Brit. J. Pharmacol. (1954), **9**, 170.

DEPRESSION OF AUTONOMIC GANGLIA BY BARBITURATES

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(RECEIVED NOVEMBER 25, 1953)

It is widely known that the administration of the barbiturates may be followed by lowering of the arterial blood pressure, both in laboratory animals (Impens, 1912; Gruber and Roberts, 1926) and in man (Mason, Baker, and Pilcher, 1930; Emge and Hoffman, 1930).

This fall in blood pressure induced by barbiturates is generally ascribed to a depression of the hypothalamic centres (Leiter and Grinker, 1934; Masserman, 1937), and the possibility of simultaneous depression of the peripheral autonomic system seems to have received little consideration.

The present work arose from consideration of the possibility that depression of vasomotor ganglia might contribute substantially to the vasodepressor effect of these drugs. On the excised sympathetic ganglion, depressant effects of pentobarbitone have been investigated by Heinbecker and Bartley (1940), Larrabee, Ramos, and Bülbring (1950), and by Larrabee and Posternak (1952). Paralysis of the superior cervical ganglion of the cat, during perfusion with fluids containing a barbiturate, is described by Brücke, Macho, and Werner (1947).

In this paper an attempt has been made to establish the extent to which various barbiturates can depress conduction through mammalian sympathetic ganglia and to elucidate the nature of the mechanisms underlying this action.

METHODS

Cats, premedicated with atropine sulphate (1 mg./kg.) and anaesthetized with ether followed by intravenous chloralose (80 mg./kg.), were used in most of the experiments though spinal animals were sometimes used. Arterial blood pressure was recorded from the carotid or femoral artery by a mercury manometer.

Electrical stimulation of the cervical sympathetic was by the application of square-wave pulses (usually of 0.1 msec. duration) through shielded platinum electrodes; the frequency of such pulses will be indicated for each experiment. The contractions of the nictitating membrane were recorded in the usual way.

Intra-arterial injections close to the superior cervical ganglion, and photographic recordings of action and polarization potentials, were made by the technique of Paton and Perry (1953).

The superior cervical ganglion was perfused by the methods of Kibjakow (1933) and Feldberg and Gaddum (1934); the perfusion fluid was warmed by passing the terminal part of the polythene tubing up the animal's oesophagus (MacIntosh, unpublished). The acetylcholine content of the effluent was assayed on the blood pressure of eviscerated eserinized cats under chloralose anaesthesia (for details, see MacIntosh and Perry, 1950). The perfusing fluid was Locke's solution containing eserine (1 in 200,000) and double the usual quantity of glucose (2 g./l.).

The anaesthetic potencies of several of the barbiturates were determined by Butler's method (1942). Solutions of the sodium salts of the drugs were injected into the tail veins of albino mice. The anaesthetic doses for about 35% and for about 65% of mice, respectively, were found by trial and error. Fifty mice were then used to determine anaesthetic potency—25 being given one of these doses and 25 the other. The median anaesthetic dose (AD50) was estimated by interpolation on log/probability graph paper.

Solutions of sodium salts of the barbiturates were freshly made up before each experiment. Where the sodium salt was unobtainable the acidic form was dissolved in an appropriate quantity of 0.1 N-sodium hydroxide. Amylobarbitone sodium was chosen for all qualitative work and as the standard drug for purposes of comparison in the quantitative experiments.

RESULTS

Action of Barbiturates on Ganglia

Vasomotor Ganglia

That amylobarbitone depresses vasomotor ganglia is evident from the fact that the pressor effects of nicotine are diminished by the barbiturate, while those of adrenaline are not. This is illustrated in Fig. 1. Fig. 1a shows the pressor responses induced in an atropinized spinal cat by intravenous injections, at 3 min. intervals, of 0.4 mg. nicotine acid tartrate. At the point marked by the arrow, 10 mg. amylobarbitone sodium was given intravenously in the interval between two consecutive nicotine injections. The nicotine-

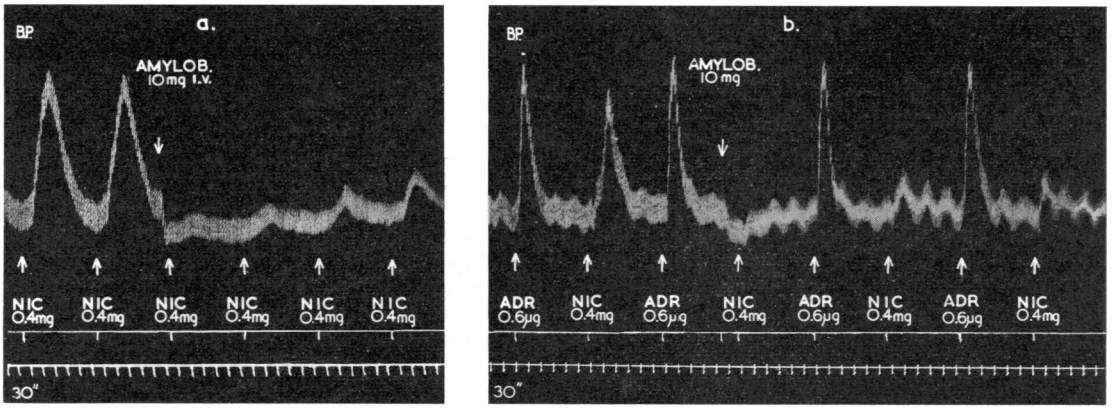

FIG. 1.—(*a*) Cat, 2.5 kg., spinal, atropinized. Blood pressure record. Pressor responses following i.v. injections of 0.4 mg. nicotine acid tartrate at 3 minute intervals. Upper arrow, 10 mg. amylobarbitone sodium i.v. (*b*) Cat, 2.7 kg., spinal, atropinized. Alternate i.v. injections of 0.6 µg. adrenaline and 0.4 mg. nicotine. Upper arrow, 10 mg. amylobarbitone sodium i.v.

induced responses were at first almost completely abolished, but later tended to recover.

That this depression of the nicotine response was not exercised on the smooth muscle of the arteriolar walls is apparent from Fig. 1b. Here, in another experiment, the pressor responses were induced by alternate injections of 0.4 mg. nicotine acid tartrate and 0.6 µg. adrenaline. Following the injection of 10 mg. amylobarbitone sodium, the nicotine pressor response was greatly reduced as before, while that of adrenaline was unaltered. Similar results were obtained with (–)-noradrenaline.

Superior Cervical Ganglion

Fig. 2 shows that amylobarbitone depressed the contraction of the nictitating membrane from electrical stimulation of the preganglionic fibres of the cervical sympathetic nerve, and that this action was exerted at the ganglion. An injection of 3 mg. tetraethylammonium iodide caused a reduction in the height of the contraction from electrical stimulation of the preganglionic fibres, but did not reduce the response to injected adrenaline. Exactly similar results followed the intravenous injection of 8 mg. amylobarbitone sodium—indicating that the site of the depressant action of the barbiturate was probably the ganglion.

During continuous preganglionic stimulation TEA intravenously produced temporary ganglion block with relaxation of the membrane, and amylobarbitone sodium intravenously gave a similar, though more prolonged, relaxation (Fig. 3a). An injection of 10 mg. amylobarbitone sodium given during postganglionic stimulation produced, however, no relaxation and, indeed, the contraction of the membrane appeared to be slightly increased (Fig. 3b). Thus amylobarbitone does not exert its effect by depressing conduction in the postganglionic nerve fibres.

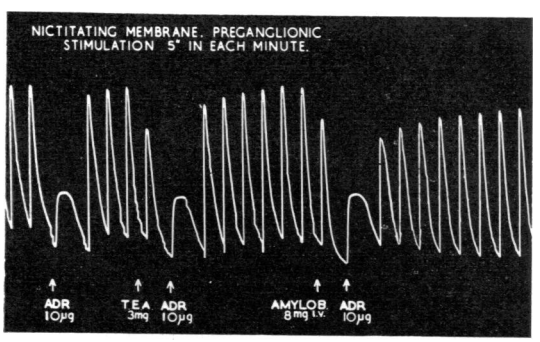

FIG. 2.—Cat, 3.0 kg., atropinized, chloralose. Nictitating membrane contractions. Periodic preganglionic stimulation (70 shocks/sec.) applied for 5 sec. in each minute. At first, third and fifth arrows the electrical stimulus was temporarily withheld and 10 µg. adrenaline given i.v. Second arrow, 3 mg. tetraethylammonium iodide i.v.; fourth arrow, 8 mg. amylobarbitone sodium i.v.

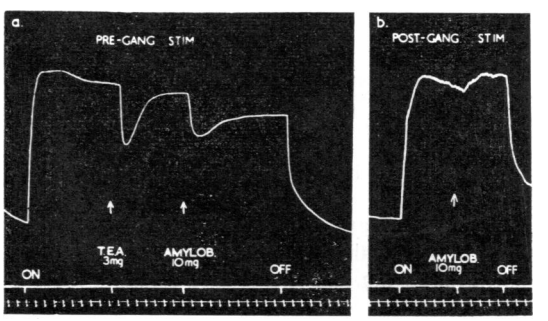

FIG. 3.—Cat, 2.6 kg., atropinized, chloralose. Nictitating membrane contraction. (*a*) Preganglionic stimulation, 10 shocks/sec. First arrow, 3 mg. tetraethylammonium iodide i.v.; second arrow, 10 mg. amylobarbitone sodium i.v. (*b*) Postganglionic stimulation, 10 shocks/sec. At arrow, amylobarbitone sodium, 10 mg. i.v.

That the preganglionic fibres are not involved in this depressant process was shown by the failure of amylobarbitone to alter or reduce the preganglionic action potentials, induced by stimulation of the preganglionic trunk low down in the neck and picked up from a point close to the lower pole of the ganglion.

It thus seems clear that amylobarbitone exerts its depressant action in, or very close to, the ganglion.

Effects of Larger Doses.—The ganglion-depressant effects of small doses of amylobarbitone appeared to be fairly transient, although cumulative effects following repeated doses became obvious by a gradual failure of the nictitating membrane contraction to return to its original level. Some experiments were accordingly done where large doses of amylobarbitone—comparable to those known to produce general anaesthesia in the cat—were used, and where the blood pressure changes were recorded. Fig. 4 illustrates such an

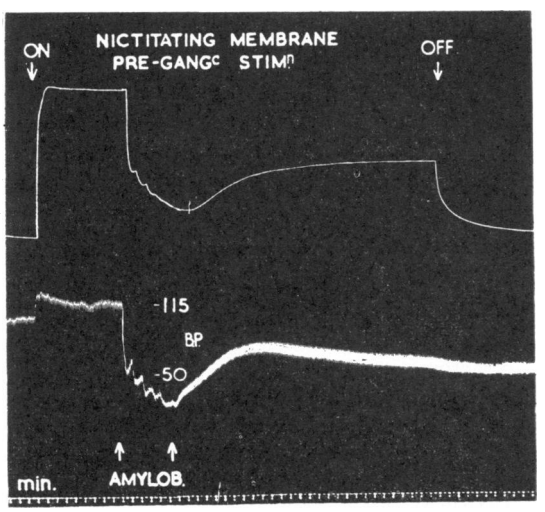

FIG. 4.—Cat, 2.5 kg., atropinized, chloralose, artificial respiration. Upper tracing, contraction of right nictitating membrane. First arrow, preganglionic stimulation commenced (10 shocks/sec.). Lower tracing, simultaneous recording of left femoral blood pressure. Lower arrow, slow i.v. injection of 50 mg./kg. amylobarbitone sodium (in 10 ml. saline). Time marker, 1 min.

experiment on an atropinized cat, anaesthetized with chloralose (70 mg./kg.) and maintained on artificial respiration to eliminate possible anoxaemia. When the blood pressure was about 115 mm. Hg, 50 mg./kg. amylobarbitone sodium—the dose recommended for intravenous anaesthesia in the cat (Garry, 1930)—was injected over a period of 5 min. This resulted in an immediate and profound reduction both in the blood pressure and in the nictitating membrane contraction. After half an hour the blood pressure had become stabilized at about 50 mm. Hg, while the contraction of the nictitating membrane had assumed a new level at about one-half its original height. Thus anaesthetic doses of amylobarbitone produce a marked and prolonged reduction in blood pressure, accompanied by a significant reduction in transmission through the sympathetic ganglion.

It is interesting to note, as was shown by similar experiments of this type, that chloralose in equivalent anaesthetic doses was almost devoid of such effects. This may well explain the particular suitability of chloralose as a laboratory anaesthetic, since it is likely to leave the efferent pathways of cardiovascular and other reflexes relatively intact.

Other Peripheral Actions of Amylobarbitone

As some earlier workers had described certain of the depressant effects of barbiturates as "atropine-like," this possibility was tested for on the cat blood pressure. The depressor effect of various doses of acetylcholine was not in the least impaired by an intravenous dose of 10 mg. amylobarbitone sodium.

Two preparations—the sciatic nerve-gastrocnemius in the spinal cat, and the isolated phrenic nerve-diaphragm of the rat—were used to determine whether amylobarbitone had any neuromuscular-blocking activity. No such activity could be demonstrated even with large doses.

Mechanism of Ganglionic Depression by Barbiturates

Depression of ganglionic transmission could result from (i) a reduction in excitability of the ganglion cells to acetylcholine, (ii) prolonged depolarization of the ganglion cells, (iii) interference with the release of acetylcholine by the preganglionic terminals, or (iv) a combination of two or more of these factors.

Excitability of Ganglion Cells.—The effect of amylobarbitone on the contractions of the nictitating membrane evoked by stimulation of the ganglion by intra-arterial injections of acetylcholine and potassium chloride was determined. The injections were made retrogradely into the cut stump of the external carotid artery (Paton and Perry, 1953) and, in Fig. 5, 4 mg. potassium chloride and 100 μg. acetylcholine were given alternately, each in 0.2 ml. isotonic saline. At the upper arrow 20 mg. amylobarbitone sodium was injected into the *femoral vein.* Responses of the nictitating membrane to acetylcholine were reduced, but those to potassium were augmented.

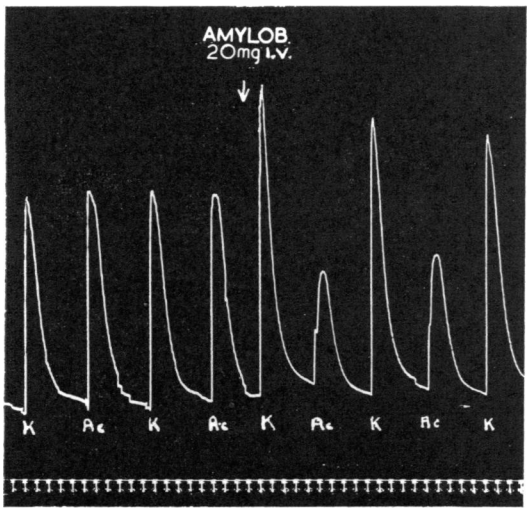

FIG. 5.—Cat, 3.8 kg., atropinized, chloralose. Contractions of the nictitating membrane. Retrograde injections into the cannulated external carotid artery of 4 mg. potassium chloride (K) and 100 μg. acetylcholine chloride (Ac), respectively. Upper arrow, 20 mg. amylobarbitone sodium i.v. Time in ½ min.

Thus, amylobarbitone acts, in part at least, by reducing the excitability of the ganglion cells to acetylcholine. The direct stimulant action of potassium ions is not similarly impaired and is, indeed, increased—perhaps by a temporary shift in the sodium-potassium balance following the sudden introduction of sodium ions with the injected barbiturate.

Polarization Changes in the Ganglion.—Since Paton and Perry (1953) have shown that certain ganglion-blocking agents produce maximal, or near maximal, depolarization of the ganglion cells, the possibility that amylobarbitone did so too was investigated. Fig. 6 shows four oscillograms from an experiment in which the technique of these workers was used.

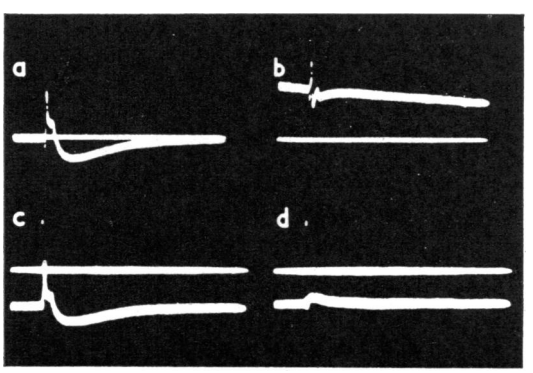

FIG. 6.—Oscillograms of ganglionic action potentials. Horizontal undeflected beam represents arbitrary reference line. Approximate duration of scan, 500 msec. (*a*) Normal action-potentials evoked by single preganglionic shock. (*b*) Effect of 100 μg. acetylcholine injected intra-arterially. (*c*) Record of normal potentials, showing stimulus artifact. (*d*) Effect of 2 mg. amylobarbitone injected intra-arterially.

In each instance the reference line was produced by one beam of the cathode-ray tube which was deflected by the horizontal time-base only. The other beam displayed amplified potentials picked up between two electrodes, one placed on the lower pole of the body of the ganglion and the other on the cut postganglionic trunk; the latter electrodes served as the "indifferent" electrode. Negativity of the ganglion with respect to the cut postganglionic trunk is represented by upward deflections of the beam.

Each oscillogram shows the brief action-potential complex initiated by a single shock applied to the preganglionic trunk. A depolarizing substance such as acetylcholine causes the whole beam to be shifted in a negative direction while the action potential complex from the preganglionic stimulus is partially suppressed (Fig. 6b). After the intra-arterial injection of 2 mg. amylobarbitone sodium, the negative spike potential and after-positivity are almost abolished (Fig. 6d), but there is no significant change in the polarization of the ganglion. Thus the ganglion-depressant action of the barbiturate is not contributed to by depolarization phenomena.

Release of Acetylcholine.—The superior cervical ganglion was perfused with eserinized Locke solution and the preganglionic trunk stimulated continuously at 10 shocks/sec. At a predetermined time after the commencement of stimulation a dose of 50 mg./kg. of amylobarbitone sodium in eserinized Locke was injected into the perfusion tubing. Samples of the effluent from the ganglion were collected at two-minute intervals and assayed for total acetylcholine content. The amylobarbitone in the effluent did not inactivate the acetylcholine nor did it exert "atropine-like" effects during the assay. Fig. 7 shows the results from a typical experiment. Amylobarbitone produced a marked interference with ganglionic transmission, but the graph of acetylcholine output was indistinguishable from that obtained in the absence of amylobarbitone.

It is concluded that anaesthetic concentrations of amylobarbitone produce marked impairment of transmission in the perfused ganglion but that this is not contributed to by an interference with acetylcholine release. In other experiments, however, high concentrations of amylobarbitone, exceeding the anaesthetic level by some eight times, did cause an interference with acetylcholine release.

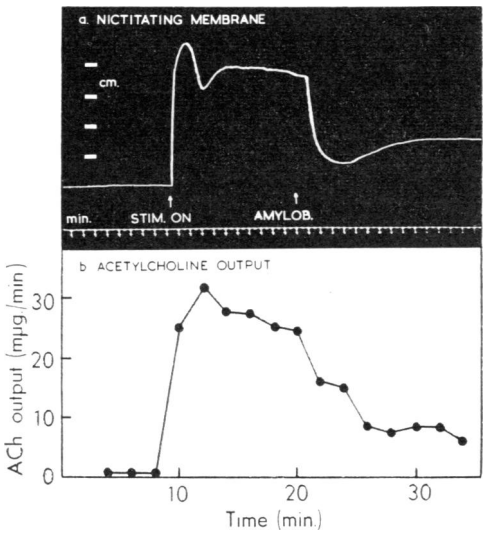

FIG. 7.—Perfusion of superior cervical ganglion with eserinized Locke solution. Upper part (*a*), record of nictitating membrane contraction. Continuous preganglionic stimulation from first arrow. Second arrow, 0.5 mg. amylobarbitone sodium in 10 ml. Locke injected into perfusion tubing. Time marker, 1 min. Lower part (*b*), graph of corresponding output of acetylcholine (mμg./min.) from ganglion, on same horizontal time scale.

Quantitative Comparison of Barbiturates as Ganglionic Depressants

Quantitative differences in the potency of the barbiturates on the ganglion are, of course, to be expected on the grounds of their varying potencies as central depressants. Whether their potencies as ganglionic depressants could be directly correlated with their C.N.S. potencies was of considerable interest.

Pentobarbitone and amylobarbitone were chosen for preliminary quantitative comparisons, since, though these drugs are isomeric, they have different potencies as C.N.S. depressants, amylobarbitone possessing about two-thirds the potency of pentobarbitone as a hypnotic in animals.

Comparisons on Vasomotor Ganglia

Preliminary experiments consisted in comparing the effects of barbiturates on the pressor responses induced in the spinal, atropinized, cat by periodic injections of choline *m*-bromophenyl ether bromide. This substance was shown by Hey (1952) to possess a nicotine-like stimulant activity on ganglia at least 10 times greater than that of nicotine itself, but with little tendency to produce secondary nicotine-like paralysis. It is thus a very suitable agent for inducing repeated pressor responses by ganglionic stimulation.

Fig. 8 shows part of a recording of the arterial blood pressure taken from such an experimental comparison. Each signal mark indicates the point of injection of 10 μg. of the choline ether into the femoral vein, resulting in pressor responses at approximately 3 min. intervals. At the first arrow, 5 mg. pentobarbitone sodium was injected intravenously ; this resulted in a reduction in the height of the succeeding pressor responses by about one-third, with gradual recovery on subsequent injections of the nicotine-stimulant drug. At the second arrow an injection of 5 mg. amylobarbitone sodium caused a much more marked reduction, with slower recovery. Thus, from this experiment, it appeared that pentobarbitone, though a stronger hypnotic than amylobarbitone, was a weaker ganglionic depressant.

Comparisons on Superior Cervical Ganglion

Factors Affecting Sensitivity of Ganglion to Drugs.—Potency tests of drugs producing block of the superior cervical ganglion are most commonly made by comparing their actions in relaxing the contraction of the nictitating membrane during continuous preganglionic stimulation at frequencies of around 10 shocks per second (e.g., Paton and Zaimis, 1951 ; Wien, Mason, Edge, and Langston, 1952). This method is not, however, very suitable for comparisons of the barbiturates: these produce cumulative effects during an experiment, so that relaxations of the nictitating membrane may not be followed by complete restoration of the contraction to its original level. A method was therefore evolved in which periodic stimulation of the preganglionic trunk was

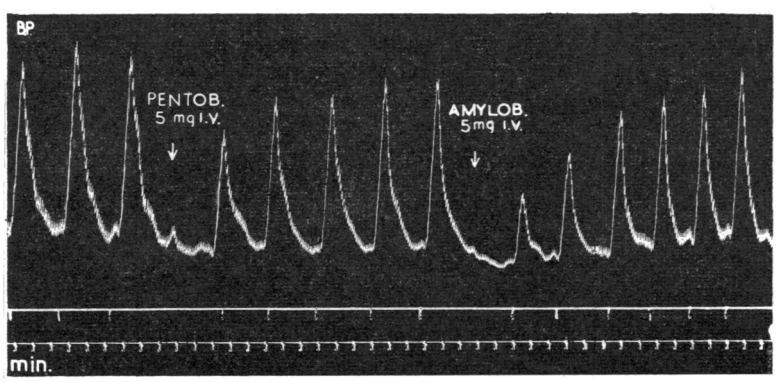

FIG. 8.—Cat, 3.7 kg., spinal, atropinized. Blood pressure. Each signal mark indicates the i.v. injection of 10 μg. choline *m*-bromophenyl ether bromide. First arrow, 5 mg. pentobarbitone sodium i.v. Second arrow, 5 mg. amylobarbitone sodium i.v.

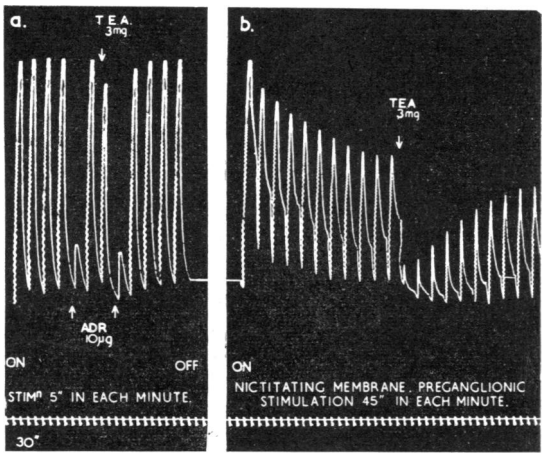

FIG. 9.—Cat, 2.6 kg., atropinized, chloralose. Contractions of nictitating membrane in response to preganglionic stimulation (70 shocks/sec.). (*a*) Stimulation for 5 sec. in each minute. Lower arrows, injections of 10 μg. adrenaline i.v. Upper arrow, 3 mg. tetraethylammonium iodide i.v. (*b*) Stimulation for 45 sec. in each minute. Upper arrow, 3 mg. tetraethylammonium iodide i.v.

used and the *proportionate* reduction in the contraction-height of the membrane following a dose of barbiturate gave a measure of the response. Absolute heights of contraction, and fluctuations of the base-line during experiments, are thus less important.

Compared with hexamethonium, or even tetraethylammonium, barbiturates are relatively weak ganglionic depressants. In attempting to assess barbiturate effects a sensitive method was therefore required. The sensitivity of the superior cervical ganglion to blocking agents can be greatly influenced both by the duration and frequency of a tetanic stimulus. The enhancement of sensitivity following increased frequency of stimulation has been remarked upon by Paton and Zaimis (1951), and the use of frequencies up to 50 shocks per second has recently been advocated (Morrison and Paton, 1953). In the present work frequencies of 50 or 70 per second were used.

The *duration* of the periods of tetanus also influences the sensitivity to a blocking agent, as shown in Fig. 9. When the stimulus is applied for 5 sec. in each minute TEA has a much smaller effect than when the stimulus is applied for 45 sec. in each minute. Stimulation for 45 sec. in each minute thus renders the preparation very responsive to blocking agents, and is therefore the most suitable for comparing weak ganglionic depressants. Its use for comparing amylobarbitone sodium and TEA iodide is shown in Fig. 10. This record clearly shows the reduction in height of the contractions attributable to several injections of the two substances. In each instance the drugs were injected 30 sec. after beginning a period of stimulation (and, therefore, 15 sec. before the stimulus ended), and the measure of response was the reduction in height of the ensuing contraction expressed as a percentage of the previous contraction. The results of this experiment are shown graphically in Fig. 11, which indicates that the two dose-response curves are reasonably linear and parallel. The activity of amylobarbitone sodium works out at a little under one-quarter that of TEA iodide, on either a weight-for-weight or a molar basis.

Comparison of Fifteen Barbiturates with Amylobarbitone.—The same method has been applied to some fifteen of the commoner barbiturates using amylobarbitone sodium throughout as the standard drug. It was usually possible to compare three or four drugs on the same animal, and wherever possible at least two doses of the standard and of the unknown were given. With certain drugs, such as the thiobarbiturates, which exhibit very weak ganglionic effects, but powerful central effects, it was only feasible to administer one dose and to match this against two or more doses of the standard. Otherwise, the total dose of thiobarbiturate would have exceeded the full anaesthetic dose before much useful information could be gained.

The dose-response curves obtained in each experiment appeared to be reasonably parallel and

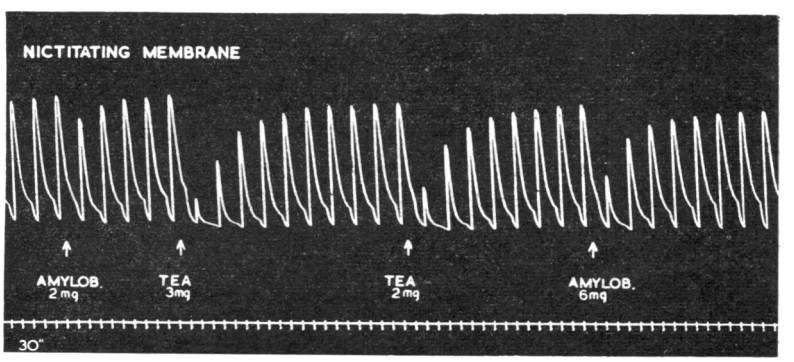

FIG. 10.—Cat, 2.7 kg., atropinized, chloralose. Recording of contractions of the nictitating membrane evoked by periodic stimulation of the preganglionic trunk for 45 sec. in each minute (as in Fig. 9*b*). The four arrows indicate, respectively, intravenous injections of 2 mg. amylobarbitone sodium, 3 mg. and 2 mg. tetraethylammonium iodide, and 6 mg. amylobarbitone sodium.

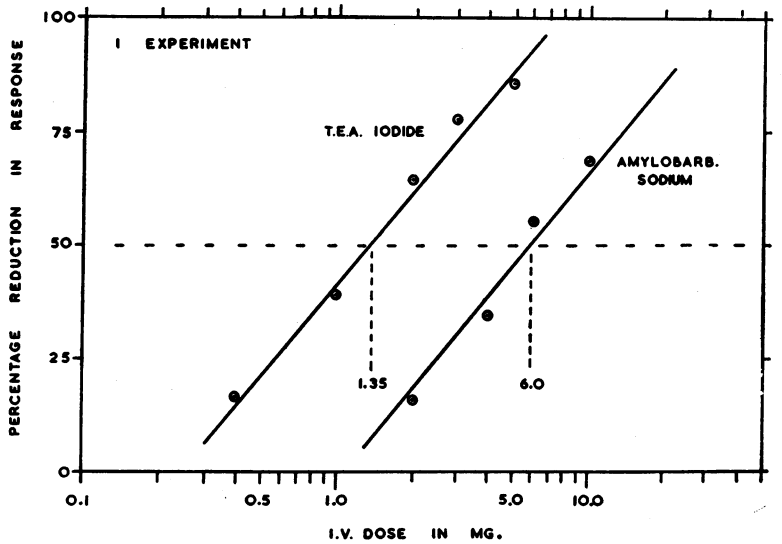

FIG. 11.—Graph of results from the experimental comparison (Fig. 10) of the ganglion depressant activities of tetraethylammonium iodide and amylobarbitone sodium. Ordinate, percentage reduction of nictitating membrane contraction. Abscissa, dose (mg.) in logarithmic scale.

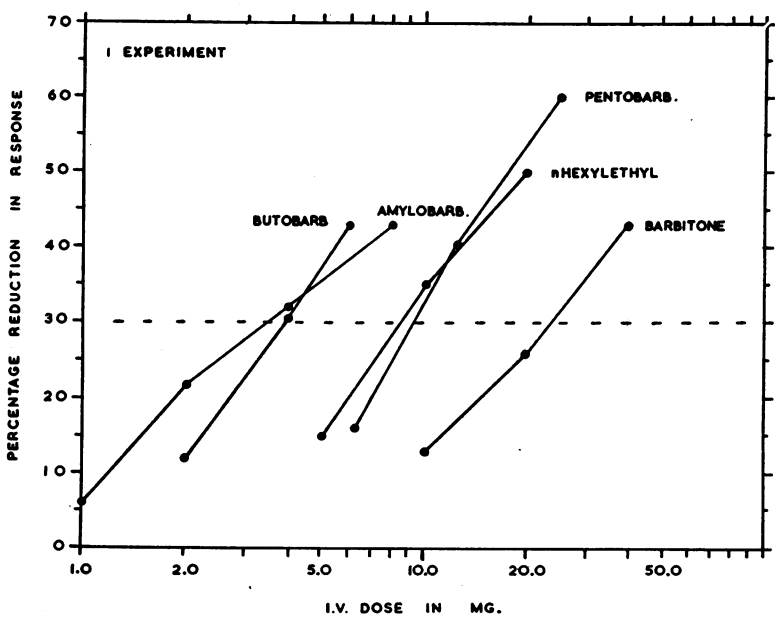

FIG. 12.—Graph showing dose-response curves obtained from an experimental comparison of the ganglion-depressant activities of the sodium salts of amylobarbitone, butobarbitone, pentobarbitone, barbitone, and *n*-hexyl-ethyl barbituric acid. Abscissa and ordinate as in Fig. 11.

activity ratios were assessed from them by graphical methods. Examples of typical dose-response curves obtained from a comparison, in the same animal, of amylobarbitone, butobarbitone, pentobarbitone, 5-*n*-hexyl-5-ethyl barbituric acid, and barbitone are shown in Fig. 12. Relative to amylobarbitone, butobarbitone is equiactive on the ganglion, pentobarbitone and the *n*-hexyl derivative are a little over one-third as active, and barbitone about one-fifth.

The results of such comparisons of fifteen barbiturates with amylobarbitone are presented in Table I, column A. Certain of these results have already been published (Exley, 1952). In each instance the potency on the ganglion is expressed, on a weight-for-weight basis, in terms of amylobarbitone = 100.0. Amylobarbitone and butobarbitone are the most active ganglionic depressants. Pentobarbitone, which is a more potent anaesthetic agent than amylobarbitone, has 42% of its activity on the ganglion—a figure which, as revealed by the standard error, differs significantly from that of amylobarbitone. Barbitone and phenobarbitone show relatively low activities of 17 and 14 respectively, while thiopentone and thialbarbitone come low down the list with activities of about 13 and 7 respectively.

Relationship of Ganglionic to Central Effects

It was of interest to compare figures for relative activities on the ganglion with those relating to C.N.S. depression. For this purpose the median anaesthetic doses as determined intravenously on mice by Butler (1942) were used. From Butler's data relative anaesthetic potencies of the drugs listed in Table I have been calculated in terms of amylobarbitone = 100.0, and are shown in column B. (The intravenous anaesthetic dose for thialbarbitone was not given by Butler and so was determined by the writer.)

TABLE I

RELATIVE GANGLION-DEPRESSANT AND INTRAVENOUS ANAESTHETIC POTENCIES OF THE COMMONER BARBITURATES

Except for thialbarbitone, anaesthetic potencies given in this table were calculated from data of Butler (1942).

Barbiturate	No. of Expts.	A Mean Potency on Sup. Cerv. Gang. ±S.E.	B Relative Anaesth. Potency	Ratio A/B
Amylobarbitone		100·0	100	1·00
Butobarbitone	8	99·1±6·2	71	1·40
n-Hexylethyl barbituric acid	6	43·4±2·7	69	0·63
Pentobarbitone	7	42·5±1·5	164	0·26
*iso*Propylallyl barbituric acid	3	32·8±3·1	93	0·35
Hexobarbitone	3	31·7±2·9	186	0·17
*iso*Butylallyl barbituric acid	3	31·6±3·8	86	0·37
Cyclobarbitone	3	31·0±5·7	49	0·63
Methylphenobarbitone	3	28·0±3·6	108	0·26
Quinalbarbitone	3	27·9±1·5	193	0·15
Barbitone	6	17·1±1·3	23	0·74
*iso*Propylethyl barbituric acid	3	16·2±1·1	49	0·33
Allobarbitone	3	15·7±0·8	74	0·21
Phenobarbitone	4	14·2±0·6	40	0·35
Thiopentone	3	12·8±1·2	450	0·03
Thialbarbitone	3	7·1±0·4	146	0·05

It is interesting to note, from the figures given in columns A and B for the different drugs, that ganglionic and anaesthetic potencies appear to show no direct correlation one with the other. The relationship of ganglionic to central nervous depressant activities is expressed in the fifth column of Table I as the ratio A/B. Such treatment of the data has the added advantage of cancelling out molar differences. The highest A/B ratio is given by butobarbitone (1.40), and the lowest by thiopentone (0.03).

All these determinations of the activity of the barbiturates were made after intravenous injections, and it was considered possible that some of the observed differences might be explicable by physical factors—such as the rate of distribution of the various drugs within the body tissues. However, when the drugs were injected directly into the arterial blood supply of the ganglion, the order of their relative ganglion-depressant potency was unchanged.

Investigation of Homologous Series of Barbiturates

Inspection of the figures for the ratio A/B, in Table I, affords very little indication of the type of chemical structure to be associated with either increased or decreased ganglionic as compared with central depressant activity. In general, it is apparent that higher values for the ratio are given by drugs such as butobarbitone, barbitone and the *n*-hexyl derivative, which contain straight alkyl chains substituted in position-5 of the barbituric acid ring. Derivatives with branched alkyl chains or complex groupings in position-5, or incorporating structural modifications of the barbituric acid ring, tend to exhibit lower values for the ratio.

It was therefore thought that an investigation of homologous series of primary alkyl-ethyl and primary di-alkyl barbituric acids might yield information on the relationships between structure and ganglion-depressant activity.

Data for certain members of these two series—barbitone, butobarbitone and *n*-hexyl-ethyl barbituric acid—are already available from Table I.

Other barbiturates containing the following substituents in position-5 were specially synthesized for this investigation:

n-propyl-ethyl ; *n*-amyl-ethyl ;
di-*n*-propyl ; di-*n*-butyl ; di-*n*-amyl.

These derivatives were compared with amylobarbitone on the superior cervical ganglion of the cat by the method previously described. The median anaesthetic dose for each was also determined on mice, by Butler's method. Anaesthesia by the di-*n*-butyl and di-*n*-amyl derivatives occurred immediately after injection into the tail vein, and was of very short duration. The figure obtained for the di-*n*-amyl derivative was less accurate because of this short duration (less than three minutes) and because the drug showed convulsant tendencies with doses near to the median. These two substances obviously possess low therapeutic coefficients, since, with doses but slightly in excess of the AD50, the animals commonly died.

Results of these tests are presented in Table II and, together with those for barbitone, butobarbitone and *n*-hexyl-ethyl barbituric acid (Table I), are shown graphically in Fig. 13. Whilst relative ganglionic and anaesthetic potencies tend to run roughly parallel for the alkyl-ethyl series—reaching a peak at the *n*-amyl-ethyl member, in the

TABLE II

RELATIVE GANGLION-DEPRESSANT AND INTRAVENOUS ANAESTHETIC POTENCIES OF CERTAIN ALKYL-ETHYL AND DI-ALKYL BARBITURIC ACIDS

Substituent in Position-5 of Barbituric Acid	A Mean of Three Potency Determinations on Sup. Cerv. Gang. ±S.E.	AD50 (mg./kg.)	B Relative Anaesth. Potency	Ratio A/B
*iso*Amyl-ethyl (amylobarbitone)	100·0	52	100	1·00
n-Propyl-ethyl	33·1±2·8	126	41	0·81
n-Amyl-ethyl	125·7±1·1	47	111	1·13
di-*n*-Propyl	48·2±3·6	136	38	1·27
di-*n*-Butyl	112·8±1·7	45	116	0·97
di-*n*-Amyl	16·4±2·8	c25	c208	c0·08

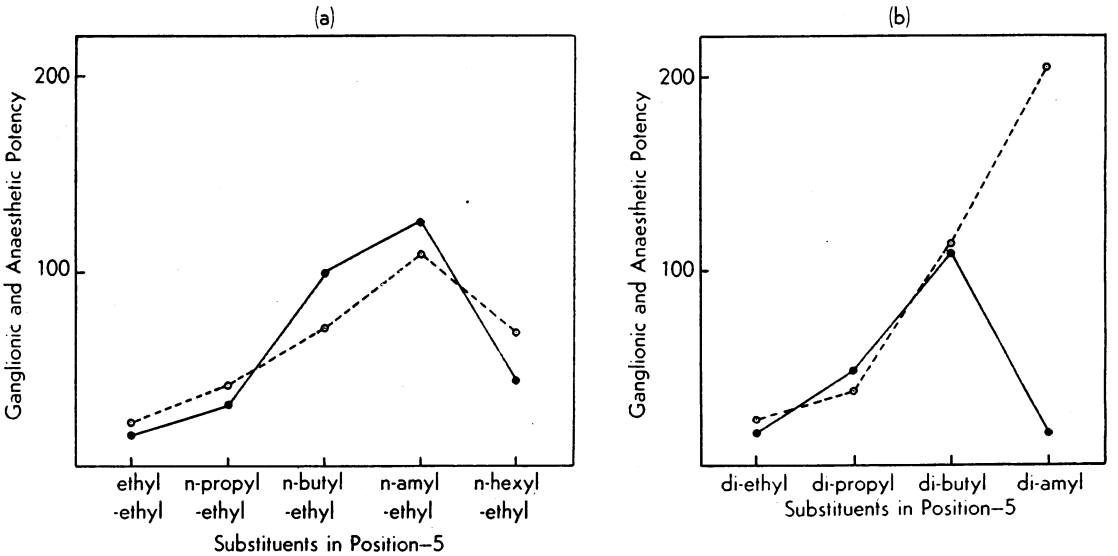

FIG. 13.—Graphs showing properties of homologous series of (*a*) primary 5-alkyl-5-ethyl barbituric acids, and (*b*) primary 5:5-di-alkyl barbituric acids. Horizontal axes, substituent groupings in position-5. Vertical axes, ganglion-depressant and anaesthetic potency, relative to amylobarbitone = 100.0. Continuous lines, ganglion-depressant potency. Interrupted lines, anaesthetic potency.

di-alkyl series these two properties show a marked divergence at the di-amyl member.

It is of interest to note that, in both series, the highest values for the ratio A/B are given by those members containing a substituent with a total of six carbon atoms—*n*-butyl-ethyl and di-propyl, respectively. A very low value for this ratio is given by the di-amyl member (0.08) which, in this respect, and in virtue of its high anaesthetic potency, shows some resemblance to the thiobarbiturates.

DISCUSSION

Investigation of the properties of amylobarbitone as a ganglion-paralysing agent has revealed several interesting likenesses to tetraethylammonium. Both drugs reduce the excitability of the ganglion cells to acetylcholine, but not to potassium chloride. Neither drug depolarizes the ganglion cells. Both, in ordinary doses, are devoid of atropine-like or neuromuscular-blocking activity.

The hypothesis that barbiturates prevent the release or synthesis of acetylcholine in nervous tissue (Dalton, 1950) has a particular appeal on account of its simplicity ; but the evidence afforded by studies on isolated brain tissue gives only equivocal support to it (Tobias, Lipton, and Lepinat, 1946 ; Richter and Crossland, 1949 ; McLennan and Elliott, 1951). Caution should, of course, be exercised in drawing any analogies between synapses in the central nervous system and those in sympathetic ganglia ; but, in the latter at least, amylobarbitone does not interfere with the release of acetylcholine except in concentrations far in excess of those which produce general anaesthesia in the intact animal.

Trethewie's claim (1942), that amylobarbitone diminished the release of acetylcholine from the perfused guinea-pig stomach and intestine during electrical stimulation of the vagus nerve, should be interpreted with care. The acetylcholine which he collected may have originated chiefly from postganglionic parasympathetic nerve terminals ; if the barbiturate did, in fact, cause interruption of nerve impulses at the level of *ganglionic synapses* then the output of acetylcholine from the postganglionic terminals might well have been profoundly reduced. But this does not imply that the barbiturate had affected acetylcholine release or synthesis at the vagal ganglia.

Tetraethylammonium salts are often presumed to produce ganglionic block by " receptor competition " with acetylcholine (Acheson and Pereira, 1946). It would seem unlikely that barbiturates have a similar mode of action, since their chemical structures bear little resemblance either to acetylcholine itself or to the quaternary ammonium blocking agents. On the other hand, barbitone and phenobarbitone have been shown by Fernando (1952) to block the action of acetylcholine on the heart of *Venus mercenaria,* and Welsh and Taub (1953) have since suggested that the " carbonyl "

groupings within the barbiturate molecule might form multiple hydrogen bonds with acetylcholine receptor substance.

The diminished excitability of the ganglion cells to acetylcholine, produced by amylobarbitone, might, however, be due to a less specific "blanketing" effect of the drug on the cell surface, or to a straightforward interference with essential cellular metabolic processes, such as has been described for narcotics on isolated brain tissue by Quastel and Wheatley (1932), and others. For the depression of spinal cord motor-neurone synapses by pentobarbitone, Eccles (1946) has suggested an increased stability of the nerve cell membranes to electrical changes. But, whatever the true explanation of the general phenomenon, it is felt that the term "ganglion-depressant" should be used in preference to "ganglion-blocking" in describing the effects of barbiturates upon autonomic ganglia.

It is possible that barbiturates may exert their effects on the brain by interfering with transmission across the synapses of the various internuncial neurones. The absence of similar central effects with tetraethylammonium might be indicative of fundamental differences between the mechanisms of synaptic transmission in sympathetic ganglia and in the brain, or it might derive from a failure of lipoid-insoluble quaternary salts to reach the central synapses.

The lack of a definite correlation between ganglionic and central nervous depressant potencies of the various barbiturates is difficult to explain. Ganglion-depressant potencies cannot be related, for example, to oil/water distribution coefficients of the drugs (Tabern and Shelberg, 1933); to rates of hydrolysis of the sodium salts at the *p*H of the blood (Bush, 1937); to speed of onset of anaesthesia after intravenous administration (Butler, 1942), or to the degree of binding of the drugs to blood proteins (Goldbaum and Smith, 1948). If Butler's figures can be taken as representing the relative rates of fixation of the drugs to nervous tissue, then drugs such as hexobarbitone or thiopentone, which exhibit both short lags and high anaesthetic potencies, might be expected to exhibit high ganglion-depressant potencies. However, the data of Tables I and II suggest that this is not so. Indeed, it is fairly obvious that drugs showing high ganglion-depressant activities fall into the intermediate group of barbiturates—that is, those with medium anaesthetic potencies and moderate anaesthetic lags.

A further possibility is that the variations in ganglion-depressant activity seen after intravenous injection might be influenced by differences in the rate at which the drugs pass from the blood stream into certain body tissues. However, whilst Brodie and his co-workers (1950, 1952) have shown that thiopentone is rapidly taken up by the fat depots of the body, thereby accounting for the short duration of action of the drug, they were unable (1953) to demonstrate a similar effect with pentobarbitone—which tends to remain equally distributed throughout the tissues. Thus variation from differences in distribution would seem to apply only to the thiobarbiturates. Further evidence that distribution differences do not contribute to the variations in ganglion-depressant potency is afforded by the fact that barbiturates injected directly into the blood supply of the ganglion exert their effects before entering the general circulation, yet they still show differences in activity similar to those found after intravenous administration.

From a consideration of the various possibilities outlined above we are forced to conclude that stereo-chemical rather than physico-chemical factors are primarily responsible for variations in ganglion-depressant activity among the barbiturates.

An important clinical implication of the results presented in Table I relates to surgical anaesthesia. It will be noted that the ratios of ganglionic to anaesthetic potency are low for the three commonly used intravenous anaesthetic agents hexobarbitone, thiopentone and thialbarbitone. Indeed, the ratio for thiopentone (0.03) is the lowest recorded. Low ratios such as these indicate low autonomic depressant activity so that the fall in blood pressure occurring during induction of anaesthesia with these drugs would be expected to be trivial. Clinical experience confirms this expectation; the fall in blood pressure with thiopentone is rarely serious and is usually less than that with intravenous hexobarbitone.

In contradistinction, drugs like amylobarbitone and butobarbitone, which show high A/B ratios, would be expected to produce more marked autonomic depression during induction of anaesthesia. Again, clinical experience confirms that serious falls in blood pressure, sometimes amounting to circulatory collapse, may occur with intravenous amylobarbitone sodium (Zerfas, 1930). It is suggested, therefore, that the routine laboratory testing of a new barbiturate, proposed for use as an intravenous anaesthetic, might profitably include an assessment of its ganglion-depressant potency.

A further practical point arising from this work concerns the choice of suitable barbiturates for the sedative treatment of conditions such as hyper-

tension and peptic ulceration, where depression of autonomic activity seems to be particularly desirable. On the basis of the results reported here, it is possible that, in this respect, butobarbitone and amylobarbitone might offer slight advantages over other members of the series. But the lack of any clearly defined structural requirements for autonomic depressant activity, as compared with central depressant activity, offers little help in the synthesis of new barbiturates with greater ganglion-depressant activities.

Finally, it is clear from these animal experiments that depression of sympathetic ganglia may play some part in contributing to the lowering of arterial blood pressure in patients subjected to the so-called "Sodium Amytal Sedation Test." The observations of Cady, Horton, and Adson (1936), that amylobarbitone is more effective than intravenous thiopentone in such a test, is now explicable on the basis of the respective ganglion-depressant activities of the two drugs.

Summary

1. Small intravenous doses of amylobarbitone sodium produce transient depression of sympathetic ganglia in cats. This is demonstrated both upon vasomotor ganglia stimulated by nicotine, and upon the superior cervical ganglion stimulated electrically.

2. The site of depression is the ganglionic synapse: neither the preganglionic and postganglionic nerve trunks nor the effector organs are involved.

3. Amylobarbitone, like tetraethylammonium, reduces the excitability of the ganglion cells to acetylcholine but not to potassium. It does not depolarize the ganglion, nor does it interfere with acetylcholine-release.

4. Intravenous anaesthetic doses of amylobarbitone cause a marked and prolonged fall in arterial blood pressure accompanied by simultaneous impairment of transmission through the superior cervical ganglion.

5. In doses which produce easily demonstrable ganglionic depression, amylobarbitone shows neither atropine-like nor neuromuscular-blocking properties.

6. A sensitive method is described for the comparison of weak ganglion-depressant agents in cats. The method involves periodic, instead of continuous, stimulation of the cervical sympathetic nerve and permits the determination of dose-response relationships for several drugs in the same animal.

7. Comparisons of amylobarbitone sodium and tetraethylammonium iodide as ganglion-depressants show that amylobarbitone has about one-quarter the potency of tetraethylammonium.

8. The ganglion-depressant activities of 15 of the commoner barbiturates were compared with that of amylobarbitone. The most active drugs are butobarbitone and amylobarbitone; the least active are the thiobarbiturates. Homologous series of primary alkyl-ethyl and di-alkyl barbituric acids were similarly examined.

9. Quantitative differences in ganglion-depressant activity are still demonstrable when the drugs are injected directly into the arterial supply to the ganglion.

10. Ganglion-depressant potencies show little correlation with central nervous depressant potencies. Possible explanations for the variability of ganglion-depressant activity are discussed.

11. It is argued that the fall in blood pressure which may follow administration of barbiturates to man is contributed to by a depression of the vasomotor ganglia. This is particularly likely to be so in people subjected to the "Sodium Amytal Sedation Test."

My thanks are due to Professor W. A. Bain for his encouragement and helpful suggestions throughout this work; to Drs. W. Feldberg and W. L. M. Perry for their kindness in enabling me to record ganglionic action potentials at the National Institute for Medical Research, and for familiarizing me with the technique of ganglion perfusion; to Messrs. May & Baker Ltd., Dagenham, who kindly synthesized certain barbiturates for me; and to the Medical Research Council for an expenses grant which covered part of the cost of this work.

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