula in an external jugular vein.

Action on the myoneural junction. Twitches of the tibialis anterior muscle were elicited by supramaximal rectangular shocks of 0·4 msec duration applied to the peripheral end of the cut sciatic nerves in chloralose anaesthetised cats. Recordings were made kymographically. Blood pressure was recorded from the carotid artery with a mercury manometer. Drugs were injected intravenously through a cannula in a jugular or a femoral vein.

DRUGS AND SOLUTIONS

The following drugs were used: 2-amino-4-methyl-6-phenylamino-1,3,5-triazine methane sulphonate, mephenesin, meprobamate, phenytoin sodium, pentobarbitone sodium, leptazol, strychnine hydrochloride, nicotine tartrate, bemegride. All drugs were either dissolved in physiological saline or suspended in 5% solution of gum acacia in physiological saline. For basic and acidic drugs the doses given in the text refer to the free bases and acids respectively.

Results

Paralysing activity and acute toxicity in mice. The effectiveness of CB.2487 and the reference drugs mephenesin and meprobamate in paralysing and killing animals following oral, intraperitoneal and intravenous administration is summarised in Table 1.

The paralysis caused by CB.2487, mephenesin and meprobamate was neither preceded nor followed by excitation, nor was there evidence of respiratory distress at paralysing doses. However, if the dose was increased, death resulted from respiratory failure. It is clear that the

TABLE 1. PARALYSING ACTIVITIES OF CB.2487, MEPHENESIN AND MEPROBAMATE ADMINISTERED ORALLY, INTRAPERITONEALLY AND INTRAVENOUSLY IN MICE

Compound	Route	Paralysing activity		Acute toxicity
		ED50 (95% Fiducial limits) mg/kg	Approx. duration of paralysis at ED50 (min)	LD50 (95% Fiducial limits) mg/kg
CB.2487	Oral	134·3 (97·68–184·7)	10–15	>500
	i.p.	74·1 (65·0–84·5)	2–3	358·1 (326·5–392·0)
	i.v.	41·7 (36·7–48·6)	0·5–1	208·9 (198·6–219·8)
Mephenesin ..	Oral	162·2 (132·9–197·9)	8–12	>500
	i.p.	112·2 (106·9–117·9)	1·5–3	464·5 (441·1–489·5)
	i.v.	30·9 (28·3–34·6)	0·5–1	186·2 (166·3–203·7)
Meprobamate ..	Oral	>200	–	>500
	i.p.	174·2 (151·7–206·2)	20–35	>500

paralysing activities and acute toxicities of CB.2487 and mephenesin are quantitatively similar following oral and parenteral administration to mice.

Anticonvulsant activity in mice. Compound CB.2487, 200 mg/kg intraperitoneally, showed little or no antagonism to leptazol- or nicotine-induced convulsions, indicating that the drug did not exert a prime action of a known type on the higher centres of the central nervous system. However, CB.2487, in doses below its paralysing dose and well below its lethal dose, did antagonise strychnine. The intraperitoneal PD50 values for CB.2487 and mephenesin in preventing strychnine induced lethality were 33·1 (27·2–40·3) and 144·5 (110·6–187·0) mg/kg respectively. The

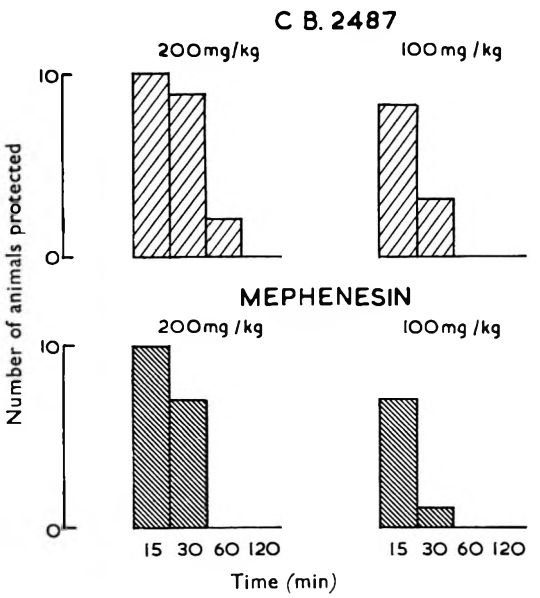

FIG. 1. Effects of CB.2487 and mephenesin, administered orally to groups of 10 mice, in preventing death induced by the intraperitoneal injection of 1·6mg/kg strychnine. The ordinate gives the interval between the administration of drug and the injection of strychnine.

potency and duration of antagonism to strychnine of CB.2487 following oral administration were marginally greater than mephenesin, but both compounds exhibited only brief activity by this route (Fig. 1).

Because of the low oral activity it was decided to revert to the intraperitoneal route to examine the nature of the strychnine antagonism. The effect of geometrically increasing doses of CB.2487 on the intraperitoneal lethality of strychnine was determined and the resulting log dose/probit mortality plots are illustrated in Fig. 2. The results show that the effects of CB.2487 are surmountable by strychnine. Furthermore, since the dose-response lines do not deviate significantly from parallelism and the log interval space between these lines is constant, these results suggest competitive antagonism between the drugs (Ariëns, Rossum & Simonis, 1956).

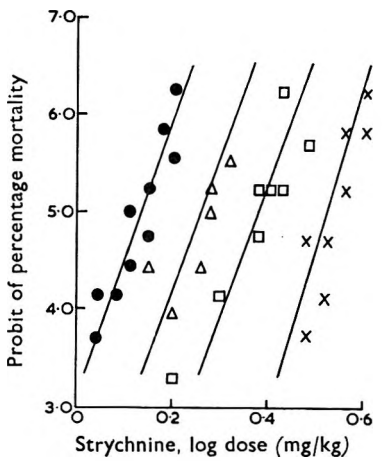

FIG. 2. Log dose/probit mortality plots for strychnine in the presence of geometrically increasing doses of CB.2487. Both drugs were injected intraperitoneally into mice. ●—● No antagonist. △—△ 25 mg/kg CB.2487. □—□ 50 mg/kg CB.2487. ×—× 100 mg/kg CB.2487.

The comparative PD50 values mg/kg (95% fiducial limits) of CB.2487, mephenesin and phenytoin, administered orally against maximal electric shock in mice, are: 190·0 (165–218), 198·0 (157·5–254) and 4·70 (3·56–6·21) respectively. Both CB.2487 and mephenesin exhibited weak activity in this test compared with the activity of phenytoin, a drug known to be potent in mice and rats in this test (Goodman, Toman & Swinyard, 1948).

In a further experiment, the durations of activities of these three compounds were investigated (Fig. 3). Again CB.2487 and mephenesin had fleeting activity even at high doses whereas the effect of phenytoin at 10 mg/kg lasted for more than 2 hr.

Antagonism of fighting behaviour in mice. The oral ED50 values mg/kg (95% fiducial limits) of CB.2487, mephenesin and meprobamate in suppressing fighting episodes in mice are 127 (95·8–169), 125 (91·3–174) and 61·7 (39·2–96·8) respectively. Only compounds which suppress

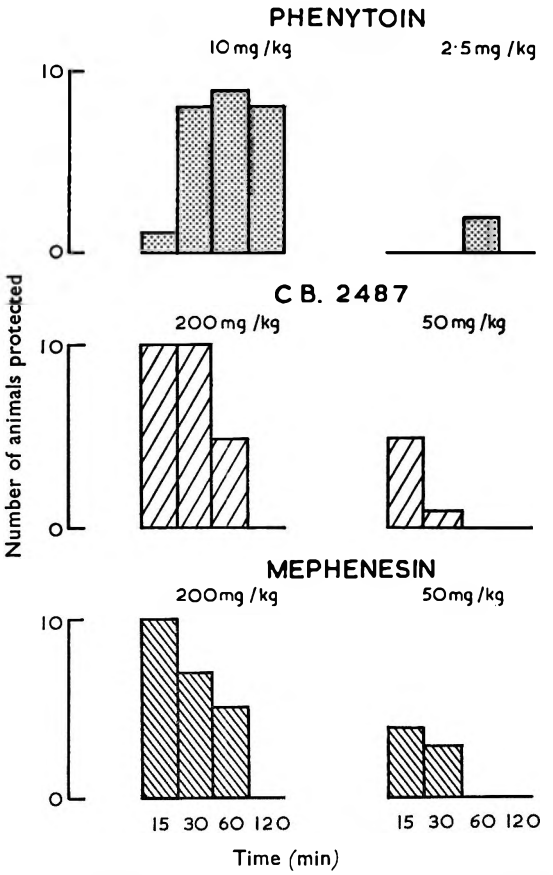

FIG. 3. Effects of CB.2487, mephenesin and phenytoin, administered orally to groups of 10 mice, in preventing seizures induced by maximal electric shock. The ordinate gives the interval between the administration of drug and the application of the shock.

fighting behaviour at doses substantially less than those causing paralysis (see Table 1) can be considered to have a selective action. The ratio, ED50 causing paralysis/ED50 in suppressing fighting episodes, is a convenient means of assessing selectivity, a high ratio indicating high selectivity. For CB.2487 the figure is 1·058, for mephenesin, 1·296 and for meprobamate $>3·241$.

It is clear that only meprobamate has a selective action. Suppression of fighting episodes by CB.2487 and mephenesin occurred at, or near to, doses which induced much depression of motor activity. Again it is seen that CB.2487 resembles mephenesin and both these compounds may be easily differentiated from meprobamate.

Antagonism of drug effects in mice by bemegride. The effects of bemegride on the duration of paralysis induced by CB.2487, mephenesin or pentobarbitone are illustrated in Fig. 4. Bemegride had little or no

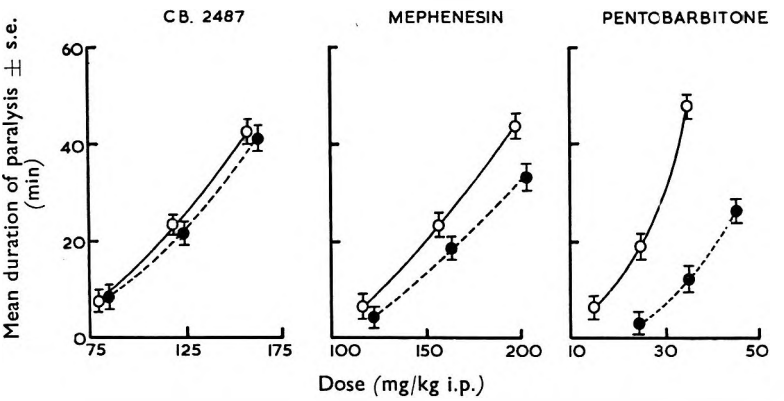

FIG. 4. Effects of bemegride on the durations of paralysis induced by CB.2487, mephenesin and pentobarbitone in the mouse. ○—○ Drug alone ●---● Drug + bemegride, 10 mg/kg i.p.

effect on the paralysis induced by CB.2487 or mephenesin but had the expected antagonism to the barbiturate. Since bemegride is believed to be a selective barbiturate antagonist (Shaw, Simon, Cass, Schulman, Anstee & Nelson, 1954) it is probable that neither CB.2487 nor mephenesin possesses barbiturate-like activity.

Action on spinal reflexes. In cats anaesthetised with chloralose CB.2487, 5 to 10 mg/kg intravenously, selectively depressed contractions of the quadriceps femoris muscle elicited through a multisynaptic pathway (crossed extensor reflex). The degree of depression was dependent upon the dose injected. At these dose levels CB.2487 did not depress contractions of the quadriceps muscle elicited through a monosynaptic pathway (patellar reflex). In agreement with published reports (Kaada, 1950; Taverner, 1952) mephenesin also selectively blocked the multisynaptic

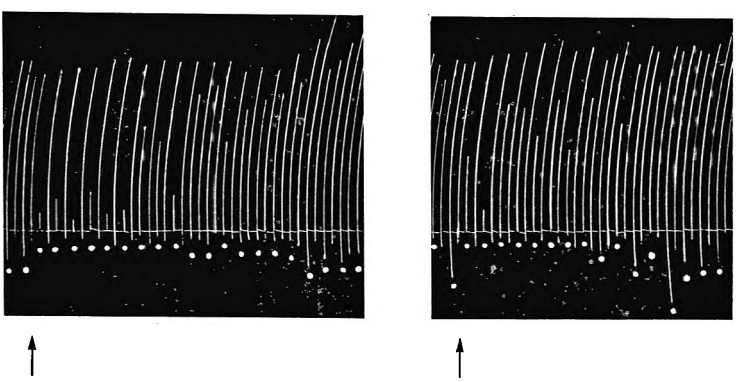

FIG. 5. Cat, light chloralose anaesthesia. Alternate crossed extensor and patellar reflex contractions of the quadriceps femoris muscle every 30 sec (i.e. 1 min between reflex contractions of the same type). Crossed extensor reflexes indicated by white dots. Left-hand panel, 10 mg/kg, CB.2487; right-hand panel, 10 mg/kg, mephenesin. Both drugs injected intravenously.

reflex, being about 0·6 times as active as CB.2487; equipotent doses of these compounds had similar durations of action. The results are summarised in Table 2 and one experiment is illustrated in Fig. 5. At the dose levels examined neither drug had any significant effect on arterial blood pressure.

TABLE 2. THE DEPRESSANT EFFECTS OF CB.2487 AND MEPHENESIN ON THE CROSSED EXTENSOR REFLEX (QUADRICEPS FEMORIS MUSCLE) IN ANAESTHETISED CATS

Compound	Dose mg/kg i.v.	Mean maximal depression (%) of quadriceps twitch ± s.e.	Mean duration of effect (min) ± s.e.
CB.2487	2·5	0 (2)	0
	5·0	31·6 ± 13·5(3)	3·7 ± 2·7
	7·5	53·0	4·0
	10·0	90·6 ± 4·2 (4)	12·5 ± 1·2
	20·0	100 (2)	24·0 ± 2·0
Mephenesin	2·5	0 (2)	0
	5·0	7·9 ± 3·1 (2)	0
	10·0	55·5 ± 16·4 (5)	4·6 ± 1·6
	20·0	85·9 ± 12·4 (3)	13·7 ± 2·3

Figures in parentheses indicate number of determinations. Duration was taken as the time between dosage and recovery of the reflex response. The point of recovery was arbitrarily chosen as the first of three consecutive responses having 75% or more of the mean value of three consecutive responses immediately before dosage.

It has already been shown that neither CB.2487 nor mephenesin affected the monosynaptic patellar reflex at doses which abolished or substantially reduced the crossed extensor reflex. In fact, the patellar reflex was found to be very resistant to both drugs, no effect being seen when the dose was increased to 8 times that required to reduce the crossed extensor reflex.

In spinalised animals CB.2487 or mephenesin, 10 to 20 mg/kg intravenously, still suppressed the crossed extensor reflex. However, the effect of these drugs on the patellar reflex was variable; usually there was no effect but enhanced contractions were produced in some preparations.

In a further series of experiments using cats anaesthetised with chloralose, the effects of CB.2487 and mephenesin on a multisynaptic flexor reflex were compared. The reflex chosen involved contraction of the tibialis anterior muscle. Both drugs were found to suppress this reflex at slightly higher dosage than that needed to suppress the crossed extensor reflex. Again CB.2487 was more active than mephenesin but at equipotent doses had about the same duration of action. The results are summarised in Table 3.

In experiments to investigate the effect of CB.2487 on spinal reflexes augmented by strychnine, it was found that intravenous doses of strychnine, 10 to 30 μg/kg, had no effect on the patellar reflex but caused marked potentiation of the crossed extensor reflex. This preferential effect of strychnine has been previously observed by Kaada (1950), Naess (1950) and Bernhard, Taverner & Widen (1951). At higher dosage, 40 μg/kg, strychnine also enhanced the patellar reflex contractions. When this did occur, generalised muscular activity was also evident. In cats given strychnine, 20 μg/kg intravenously, to augment the crossed extensor

TABLE 3. THE DEPRESSANT EFFECTS OF CB.2487 AND MEPHENESIN ON A FLEXOR REFLEX (TIBIALIS ANTERIOR MUSCLE) IN ANAESTHETISED CATS

Compound	Dose mg/kg i.v.	Mean maximal depression (%) of tibialis twitch ± s.e.	Mean duration of effect (min) ± s.e.
CB.2487	5	25·7	2·0
	10	57·3 ± 11·9 (2)	11·0 ± 1·0
	15	91·6 ± 8·4 (2)	11·5 ± 5·5
	20	94·4 ± 4·6 (3)	19·7 ± 3·8
Mephenesin	5	16·8 ± 11·3 (2)	0
	10	29·3 ± 1·5 (3)	3·3 ± 0·9
	15	69·6 ± 3·6 (3)	7·0 ± 1·5
	20	79·8 ± 20·2 (2)	9·5 ± 2·5

Figures in parentheses indicate number of determinations. Duration was taken as the time between dosage and recovery of the reflex response. The point of recovery was arbitrarily chosen as the first of three consecutive responses having 75% or more of the mean value of three consecutive responses immediately before dosage.

reflex, CB.2487 (6·25 mg/kg intravenously) antagonised the augmentation caused by strychnine and, in higher doses, reduced or abolished the reflex itself. Mephenesin was found to have a similar action in this preparation but was only about one half as active as CB.2487.

Action on the myoneural junction. In the anaesthetised cat, CB.2487 in intravenous doses up to 40 mg/kg neither potentiated nor depressed the maximal twitches of the tibialis anterior muscle stimulated through its motor nerve. At these dose levels mephenesin was also without effect on this preparation. Clearly CB.2487 and mephenesin do not exhibit neuromuscular blocking activity in intravenous doses approximately 4 times those required to depress reflex contractions of the tibialis anterior muscle elicited through a multisynaptic pathway.

Discussion

2-Amino-4-methyl-6-phenylamino-1,3,5-triazine (CB.2487) closely resembles mephenesin in its effects in the mouse. Both drugs cause obvious reduction in motor activity followed by paralysis without respiratory arrest. The impression that CB.2487 was mephenesin-like was further substantiated when it apparently antagonised competitively strychnine-induced toxicity, indicating that its prime locus of action was on the spinal cord. A more detailed comparison of CB.2487 and mephenesin confirmed their similarity; furthermore, it was also shown that CB.2487 could be distinguished from drugs such as meprobamate and pentobarbitone. Since CB.2487 closely resembles mephenesin in its action and since the pharmacology of mephenesin has been well documented (Berger, 1947; Kaada, 1950; Taverner, 1952) it seems probable that the effects of CB.2487 also depended on an action on the spinal cord and the drug would not be of value as a sedative or tranquilliser.

The most pronounced action of CB.2487 is its ability, in relatively small doses, to depress the crossed extensor and flexor reflexes in the anaesthetised cat. Similar results were obtained in the spinal animal as well as in the intact anaesthetised animal, showing that the effect of CB.2487 is not a consequence of an action on the brain. An action on the neuromuscular

junction, on skeletal muscle, on the muscle spindles, and on conduction in peripheral nerves (including the lower motoneurones, the γ-efferents and the Group Ia afferents) was ruled out when it was observed that CB.2487 did not depress contractions of the tibialis anterior muscle elicited by stimulation of the sciatic nerve or contractions of the quadriceps femoris muscle elicited by tapping the patellar tendon. These results therefore confirmed the location of action of CB.2487 in the spinal cord, typing the drug as a centrally acting muscle relaxant.

The selective depression of multisynaptic reflexes and not a monosynaptic reflex by CB.2487 in the cat, together with the lack of action of this compound in antagonising convulsions in mice induced by leptazol and nicotine strongly suggests that its site of action is not on the anterior horn cell of the spinal cord, since these cells and the lower motoneurones constitute the final common path in all these cases. In fact these results indicate the site of action of CB.2487 to be at some point in the interneurone chains that form part of the pathways involved in the crossed extensor and flexor reflexes. Only a speculative view can be presented on the possible mechanism by which CB.2487 exerts its effects; however, the interaction of CB.2487 with strychnine suggests a close relationship to the effects of inhibitory transmitter. Inhibition in the spinal cord might be brought about by a drug which mimicked the action of, increased the release of, or prevented the destruction of inhibitory transmitter, which stimulated inhibitory neurones or which lowered their threshold to normal physiological stimuli. According to Eccles (1964), strychnine acts by competing with the inhibitory transmitter for the same receptors on the post-synaptic cell. If CB.2487 mimicked the action or prevented the destruction of inhibitory transmitter, the apparently competitive relationship between it and strychnine could be explained on this basis. There is evidence (Curtis, 1959), as yet not fully substantiated (Anderson, Eccles, Løyning & Voorhoeve, 1963; Crawford, Curtis, Voorhoeve & Wilson, 1963), that the same transmitter is responsible for inhibition at all inhibitory synapses. If this were so then the anterior horn cells would presumably be sensitive to any substance which resembled inhibitory transmitter, yet the monosynaptic pathway was not blocked by CB.2487. The same considerations apply to the possibility that CB.2487 activates inhibitory neurones; all post-synaptic cells would be depressed, including the anterior horn cells, unless the drug selectively stimulates some inhibitory neurones but not others. Clearly without further knowledge about the identity of central transmitters and the mechanism of action of strychnine it is impossible to determine the site and mechanism of action of CB.2487 more precisely.

Possible clinical uses of centrally acting muscle relaxants include the relief of chronic spastic conditions of voluntary muscles, involving for example, hemiplegia, disseminated sclerosis, the many spastic states following brain injuries in childbirth and tetanus. To be of value in such conditions a centrally acting muscle relaxant drug would have to be long acting, selectively affect the spastic muscle and preferably be effective by mouth. Noticeable features of the pharmacological properties of

CB.2487 are the brief duration of activity and the large difference between the oral and parenteral activities, the former being much lower. The poor oral activity and brief duration of action make CB.2487 a poor prospect for use in any of these clinical conditions.

Acknowledgements. I wish to thank Miss J. I. Pearson and Mr. D. Rowe for technical assistance.

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Some aggregation effects observed with an emulsion dispersed in saline containing cetrimide*

M. J. GROVES

Aggregation behaviour has been observed when an emulsion containing liquid paraffin and cetostearyl alcohol as disperse phase is diluted in saline containing cetrimide below the critical micelle concentration. This aggregation has been examined with the Coulter Counter. Under standardised conditions the apparent size of the aggregated particles is directly related to the logarithm of the molar concentration of the cetrimide present over three orders of magnitude. Above the critical micelle concentration the particles appear to be deaggregated.

EMULSIONS most frequently employed as pharmaceutical semi-solid vehicles are more strictly to be thought of as dispersions or solid-liquid suspensions. The optical size analysis of a sample of a typical pharmaceutical emulsion, cetrimide cream, B.Vet.C. 1953, exposed to ultrasonic irradiation, suggested that substantial numbers of sub-micron particles were present. This was confirmed by electron microscopy and a centrifugal method, as described by Freshwater, Groves & Scarlett (1965). Both these methods are likely to underestimate the numbers of particles in the top size range and another method was sought to confirm an optical microscope analysis. Although it is generally accepted that the Coulter Counter is of doubtful value in sizing particles below 0·5 μ, the instrument has been used for emulsion systems (Higuchi, Okada & Lemberger, 1962; Wachtel & La Mer, 1962; Swift & Friedlander, 1964; Rowe, 1965) and appeared to be ideal for the system under examination.

The principle and operation of the Counter has been described by others (Coulter, 1956; Kubitschek, 1960) but any sample for analysis has first to be diluted with electrolyte. Visual examination of dilutions of the cetrimide cream formulation in a wide range of concentrations and types of electrolyte showed that much flocculation was occurring, the rate appearing to be related to the concentration of electrolyte. However, the addition of cetrimide to 0·154 M sodium chloride solution (physiological saline) gave the least flocculation. As the work proceeded it became clear that, under some conditions, aggregation could take place. The conditions enabling the Counter to be applied to the particle size analysis of other cetrimide emulsion systems have been investigated.

Experimental

Materials

All materials were of commercial pharmaceutical quality. The cetrimide sample was a purified pharmaceutical grade consisting principally of tetradecyltrimethylammonium bromide. The critical micelle concentration (CMC) of this sample in 0·154 M sodium chloride solution was measured by a number of methods (Table 1) and taken as 0·003 M.

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* Presented in part at a Coulter Users Meeting, 1st October, 1965, Nottingham.

TABLE 1. THE CMC OF CETRIMIDE IN 0·154 M SODIUM CHLORIDE MEASURED BY DIFFERENT METHODS (AT 25° C)

Method	CMC (M)
Dropweight surface tension	0·0015
Titration Sky Blue FF[1]	0·0025
Adsorption spectrum Sky Blue FF[2]	0·003

[1] Corrin & Harkins (1947).
[2] Corrin & Harkins (1946).

The emulsion under examination was a sample of cetrimide cream, B.Vet.C. 1953, which had been subjected to ultrasonic irradiation before cooling to room temperature. The resulting dispersion was allowed to age for six months before examination.

INSTRUMENTATION

All particle counts were made on a Coulter Counter Model A (Industrial) fitted with 30 and 70 μ orifice tubes previously calibrated with polystyrene and polyvinyltoluene latices and pollens of known diameter.

DILUTION MEDIA

Since dilution media for use with the Counter require to be substantially free of extraneous particulate contamination, a commercially available physiological saline solution for injection (Groves & Major, 1964; Groves, 1965) was employed. Quantitative dilutions of a filtered concentrated solution of cetrimide in saline were prepared as required.

DILUTION PROCEDURE

To reduce the particle concentration of an emulsion to a suitable level for counting it is necessary to make the dilution in at least two stages, as noted by Higuchi & others (1962) and Rowe (1965). Since the CMC of cetrimide was known to be approximately 0·003 M in 0·154 M sodium chloride (Table 1) the primary dilutions were prepared in saline containing either 0·03 M or 0·0003 M cetrimide. A 50 mg sample of the cetrimide cream was accurately weighed on a watch glass and triturated with 100 mg glycerin using a camel hair brush. The dispersion was then slowly diluted with the required cetrimide-saline before making up to 50 ml. The primary dilutions were kept in plastic stoppered tubes on a slowly rocking table at ambient room temperature (24–28°) until required for analysis. This technique was found to be sufficient to prevent creaming of a diluted preparation without undue aeration.

Secondary dilutions were prepared by pipetting 1·5 ml of the primary dilution into 100 ml of the appropriate cetrimide-saline solution contained in a 150 ml beaker on the Counter orifice stand.

COUNTING PROCEDURE

Reproducible results could not be obtained in the system under examination unless the secondary dilution was kept stirred, presumably owing to

creaming and flocculation. Since the stirrer fitted to the Counter was difficult to adjust to the slow constant speed needed to avoid stirring air bubbles into a solution otherwise prone to foaming, a constant speed stirrer rotating at 187·5 rpm was used. The stirrer was started immediately after preparation of the secondary dilution and counting begun a known time after dilution, starting at the highest instrument threshold and reducing this by 0·5 μ intervals until the complete size range had been covered. Background counts were taken using the same procedure.

Results

Primary dilution in 0·03 M cetrimide

Using the 30 μ orifice tube the size distribution obtained with both primary and secondary dilutions in 0·03 M cetrimide is superimposed on the results obtained by other particle sizing methods (Freshwater & others, 1965) (Fig. 1). Reasonable agreement with the optical size analysis is

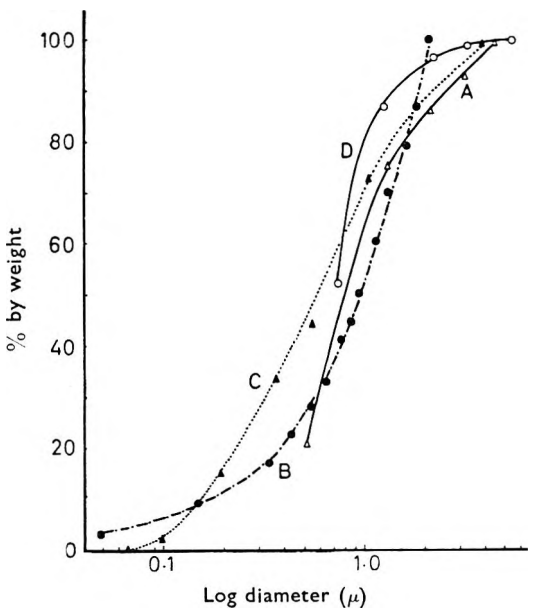

FIG. 1. The particle size distribution of a sample of cetrimide cream B.Vet.C. (1953) by different methods. A, optical microscope. B. electron microscope. C, centrifugal photosedimentometer. D, Coulter Counter.

apparent. From number size distribution the number volume mean diameter, d_{vn}, can be calculated as:

$$d_{vn} = \left[\frac{\Sigma nd^3}{\Sigma n}\right]^{1/3} \qquad \text{(Heywood, 1963)}$$

As indicated in Fig. 1, d_{vn} is 0·88 μ. Making the identical analysis, but using a 70 μ orifice, the lowest limit of particle diameter detected was

1·5 μ and this gave a d_{vn} value of 2·45 μ. Analyses with the 70 μ orifice tube, and using secondary dilutions of 0·003, 0·0003, and 0·00003 M cetrimide stirred for 1 hr, gave a d_{vn} value of 2·4 μ in all instances. This indicates that under these conditions no significant change in the state of the dispersion has occurred.

Primary dilution in 0·0003 M cetrimide

It was found experimentally that aggregation was occurring in the secondary dilutions prepared from a 0·0003 M cetrimide-saline primary dispersion. A series of size distributions were obtained using the 70 μ orifice tube after 1 hr stirring in the presence of different concentrations of cetrimide. Some of these distributions are compared in Fig. 2 with

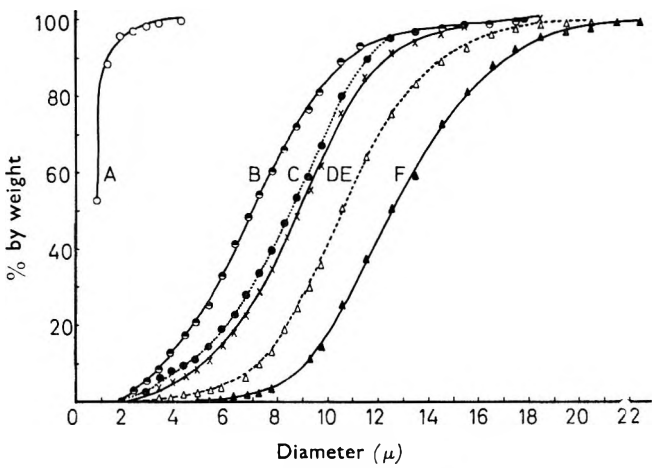

Fig. 2. The change in particle size distribution of the cetrimide cream sample with concentration of cetrimide present in the saline (Coulter Counter). A, 30 μ orifice tube, 1° and 2° dilutions 0·03 M cetrimide. B–F, 70 μ orifice tube, 1° dilution = 0·0003 M cetrimide. Cetrimide 2° dilution: B = 0·03 M; C = 0·003 M; D = 0·0015 M; E = 0·00015 M; F = 0·00003 M.

the result obtained using the 30 μ orifice and 0·03 M cetrimide-saline. The apparent particle size is appreciably larger than the size indicated in Fig. 1. In addition, it will be seen that the apparent size increases as the concentration of the cetrimide present in the secondary dilution is reduced. It seemed likely that the size of aggregates was being measured by the instrument. For the purpose of direct comparison, each size distribution was expressed as a number volume mean diameter and plotted as a function of the logarithm of the molar concentration of cetrimide present in the secondary dilution, Fig. 3. The inflexion point shown in Fig. 3 corresponds to a concentration of 0·0003 M cetrimide, the concentration of the primary dilution. On a logarithmic scale there is a linear correlation between the apparent mean "particle" (or aggregate) size and concentration over three orders of magnitude. There is no evidence of an inflexion at the CMC (0·003 M).

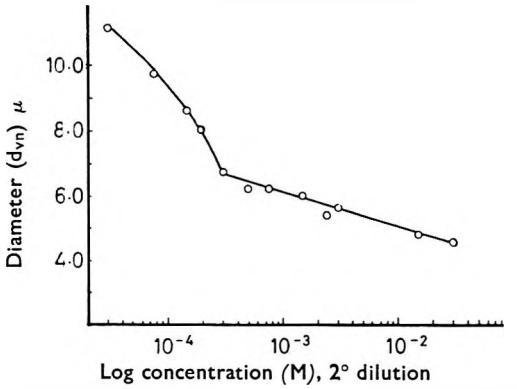

FIG. 3. The change of apparent mean diameter of emulsion particles with cetrimide concentration. 1° dilution = 0·0003 M cetrimide. 2° dilutions as shown.

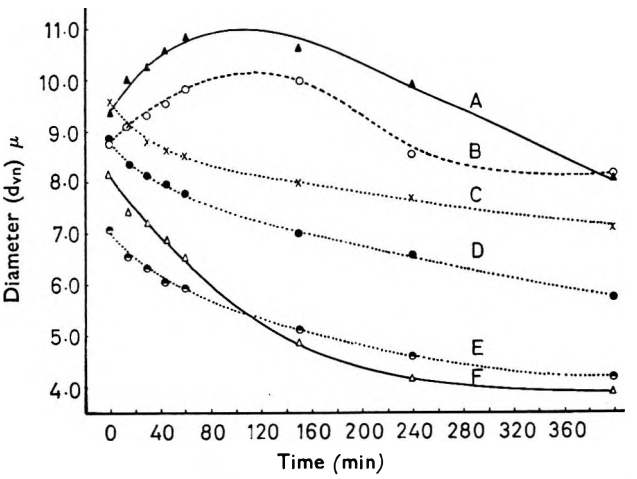

FIG. 4. The change of apparent particle size as a function of cetrimide concentration and time. 1° dilutions = 0·0003 M cetrimide. 2° dilutions: A = 0·00003 M; B = 0·000015 M; C = 0·0003 M; D = 0·00015 M; E = 0·003 M; F = 0·03 M.

The sizing technique described above required about 30 min for completion and was therefore not suited to a study of the rate at which the aggregation was proceeding. Accordingly, the procedure was abbreviated to enable an analysis to be made in 4–5 min by counting at wider size intervals. The results of this experiment are shown in Fig. 4 for dilutions maintained at constant temperature (25°) and constant rates of stirring for up to 400 min.

In all instances replicate experiments gave substantially identical results. Results shown are therefore those from one run under each set of conditions.

Discussion

These experiments suggest that a valid estimate of the size distribution

of emulsion particles greater than 0·5 μ can be obtained with the Coulter Counter if the primary dilution is made in saline containing the emulsifier above its critical micelle concentration. The mean particle size increases if the emulsifier is below the CMC, indicating that aggregation is taking place. Rowe (1965) has independently described the use of electrolyte containing anionic or non-ionic emulsifiers. No reason was given for this but he does not appear to have encountered aggregation. On the other hand, Higuchi and his associates (loc. cit.) found that aggregation of hexadecane emulsions and polymer latices occurred in concentrations of sodium lauryl sulphate and dioctylsodiumsulphosuccinate above the CMC and not below. Using the creaming rate as an indication of aggregation, Cockbain (1952) reported that deaggregation took place below the CMC of soap-stabilised emulsions. Higuchi, Rhee & Flanagan (1965) also noted that a small amount of myristyl-γ-picolinium chloride increased the aggregation rate of polymer latex particles in the presence of dilute salt concentrations. Retardation was observed near, or above, the expected CMC for this cationic surface-active agent.

It would be anticipated that the presence of electrolyte decreases the thickness of the electrical double layer around each emulsion particle and hence affects the stability of the dispersion. Aggregation is therefore possible during a Coulter Counter size analysis which must be made in the presence of electrolyte. The fact that a cationic emulsion is readily susceptible whereas anionic systems are not, may be due to either the difference in thickness of the double layer or the strength of the van der Waals' forces involved at the interface.

Van den Tempel (1953) reviewed the flocculation and aggregation behaviour of oil-in-water dispersions. Higuchi & others (1962) noted that the Counter was suitable for investigating aggregation phenomena and this has been adequately confirmed (Higuchi, Okada, Stetter & Lemberger, 1963; Swift & Friedlander, 1964; Higuchi & others, 1965; Mourad & Lemberger, 1965 a, b). Following these authors, an attempt was made to treat the data in Fig. 4 according to Smoluchowski (1916), who predicted that a plot of the reciprocal of the total particle number with time should be linear. This was found to be valid for a short period of 60–100 min but was not applicable for the total period of the experiment. There are a number of important experimental differences between the present work and that of Higuchi and his colleagues, who have confined their attention to essentially monosize dispersions. As shown in Fig. 1, the present system has a wide size distribution and van den Tempel (1953) pointed out that such a system was likely to aggregate more rapidly. Friedlander (1961) showed that the decay in the total number of particles in a heterogeneous system should be of the form:

$$1/N_{\infty}^{2} \simeq t, \text{ where } N_{\infty} = \text{total number of particles and } t = \text{time.}$$

This decay law was also found to be inapplicable and, for the present, it appears that the data in Fig. 4 cannot be treated quantitatively. This may be because the data were based on counts above a threshold corresponding to 2·5 μ and it is known that the system contains particles down

to at least 0·015 μ (Freshwater & others, 1965). As previously noted, the Counter is of limited value for counting particles below 0·5 μ diameter, and in its present state of development is unable to count the true total number of particles present in the dispersion. Any deductions which can be drawn must remain qualitative only.

Essentially, Fig. 4 shows the variation of a mean aggregate diameter with time and concentration of cetrimide. It will be seen that at 0·003 M (i.e. the same molarity as the primary concentration) there is a slow fall in aggregate size and this presumably represents a change in the size of the aggregate following a change of shear rate (Swift & Friedlander, 1964). Below the concentration of the primary dilution there is at first a relatively rapid increase in aggregate size followed after some 100 min by a slow deaggregation. The marked inflexion point at 0·0003 M noted in Fig. 3 is an indication of the same phenomenon.

This, then, suggests that an equilibrium aggregate size is formed slowly in the presence of the emulsifier. As the concentration of emulsifier is increased the aggregate size decreases in a regular fashion until, ultimately, the aggregates are reduced to the primary particles of the system under investigation. There are difficulties in establishing the time when equilibrium is reached although it is clear that it is in excess of the 400 min and appears, from other evidence, to be at least two or three days. It is of interest to note from Fig. 3 that the relationship is logarithmic, supporting the observations of Rehfeld (1962) and Rowe (1965) that the activity of surfactants increases as a log function of concentration.

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Enlargement of salivary glands in rats after chronic administration of physalaemin or isoprenaline

G. BERTACCINI, G. DE CARO AND R. CHELI

Chronic administration of physalaemin and isoprenaline to rats produced a significant salivary glands enlargement which was moderate (34% fresh weight increase and 48% dry weight increase compared with control glands) after the peptide administration and striking after the amine (630% fresh weight increase and 600% dry weight increase compared with control glands). Physalaemin caused only hypertrophy of the gland, whereas isoprenaline induced a marked hyperplasia.

PHYSALAEMIN, a polypeptide recently found in the skin of the South American amphibian *Physalaemus fuscumaculatus*, (Erspamer, Anastasi, Bertaccini & Cei, 1964; Bertaccini, Cei & Erspamer, 1965) was shown to possess a powerful sialagogic activity particularly evident in dogs and rats (Bertaccini & De Caro, 1965). In these animals the threshold salivatory dose by intravenous injection was less than 1 μg/kg and its effect exceeded by 7 to 250,000 times that of the most common sialogenous drugs.

Physalaemin had no detectable effect on the autonomic nervous system but seemed to exert a direct effect on the salivary glands.

Since chronic administration of isoprenaline to rats is followed by hypertrophy of salivary glands (Selye, Veilleux & Cantin, 1961, Wells, 1962; Schneyer, 1962; Pohto & Paasonen, 1964) physalaemin was administered chronically to rats to find out if it had a similar effect and if so, what were the histological changes.

Experimental

MATERIALS AND METHODS

Young female albino rats of the Wistar strain (Morini Farm, S. Polo) weighing 110 $\pm$ 2·4 g were used. The diet consisted of common laboratory chow with water *ad libitum*. Two groups of 12 rats each, received 10 μg/kg and 100 μg/kg respectively of physalaemin, four times daily, for 12 days. Two groups of 15 rats each, received isoprenaline, 400 mg/kg once daily and 50 mg/kg twice daily respectively, for 12 days. The peptide and the amine were dissolved in 0·5 ml of distilled water and injected intraperitoneally. One group of 20 rats received only distilled water intraperitoneally. Animals were weighed every other day. Control and treated animals were killed 24 hr after the last injection. At autopsy the parotid, submaxillary and major sublingual salivary glands were dissected free of extraneous tissues and weighed together. The dry weight of the glands was determined after drying the glands in an oven for 48 hr at about 100°. From each group of rats some salivary glands

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were fixed in 10% formalin solution for subsequent staining with haematoxylin and eosin. Brain, lungs, liver, spleen and kidneys were also removed, weighed and examined histologically.

Results

All the animals increased in weight (Table 1). Rats treated with physalaemin showed a remarkable flushing of the ears, snout and paws which appeared immediately after the injection; in addition, increased salivation and occasionally nasal and lachrymal secretion were noted with the larger dose (100 μg/kg). Full recovery took 15 to 30 min.

TABLE 1. INCREASE IN TOTAL BODY WEIGHT FOLLOWING ADMINISTRATION OF PHYSALAEMIN OR ISOPRENALINE TO RATS

Treatment			Body weight		
Drug	Dose	No. of rats	Initial, g ± s.e.	Final, g ± s.e.	% Increase
None	—	20	112·5 ± 1	150 ± 2·7	34
Physalaemin ..	10 µg/kg 4 times daily	12	111 ± 1·1	148 ± 3·8	33·5
Physalaemin ..	100 µg/kg 4 times daily	12	110 ± 1·3	143 ± 3	30
Isoprenaline ..	50 mg/kg twice daily	15	111·5 ± 1	148 ± 3	32·5
Isoprenaline ..	400 mg/kg once daily	15	113·5 ± 1·5	125 ± 4·5	14

Rats treated with isoprenaline showed a profuse salivation which became evident within 5 to 10 min after the injection; saliva flowed out of the mouth moistening the fur around the lower lip and mandible. This was particularly evident in the rats treated with the higher dose of the amine (400 mg/kg) and especially during the first two or three days of treatment. In this period most of the body was moistened.

About 20 min after injection the rats lay prostrate on the floor of the cage and a generalized piloerection was also noticed. Within 3 to 6 hr however, they appeared to have recovered except for a marked thirst which lasted all day.

After a decrease in weight during the first 3 or 4 days of treatment, the general condition of the rats improved although the final increase in weight was only 14% (control rats increase = 34%).

The effects of these drugs on the fresh and dry weights of salivary glands are shown in Table 2. Physalaemin caused an increase in the wet and dry

TABLE 2. SALIVARY GLANDS WEIGHTS OF RATS AFTER TREATMENT WITH PHYSALAEMIN OR ISOPRENALINE

Treatment			Salivary glands weight in mg/kg	
Drug	Dose	No. of glands	Fresh, mg ± s.e.	Dry, mg ± s.e.
None	—	15	399 ± 13	112 ± 5
Physalaemin ..	10 µg/kg	8	535 ± 34	166 ± 16
Physalaemin ..	100 µg/kg	8	415 ± 22	114 ± 9·5
Isoprenaline ..	50 mg/kg	10	2537 ± 141	688 ± 40
Isoprenaline ..	400 mg/kg	10	2012 ± 93	538 ± 25

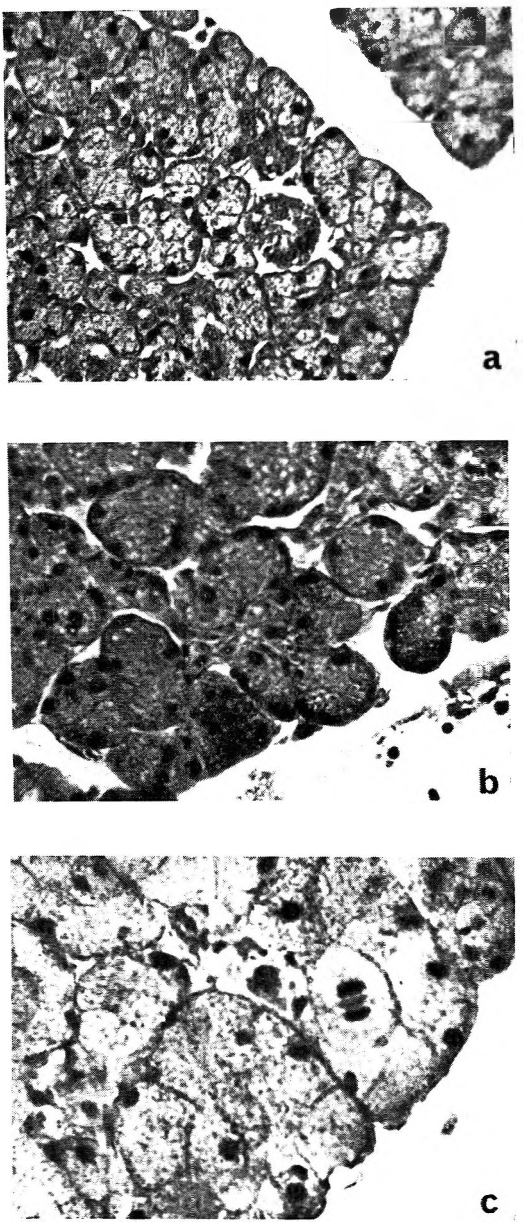

FIG. 1. Histological pattern of parotid glands from control rats (a), rats treated with physalaemin (b) and rats treated with isoprenaline (c).

weights of salivary glands which was statistically significant ($P < 0.02$) with the low dose of the peptide but very slight (4%) and non-significant ($P > 0.4$) with the dose of 100 μg/kg. Isoprenaline caused the usual striking enlargement of the glands already observed by many authors. The increase in weight of the gland was less pronounced, though evident, in the rats receiving the higher dose of the amine.

Histological examination showed differences between glands from rats treated with physalaemin and those from rats treated with isoprenaline. With physalaemin treatment hypertrophy was the major change; increase in cell size was moderate but enough to account for the increase observed in gland weight. Areas of necrosis were never seen nor were mitotic aspects visible (Fig. 1b). With isoprenaline there was not only an extraordinary increase in cell size but also some hyperplasia (Fig. 1c) as shown by the presence of numerous mitoses and of several nuclei in the phase of spirema.

On gross and histological examination the parenchymatous organs of rats treated with physalaemin were practically normal. The fresh weights did not differ from those obtained from the equivalent tissues of control animals (Table 3).

TABLE 3. FRESH WEIGHT OF DIFFERENT PARENCHYMATOUS ORGANS OF RATS AFTER TREATMENT WITH PHYSALAEMIN OR ISOPRENALINE

Treatment		Fresh weight in g per 100 g rat ± s.e.				
Drug	Dose	Brain	Lungs	Liver	Spleen	Kidneys
None	—	0·94 ± 0·2	0·78 ± 0·03	4·51 ± 0·26	0·36 ± 0·02	0·90 ± 0·04
Physalaemin ..	10 µg/kg	0·99 ± 0·07	0·76 ± 0·05	4·23 ± 0·15	0·54 ± 0·09	0·94 ± 0·05
Physalaemin ..	100 µg/kg	1·07 ± 0·14	0·90 ± 0·09	4·06 ± 0·2	0·49 ± 0·08	1 ± 0·1
Isoprenaline ..	50 mg/kg	0·98 ± 0·03	0·86 ± 0·07	5·53 ± 0·34	0·55 ± 0·13	0·97 ± 0·04
Isoprenaline ..	400 mg/kg	0·85 ± 0·05	0·74 ± 0·02	5·15 ± 0·19	0·45 ± 0·04	0·83 ± 0·03

Discussion

Physalaemin, 10 μg/kg, selectively increased the weights of salivary glands in rats, whereas a higher dose (100 μg/kg) did not.

Isoprenaline was much more effective and again the effect was more striking with the lower dose, probably due to a toxic action in the larger dose (Pohto & Paasonen, 1964). Indeed, in our experiments rats treated with 400 mg/kg of isoprenaline showed little increase in weight compared with controls. With physalaemin it is more difficult to explain the lack of effect of the higher dose of the peptide and in fact the total increase in body weight and the conditions of the organs examined were quite satisfactory.

The dose of isoprenaline (400 mg/kg) was that used by Selye & others (1961) to cause evident enlargement of the glands. Bertaccini & De Caro (1965) found in acute experiments that physalaemin was 100,000 times more potent in its salivatory effects on the rat than isoprenaline, hence the large difference in dosage.

The fact that there was also an increase in dry weight after physalaemin (10 μg/kg), suggested that there was a real increase in non-aqueous

cellular material. Histological examination also showed a moderate cellular hypertrophy. Thus physalaemin as a potent sialagogue could be expected to cause a hypertrophy from hyperfunction. In contrast the hyperplasia observed after isoprenaline is probably due to a powerful and selective stimulation of salivary gland growth (Selye & others, 1961).

The sialogenous action of physalaemin, which is even more striking in the acute experiment (Bertaccini & De Caro, 1965) must be regarded as only a side-effect. when compared with the hypotensive action of the peptide (threshold hypotensive dose 10–30 times lower than threshold salivatory dose in rats and over 1000 times lower in dogs).

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Central nervous system effects of four β-adrenergic receptor blocking agents

SIR,—Propranolol has been shown to possess marked central nervous system depressant and anticonvulsant properties. Leszkovszky (1965) suggested that these effects may be due to the presence of a naphthyl group in its molecule. We now report an investigation in which the CNS effects of three β-receptors blockers having a naphthyl group are compared to those effects produced by a highly specific β-adrenergic blocking agent (Somani & Lum, 1965) with different chemical structure, the 2-isopropylamino-1-(*p*-nitrophenyl)ethanol (INPEA). With the aim of separating CNS effects eventually related to β-inhibition from those due to independent pharmacological properties, the pure optical isomers of INPEA, the absolute configuration of which we recently determined chemically (to be published), and of which only the D(−) form has β-blocking properties, were used.

Adult NMRI mice of either sex were used. Median lethal doses were estimated by the subcutaneous route. All tests were run for 15 min after s.c. administration of drug. The convulsants or hexobarbitone were injected intravenously at the rate of 0·01 ml/sec. Hexobarbitone sodium toxicity was determined in mice pretreated with 0·2 LD50 of the drug to be tested. Ataxia was evaluated by the ability of mice to remain for 3 min on a rotating rod (7 rpm). Fighting behaviour was induced in male mice by footshock at 2 mA, 100 V stimulus intensity of 1 msec duration, and 1 shock/sec. The number of attacks in a 3 min test period subsequent to treatment with 0·2 LD50 of the agent under examination was registered and expressed as a percentage of the number of attacks observed in controls. Strychnine antagonism and nicotine antagonism were determined in mice challenged with a dose of strychnine sulphate (0·76 mg/kg), or nicotine hydrogen tartrate (2·70 mg/kg), which in controls proved to be lethal to 95–97% of the mice within 10 min; survival was considered to be a sign of protection. Leptazol antagonism was determined in animals treated with a lethal dose of the convulsant (45·0 mg/kg); inhibition of the tonic extensor phase of the hind legs was considered to be protection. Maximal electroshock seizures were elicited through cornea

TABLE 1. CNS EFFECTS OF 4 DIFFERENT β-ADRENERGIC BLOCKING AGENTS

Test	Results expressed in mg/kg	Propranolol	Pronethalol	Idrobutamine (1)	D-(−)-INPEA	L-(+)-INPEA
Toxicity, s.c.	LD50*	167·5 (187·3–149·8)	424·0 (534·2–336·5)	81·0 (94·8–69·2)	400·0 (457·6–349·7)	296·0 (327·0–267·9)
Hexobarbitone toxicity 65·6 (80·4–53·6)	LD50*	30·4 (37·4–24·7)	63·0 (74·3–53·4)	101·5 (113·9–90·5)	91·9 (101·6–83·2)	124·5 (140·1–110·7)
Effects on fighting behaviour	% of controls	31·5	2·3	124·7	115·1	98·3
Ataxia	ED50*	21·9 (29·9–16·0)	26·2 (48·0–14·3)	45·3 (50·2–40·9)	92·5 (113·6–75·3)	90·0 (125·9–64·3)
Duration of ataxia at 0·2 LD50	ET50†	138·5 (154·7–124·0)	20·6 (24·5–17·3)	0	0	0
Antagonism of tonic leptazol seizures	ED50*	18·9 (21·8–16·3)	23·6 (28·9–19·3)	>40<50	0	0
Antagonism of tonic electroshock seizures	ED50*	13·9 (18·4–10·5)	19·0 (26·0–13·9)	43·8 (48·7–39·3)	0	0
Antagonism of nicotine toxicity	ED50*	1·04 (2·06–0·52)	1·90 (3·75–0·96)	3·36 (7·39–1·53)	0	0
Antagonism of strychnine toxicity	ED50*	>20<30	>100<150	>40<60	0	0

(1) (Clin. Terap., **33**, 523 (1955).
* Results calculated from experimental data by the method of Litchfield & Wilcoxon (1949).
† Results calculated from experimental data by the method of Litchfield (1949).

electrodes at 100 V, using a pulse rate of 150/sec, and a pulse width of 0·5 msec, for 0·3 sec; abolition of the tonic extensor seizures of the hind legs was used as a criterion of protection.

Table 1 shows the results and it will be seen that given in non-toxic doses, propranolol and pronethalol have CNS-depressant properties. In fact, a direct depressant action on the CNS adequately explains the reduced fighting behaviour produced by both agents as well as the increase in acute hexobarbitone toxicity caused by propranolol. INPEA or 2-s-butylamino-1-(5,6,7,8-tetrahydro-2-naphthyl)ethanol hydrochloride (idrobutamine) on the other hand, evoke some central excitant effects in these tests. Propranolol, pronethalol and idrobutamine are capable of preventing death from strychnine-, nicotine-, or leptazol-induced convulsions and in modifying the pattern of maximal (tonic-clonic) electroshock convulsions. The compounds can be ranked in the following approximate order of decreasing activity: pronethalol, propranolol, idrobutamina. Protection against leptazol toxicity is not effected by elevation of the threshold for convulsion seizures nor so much by modification of the pattern of maximal (tonic-clonic) seizures induced by the convulsant, but rather by preventing death that normally occurs after repeated tonic episodes. Protection from nicotine toxicity is produced by prevention of the terminal convulsions; the typical tremors produced by the central action of nicotine are not abolished and the antagonistic action appears to be unrelated to sedation since non-sedative doses are highly effective. The protective effects of the compounds on spinal cord (strychnine-poisoning) are compatible with those on higher centres, but lower doses are effective centrally. Just as with CNS depression, the anticonvulsant properties possessed by propranolol, pronethalol and, to a much lesser degree idrobutamine, are not shared by INPEA which, on the contrary, causes some measure of CNS stimulation in these tests.

Thus our experimental analysis of the CNS effects of this series of β-blocking agents reveals striking differences between propranolol, pronethalol, and idrobutamine on the one hand, and INPEA on the other. By a process of exclusion it can therefore be concluded that the depressant action on the CNS or the anticonvulsant properties of the former agents, or both, may well be related to their particular chemical structure, but β-receptor blockade is not involved in these actions. Similar considerations apply to the CNS effects caused by INPEA. A complete dissociation of CNS stimulation from β-adrenergic receptor blockade is emphasised by the fact that the adrenergically inactive L-(+)-isomer is about equally active in causing CNS stimulation as is the β-adrenergic receptor blocker D-(−)-INPEA. Moreover, because of the much lower doses required to produce β-receptor blockade, there is some evidence that even the central excitatory effects of D-(−)-INPEA are unrelated to β-adrenergic blockade.

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Effect of high pressure oxygen on the duration of anaesthesia in mice

SIR,—Anaesthesia modifies the toxicity of hyperbaric oxygen (Bean, 1945). Conversely, hyperbaric oxygen may be expected to modify the action of anaesthetics, but no such experiments appear to have been reported. The only reference to such an action is that by Bean (1931) who stated that high pressure oxygen lightens anaesthesia, and that higher doses of an agent are required to provide anaesthesia in experimental animals exposed to high pressure oxygen for prolonged periods. We had also observed that animals under high pressure oxygen seemed less deeply anaesthetised than animals similarly anaesthetised in air. The present work provided quantitative data to support these impressions. Further impetus was given to this study by the finding that the biochemical action of amylobarbitone in blocking electron transport reactions *in vivo* can be reversed, at least in part, by hyperbaric oxygenation (Chance, Jamieson & Williamson, 1966).

Sleeping times in mice anaesthetised with pentobarbitone sodium were measured as a test for anaesthetic duration. The six-compartment pressure chamber has been described previously (Jamieson & van den Brenk, 1963). Twelve male albino mice, 22–27 g, were injected intraperitoneally with pentobarbitone sodium for each experiment. Each mouse was weighed and dose prescribed per unit weight. The total time taken to inject each group of mice was approximately 3·5 min. Each of the six containers inserted in the pressure vessels was centrally partitioned to house each mouse separately. Alternate cages were pressurised, the remainder being maintained under ambient air conditions as controls. Chambers were pressurised at a rate of 1·3 atmospheres per min, a maximum time of 8 min elapsing between injection and attaining the required pressure. Oxygen flowed continually through the pressurised

TABLE 1. THE EFFECT OF HYPERBARIC OXYGEN [ATMOSPHERES ABSOLUTE (ATA)] ON THE DURATION OF PENTOBARBITONE SODIUM ANAESTHESIA IN MICE

Dose pentobarbitone Na (no. of animals)		Treatment	Awakening time min ± s.e.	P value
35 mg/kg	(16)	air	46·5 ± 3·8	<·001
,,	(17)	6 ATA O_2	27·2 ± 1·3	
,,	(18)	air	40·6 ± 3·3	<·001
,,	(17)	5 ATA O_2	24·7 ± 1·4	
,,	(18)	air	37·9 ± 2·6	<·001
,,	(18)	4 ATA O_2	25·7 ± 1·2	
,,	(18)	air	35·5 ± 2·2	N.S.
,,	(18)	3 ATA O_2	31·3 ± 2·1	
50 mg/kg	(18)	air	64·1 ± 2·0	<·02
,,	(18)	3 ATA O_2	56·7 ± 1·5	

TABLE 2. CONVULSIVE TIMES IN MICE EXPOSED TO HYPERBARIC OXYGEN [ATMOSPHERES ABSOLUTE (ATA)]

Treatment (no. of animals)	Time to convulsion min ± s.e.
6 ATA O_2 (12)	10 ± 1
5 ATA O_2 (12)	30 ± 2
4 ATA O_2 (12)	45 ± 2
3 ATA O_2 (12)	(no convulsions)

chambers at a rate of 1 litre/min to maintain gas tensions and temperatures constant. The chamber temperatures were monitored individually and ranged from 19° to 24° in different experiments, a variation of ±0·5° in any one experiment. Three trials were made at each pressure and the results pooled (Table 1). The "awakening time" was the duration between time of injection and the time when the animal could stand, grip the grate in the cage, and attempt to walk. The induction of hypnosis failed in a very small number of animals in both control and pressurised groups and these mice have been excluded from the results. All other animals lost their righting reflex within 5–7 min after injection.

In preliminary experiments a dose of pentobarbitone sodium of 35 mg/kg gave satisfactory sleeping times for animals compressed to 4–6 atmospheres absolute, in that the mice awoke before severe signs of oxygen toxicity (largely based on pulmonary damage) had occurred. Pressures above 6 atmospheres were not used since pulmonary toxicity occurs too rapidly at this pressure.

The difference in duration of anaesthesia between animals breathing ambient air and oxygen at 4, 5 and 6 atmospheres respectively was highly significant (Table 1). There was a slight gradation in effect for this pressure range. At 3 atmospheres, a dose of 35 mg/kg pentobarbitone sodium produced a slight but not statistically significant difference in length of sleeping time. If the dose was increased to 50 mg/kg to prolong sleeping times and correspondingly increase the duration of exposure to 3 atmospheres oxygen, a slight but significant reversal of anaesthetic effect occurred (Table 1).

There was no clear correlation of convulsive times in unanaesthetised mice (Table 2) and duration of anaesthesia under hyperbaric oxygen, the dose-effect relationship being much steeper for oxygen convulsions than for reversal of anaesthesia in relation to oxygen pressure.

It is known that barbiturate anaesthesia can protect against convulsions and pulmonary damage produced in oxygen toxicity (Bean, 1945; Jamieson & van den Brenk, 1962), and can also potentiate a spastic motor paralysis resulting from hyperbaric oxygen (van den Brenk & Jamieson, 1964). It has now been shown that hyperbaric oxygen decreased the anaesthetic effect of barbiturate drugs.

Although the results do not exclude the possibility of chemical or metabolic inactivation of the barbiturate *in vivo* due to high pressure oxygen, it seems most likely that hyperbaric oxygen is able to directly reduce the action of barbiturates on the central nervous system by in itself altering the sensitivity of cells to the anaesthetic agent.

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Effect of reserpine on the histaminolytic activity of guinea-pig liver

SIR,—Sachdev, Aiman & Rajapurkar (1961) showed that serpentine, but not reserpine, inhibited histaminase. Serpentine was shown to potentiate the response of guinea-pig ileum, uterus and trachea to histamine in doses similar to that of aminoguanidine used by Arunlakshana, Mongar & Schild (1954). It also inhibited the action of a preparation of histaminase (Torantil).

Gaitonde, Satoskar & Mandrekar (1960) showed that injection of 10 μg of reserpine into the lateral ventricle of cats produced an inhibition of gastric acidity, while intravenously 50 μg reserpine increased gastric acidity as well as blood histamine levels. Gaitonde & Shaligram (1960) administered 500 μg of reserpine intravenously in cats under chloralose anaesthesia and showed an increase in gastric acidity from 1·06 m-equiv. to 2·77 m-equiv. (213%). The blood histamine was increased from 0·053 μg/ml to 0·183 μg/ml. Sachdev, Dave & Panjwani (1965) found that urine of guinea-pig and man given reserpine, potentiated the response of the guinea-pig ileum to histamine. Suspecting antihistaminase activity in some metabolite produced during reserpine therapy, they found that methyl reserpate, a well known metabolite of reserpine, produced a moderate inhibition of the histaminolytic activity of guinea-pig liver. The effect of reserpine administration in the usual therapeutic doses on the histaminolytic activity of guinea-pig liver has therefore been examined.

Three groups of 3 adult guinea-pigs each were fed with 0·1 mg/kg reserpine daily, for 1, 2 and 3 weeks respectively. One group was kept as a control. 3 more animals were fed with 1, 5 and 10 mg/kg of aminoguanidine respectively, for 3 days. Histaminolytic activity was estimated in the supernatant of a homogenised and centrifuged preparation of liver, taken immediately after death (Spencer, 1963). A known amount of histamine was added and the preparation incubated in a Warburg apparatus at 37°. The histamine content of the incubates at 0, 10, 20, 40, 80 min was assayed, after boiling, on the ileum of guinea-pig treated with atropine.

The time in which 50% histamine was destroyed (DT50) was calculated from the graph obtained. The average DT50 in the 3 control animals was 41 min, s.d. $\pm$ 11. This was reduced to 24 min $\pm$4 after one week of administration of reserpine, indicating an increased histaminolytic activity. However the difference was statistically insignificant. The average DT50 after 2 and 3 weeks administration of reserpine was 58 $\pm$ 14 and 76 $\pm$ 10 respectively. The number of animals in each group was too small to permit a reliable estimate of change in histaminolytic activity until after 3 week treatment when a statistically significant decrease was observed $t = 3{\cdot}948$; $P = 0{\cdot}05$. Aminoguanidine, 5 and 10 mg/kg depressed the activity significantly, but 1 mg/kg did not produce any effect.

The slight initial increase in the histaminolytic activity may have been due to adaptive induction of the enzyme by histamine or by some metabolite of reserpine. Southren, Kobayashi, Levine & Sherman (1965) have, however, shown that subcutaneous administration of 0·3 to 1·2 mg of histamine daily for 45–48 days, in women, did not affect the plasma diamine oxidase levels.

Inhibition of the histaminolytic activity of liver during reserpine administration may explain hyperchlorhydria induced by reserpine. Six cats were anaesthetised with 80 mg/kg of chloralose and given 0·25 to 0·5 mg/kg of reserpine intravenously or 0·25 to 2 mg/kg methyl reserpate; this treatment induced a prolonged increase in gastric secretion and acidity. Reserpine was more powerful and produced a 3 to 4 times increase which lasted for 4–5 hr.

Prior administration of SKF 525–A markedly inhibited the hyperchlorhydria produced by reserpine, indicating that hyperchlorhydria was probably due to a metabolite.

Efforts to isolate from the urine of animals treated with reserpine the metabolite responsible for potentiating the histamine response, have so far been unsuccessful.

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In vivo inhibition of ^{3}H-noradrenaline uptake by mouse brain slices *in vitro*

SIR,—Pretreatment of animals with reserpine decreases the capacity of tissues to accumulate tritiated noradrenaline *in vitro* (Dengler, Spiegel & Titus, 1961a; Ross & Renyi, 1966). Several other psychoactive compounds added to brain slices *in vitro* are known to inhibit the uptake of noradrenaline (Dengler, Spiegel & Titus, 1961b). However, pretreatment of mice with these compounds does not seem to decrease the noradrenaline uptake by subsequently prepared brain slices. In contrast to reserpine, most of these compounds seem to act reversibly and are probably loosely bound to the tissues. The tissue contents of these substances may therefore decrease during the *in vitro* incubation procedure by diffusion of the compounds into the incubation medium. We have tried to avoid this diffusion by using a briefer incubation period than was used in the aerlier experiments.

Mice were injected intraperitoneally with the compounds and they were killed at the time noted in Table 1. The incubation of the brain cortex slices with the tritiated noradrenaline and the extraction of the amine taken up was as previously described (Ross & Renyi, 1964), with the exception that the incubation time was only 5 min. Four animals were used for each compound. The content of the amine in the slices was expressed as nmol/g. The statistical significance was calculated according to the Student's *t*-test.

The results obtained are presented in Table 1. Compounds supposed to inhibit the noradrenaline uptake at the cell membrane level (Carlsson & Waldeck, 1965), namely, desipramine, imipramine or amitriptyline, were strong inhibitors of the uptake of tritiated noradrenaline under the conditions used. But cocaine, which when added *in vitro* is a powerful inhibitor of the noradrenaline uptake by brain slices, had only a slight effect when injected *in vivo*. This finding would seem to suggest that too small amounts of cocaine reach the mouse brain *in vivo*.

The large dose of chlorpromazine strongly inhibited the noradrenaline uptake, but the smaller dose had no effect although the animals were strongly tranquillised. This result may indicate that the phenothiazine class of tranquillisers

has the same inhibitory effect on the noradrenaline uptake as have the antidepressive agents mentioned above, but that this effect is pharmacologically masked by their more potent tranquillising action (cf. Bickel, Sulser & Brodie, 1963).

(±)-Amphetamine in a high dose which produced behavioural stimulation in mice also had a strong inhibitory action on the amine uptake in cortical slices. The lower, pharmacologically inactive dose, however, had no effect on the uptake. Whether there is any relation between the stimulatory action and the inhibition of uptake of amine by amphetamine remains to be elucidated.

The pretreatment of the mice with reserpine or tetrabenazine caused only a slight, insignificant decrease in the capacity of the tissue to accumulate noradrenaline under the brief incubation condition used in contrast to that found with longer incubations (Ross & Renyi, 1966). This result is in agreement with the view that reserpine blocks the granular storage mechanism for noradrenaline (Hillarp & Malmfors, 1963).

TABLE 1. EFFECT OF PRETREATMENT OF MICE WITH SOME PSYCHOACTIVE COMPOUNDS ON THE NORADRENALINE UPTAKE BY BRAIN CORTEX SLICES *IN VITRO*

Compound	Dose mg/kg i.p.	Time after injection hr	Noradrenaline uptake nmol/g ± s.e.
Control	—	—	0·117 ± 0·004 (n = 15)
Desipramine HCl	10	0·5	0·094 ± 0·007*
	10	1	0·086 ± 0·008†
	10	2	0·097 ± 0·004†
	10	4	0·110 ± 0·012
	2	1	0·097 ± 0·004†
Imipramine	20	1	0·091 ± 0·007‡
Amitriptyline	20	1	0·088 ± 0·003‡
Cocaine HCl	20	0·5	0·107 ± 0·002*
	20	1	0·123 ± 0·006
Chlorpromazine HCl	20	1	0·072 ± 0·007‡
	5	1	0·127 ± 0·002
(±)-Amphetamine sulphate	10	1	0·068 ± 0·009‡
	2·5	1	0·119 ± 0·003
Reserpine	5	18	0·104 ± 0·006
Tetrabenazine HCl	60	1	0·106 ± 0·005
Haloperidol	2·5	1	0·124 ± 0·003
Chlordiazepoxide	20	1	0·125 ± 0·004

* 0·05 > P > 0·01 † 0·01 > P > 0·001 ‡ P < 0·001

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The limit of sensitivity of bioassay of catecholamines on the perfused arterial segment

SIR,—The method of bioassay of catecholamines described by de la Lande & Harvey (1965) involved the injection of a solution of the catecholamine into Krebs bicarbonate solution with which a segment of the central artery of the ear of the rabbit was perfused. The volumes for injection must be small (0·4 ml or less) and approximately equal when comparisons are made between test and unknown solutions. These requirements can be avoided and a gain in sensitivity of some five fold achieved by perfusing the preparation with the solutions.

In each of six experiments, we have observed that the increase in perfusion pressure in response to an infusion of noradrenaline is sustained and is concentration dependent. The sensitivity is indicated by the average responses to noradrenaline, 0·5 ng/ml, in the above six experiments which were 5, 5, 10, 15, 19 and 20 mm of mercury respectively. The procedure does, however, raise a problem by requiring large volumes of test solution for infusion. This difficulty can be minimised by infusing, for brief periods only, i.e. 10–15 sec; this enables volumes of 1·0–1·5 ml to be tested. The response obtained is then not maximal but is reproducible and dose dependent (Fig. 1).

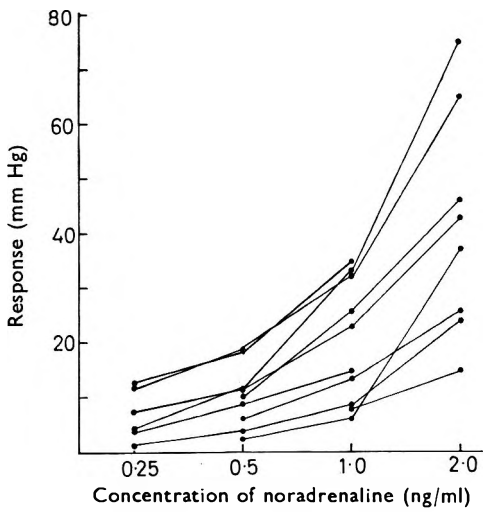

FIG. 1. The increases in perfusion pressure in response to 15 sec infusions of noradrenaline in ten arteries.

Brief infusions are given by stopping the perfusion pump during the short intervals (1–2 sec) required for the transfer of the outlet tubing between the reservoir of Krebs solution and the test solution (warmed at 37°) in a second reservoir. A dead space of 3–5 ml in the infusion system between reservoirs and preparation ensures that the record of the response to the catecholamine is unaffected by the preceding transient drops in pressure caused by brief interruptions to the perfusion.

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The effect of ascorbic acid on anaphylactic shock in dogs

SIR,—Many controversial reports about the effect of ascorbic acid on anaphylactic shock in different species are found in the literature. Recently, Dawson & West (1965a) showed that large doses, given just before the antigen challenge, protect guinea-pigs or rats from anaphylactic shock. In another paper, Dawson & West (1965b) reported that the protective action of ascorbic acid followed a direct effect on the bronchial muscle. The effect of ascorbic acid on anaphylactic shock in dogs has now been investigated.

Twenty-two dogs, 6·5 to 11·5 kg, were sensitized with horse serum (6 ml subcutaneously 5 times at 3-day intervals). About 22 days later, they were anaesthetised with intravenous chloralose (110 mg/kg) and injected intravenously with 20 ml of horse serum. Blood pressure recordings were made from the right carotid artery with a mercury manometer. Four of the dogs had no pre-treatment and served as controls. The sudden and excessive fall in blood pressure with congestion in the liver occurred immediately after the challenge with antigen in these four animals. Groups of 3 of the other 18 dogs were injected intravenously with ascorbic acid (500 mg/kg) either immediately or at 5, 10, 15, 20 or 30 min before the antigen challenge. This dose of ascorbic acid had no effect on anaphylactic shock, the blood pressure in all animals falling from about 110 to about 30 mm Hg, and most of them (85%) dying within 1 hr of challenge. Blood samples taken before and after anaphylactic shock were assayed for their histamine contents (Csaba, Szilagyi, Damjamovich & Kover, 1963), but no change was found in the amount of histamine liberated in anaphylaxis (controls increased about 50-fold whereas ascorbic acid-treated animals showed mean increases of about 41-fold).

Thus, ascorbic acid given before the antigen challenge in dogs has no protective action against anaphylactic shock and does not influence histamine release. The shock organ in dogs is the liver and not the lung as in guinea-pigs. The inability of ascorbic acid to influence the reaction in the dog thus adds weight to the hypothesis that ascorbic acid directly inhibits the bronchospasm in guinea-pigs and does not act solely through the adrenal system as some workers [for example, Guirgis (1965)] have suggested.

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Adrenergic mediation in the antagonism between desipramine and reserpine

SIR,—It is well known that tricyclic antidepressant drugs, including desipramine, counteract the hypothermia induced by reserpine (Garattini, Giachetti, Jori, Pieri & Valzelli, 1962; Askew, 1963) through an effect which is probably of central origin (Bernardi, Jori, Morselli, Valzelli & Garattini, 1966). It was also reported that desipramine potentiates the hyperthermia (Jori & Garattini, 1965) and other pharmacological responses elicited by noradrenaline (Sigg, 1959; Thoenen, Huerlimann & Haefely, 1964; Bonaccorsi, 1966; Hrdina & Garattini, 1966), probably as a result of the inhibition of the catecholamine uptake at the nerve endings (Hertting, Axelrod, Whitby & Patrick, 1961; Iversen, 1965).

This allowed the suggestion that desipramine increases the relative concentration of noradrenaline at the receptor sites and that this effect is a mechanism by which desipramine antagonises the reserpine syndrome (Matussek, Rüther & Titus, 1964; Sulser, Bickel & Brodie, 1964; Jori, Paglialunga & Garattini, 1966).

We report some preliminary experiments designed to test if the effect of desipramine on reserpine-hypothermia could be interpreted as an interaction of desipramine with the adrenergic system.

Sprague-Dawley rats were injected intravenously with reserpine (2·5 mg/kg) and kept in Makrolon cages at an environmental temperature of 20°. 16 hr later desipramine was given intravenously at a dose of 1·5 mg/kg. Adrenergic blocking agents were injected intraperitoneally 30 min before desipramine.

When desipramine was given, rats were placed in individual cages to record the body temperature continuously during 2 hr automatically (Jori & Paglialunga, 1966).

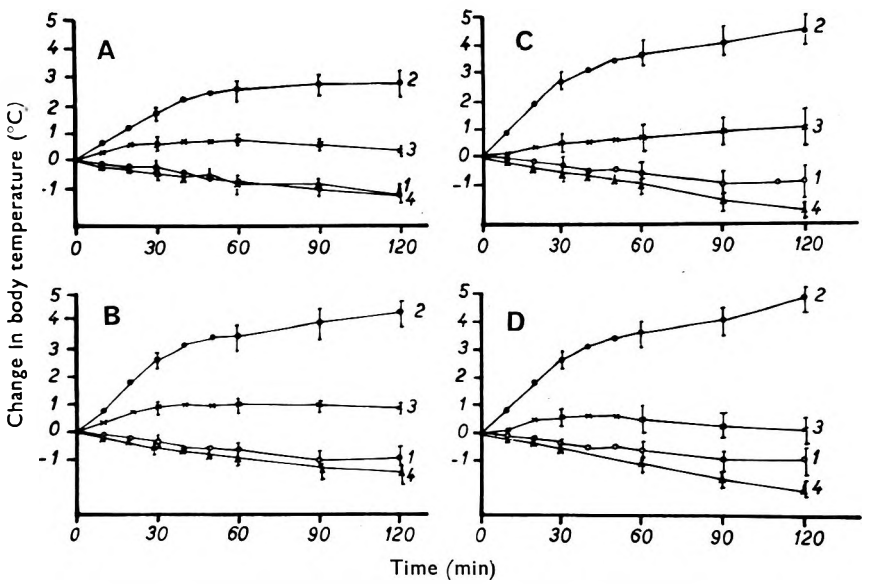

FIG. 1. Changes of body temperature in rats treated with reserpine (2·5 mg/kg i.v.) 16 hr before test. At zero time, the animals (average temperature 30 ± 1°) received saline (curves 1/A; 1/B; 1/C; 1/D) or desipramine 1·5 mg/kg i.v. (curves 2/A; 2/B; 2/C; 2/D) or phentolamine plus desipramine 2·5 mg/kg i.p. (3/A), phenoxybenzamine plus desipramine 10 mg/kg i.p. (3/B); pronethalol 15 mg/kg i.p. (3/C), desipramine plus propranolol 10 mg/kg i.p. (3/D) or phentholamine (4/A), phenoxybenzamine (4/B), pronethalol (4/C), propranolol (4/D).

The results in Fig. 1 show that the adrenergic blocking agents tested—phenoxybenzamine, phentolamine, pronethalol and propranolol—inhibit the hyperthermic effect induced by desipramine in fully reserpinised rats.

Other experiments we have made show that an infusion of noradrenaline induces a significant increase of body temperature in rats made hypothermic by reserpine.

Since the synthesis of noradrenaline is not impaired by reserpine (Hillarp & Malmors, 1964) our results are compatible with the hypothesis that desipramine increases body temperature in reserpinised animals by increasing the concentration of free noradrenaline at the receptor sites because of the inhibition of noradrenaline uptake.

This mechanism may be also relevant to the explanation of the clinical antidepressant activity of imipramine-like drugs.

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Book Review

CHEMICAL ASPECTS OF THE AUTONOMIC NERVOUS SYSTEM. By D. J. Triggle. Pp. ix + 329 (including index). Academic Press, London and New York, 1965. 75s.

In Chapters I, II and III (51 pages in all) Dr. Triggle sets out what he considers chemists should know about the workings of the autonomic nervous system and the peripheral connections of striated muscle. In Chapter IV (23 pages) he discusses, in general terms, the interaction of drugs with receptors. The next part of the book (97 pages) is devoted to cholinergic synapses; Chapter V deals with compounds believed to have a presynaptic action at the neuromuscular junction, Chapter VI with compounds which are agonists at acetylcholine receptors, Chapter VII classifies antagonists of acetylcholine, which

are then discussed in Chapter VIII ("muscarinic receptors"), Chapter IX (receptors in ganglia) and Chapter X (receptors at the neuromuscular junction). Chapter XI is a brief account of compounds which inhibit the synthesis of acetylcholine and in Chapter XII Dr. Triggle speculates about the structure of acetylcholine receptors.

The last section (144 pages) is concerned with adrenergic receptors; the possible cholinergic link in transmission (Chapter XIII), the activity of agonists (Chapter XIV), uptake, storage and release of transmitter (Chapter XV). compounds which affect these processes (Chapter XVI), reversible adrenergic blocking agents (Chapter XVII), irreversible blocking agents (Chapter XVIII), synthesis of catecholamines and inhibitors of synthesis (Chapter XIX), metabolism and inhibitors of metabolism (Chapter XX), speculations about the adrenergic receptor (Chapter XXI) and some general remarks (Chapter XXII).

The choice of material clearly reflects Dr. Triggle's own interests and what he has had to learn as a chemist working on biological problems. In the adrenergic section this makes for some very stimulating reading (especially Chapter XVIII) but in other sections it leads to a slightly unbalanced presentation. For example, considerable emphasis is placed on the presynaptic actions of compounds such as neostigmine (Chapter V) but no account is given of substrates and inhibitors of cholinesterases, although a picture of the active site of acetylcholinesterase appears in the section on the cholinergic receptor (Chapter XII). Again, the account of drug-receptor interaction in Chapter IV is presented almost exclusively from the viewpoint of Ariëns and van Rossum, with which Stephenson's ideas have been, quite incorrectly, equated.

The provision of an adequate biological background for chemists is always a problem with this kind of book. Dr. Triggle has given a full account and chemists who have done no biology may find this fairly heavy going (although pharmacists with some knowledge of physiology may find it useful). At the same time, although the biological processes are discussed in detail, no account is given of the types of experiments which actually lead to estimates of activity and no indication is given of the confidence which can be placed in such figures. The Tables often require more explanation than is given (e.g. Table X.3, is headed "neuromuscular blocking activity", but lists results on the frog rectus, frog heart and cat-blood-pressure and seems to imply that these all indicate neuromuscular blocking activity).

Many of the Figures, too, have been borrowed and retain mystifying letters not referred to in the legend: this appears to have led to the muddling of Fig. III. 1a with Fig. III. 1b. A lack of patience with tedious details is also apparent in the index (which is extremely sketchy) and in incorrect spelling. One of the most unfortunate mistakes is the spelling of A. J. Clark, who appears as "Clarke" in the preface and on pages 52 and 53, as "Clark" on page 59, and as both on page 64.

The aim of the book is to interest chemists in the ways in which drugs affect tissues and to indicate to biologists the types of chemical process which may be involved. Dr. Triggle is clearly qualified to tackle both these problems and both chemists and biologists should find the book stimulating.

R. B. BARLOW

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