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THE COMPARISON OF RESPIRATORY STIMULANT DRUGS

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Drugs stimulating respiration are usually classified as analeptics or as respiratory analeptics. This latter term is perhaps more useful since the term "analeptic" has such a wide significance that, in addition to picrotoxin and leptazol, it can also include such substances as amphetamine and ephedrine, which have an action at higher levels in the central nervous system. The method about to be described has been devised primarily for the more purely respiratory type of analeptic.

The methods usually employed to compare analeptics (using the term generally) are based upon the reduction they produce in the duration of anaesthesia. Trevan (1939) used this principle to compare the central stimulant actions of amphetamine and isomers of ephedrine in mice which had been anaesthetized with paraldehyde. He also included some experiments in which picrotoxin and leptazol were employed as the stimulant drugs, which showed that amphetamine had a greater awakening effect upon mice than either picrotoxin or leptazol, but that leptazol is slightly more effective than picrotoxin.

This type of method is very suitable for the examination of compounds of the amphetamine type in which Trevan was primarily interested, but has several disadvantages when employed to test the more predominantly respiratory stimulants.

Chakravati (1939) pointed out some of the objections to this method and attempted to overcome them. One of the main objections is the variable depth of anaesthesia resulting from the administration of a fixed dose of anaesthetic to a series of mice, and Chakravati attempted to sort out his animals according to the depth of anaesthesia. Despite this, several objections still remain. Firstly, the animals are not necessarily anaesthetized to a constant depth and, secondly, the degree of anaesthesia is not comparable with that occurring in patients when analeptics are required. When these drugs are used clinically it is usually to combat excessive degrees of anaesthesia resulting, most often, from barbiturate anaesthetics.

The criterion by which stimulant drugs will be judged in such clinical use is not so much the extent to which they will awaken the patient as the reliability with which respiration can be improved with their aid. The two effects are interrelated since the awakening effect will follow the use of a successful respiratory stimulant more quickly than if it were not employed.

A most interesting contribution was made to the study of analeptic drugs of this type by Das (1939), who gave continuous intravenous injections of anaesthetics to depress the respiration of rabbits by about 50 per cent and then observed the effects of different stimulant drugs upon that level of anaesthesia. The method we have used is similar to that of Das except that a much more critical level of anaesthesia is employed.

Experimental Procedure

Guinea-pigs weighing between 250 and 700 g. are used, in groups of eight or ten animals, the weight distribution in any set of animals being not more than ± 20 per cent different from the mean value for the group. The animal is secured prone on an operating table and the hair is removed from the outer surface of the forelimbs between the ankle and the knee. A bleb is raised on each forelimb by the injection of 0.1 c.c. of a solution of 2 per cent (w/v) procaine subcutaneously just over the route of the accessory cephalic vein. After a minute the skin in this region is plucked up with forceps and a small patch cut out with scissors, thus exposing about ¼ in. of the vein in each forelimb.

The anaesthetic is made up in such a strength that it will kill the guinea-pig when infused continuously in about ten minutes. The anaesthetics we have used are thiopentone soluble and pentobarbital soluble, both made up in solutions of 7.5 mg./c.c. The rate of continuous injection employed does not seem very important, but for these solutions 0.6 c.c./min. has been used and found satisfactory.

The anaesthetic solution is injected into the animal by means of a fine dental needle inserted in the vein and attached to a motor-driven syringe containing the solution. With a little practice this injection becomes a simple matter. As injection proceeds the depth of anaesthesia increases until there is considerable respiratory depression. The intervals between successive breaths become longer and longer. When twenty seconds have elapsed after a breath the injection is stopped. At this level of anaesthesia spontaneous recovery is very improbable. If the animal is kept warm, however, the heart will continue to beat for several minutes. The analeptic drug is next injected into the vein of the opposite limb in a similar manner, as a solution containing a half to one LD50 of the substance per c.c. In our experiments a rate of 0.6 c.c./min. was employed.

A true respiratory analeptic will cause respiration to start again in a few minutes and the amount of drug required to do this is recorded. The injection of the drug is continued until there are signs of over-stimulation, such as convulsive jerks; the injection is then stopped and the total amount of the analeptic drug given is recorded.

The guinea-pig is now placed, with the cuts on the forelimbs protected by pads of absorbent cotton wool, in a constant temperature cabinet at 30° C. and observed, until it is clear whether the animal will live or die. If the animal lives for two hours death later is very unlikely; animals surviving this period are then destroyed.

We have termed the dose required to restart respiration, and the dose required to produce over-stimulation, *A* and *B* respectively. The values of the *A* and *B* doses per unit weight can be compared for different analeptics.

RESULTS

Experiments were first made to determine for how long the injection of the anaesthetic must be continued after the cessation of respiration in order to avoid spontaneous recovery. The results obtained are given in Table I and indicate

TABLE I

THE EFFECT OF CONTINUING THE INJECTION OF PENTOBARBITAL SOLUBLE (4·5 MG./MIN.) AFTER THE LAST BREATH UPON THE MORTALITY OF GUINEA-PIGS

Time lapse after the last breath in seconds	Mortality after 1 hour
0	1/10
5	2/10
10	8/10
20	10/10
20	10/10
20	10/10

that such recovery is very unlikely when twenty seconds have elapsed after the last breath. Table II shows the mean doses of the two anaesthetics required to cause respiratory arrest in seven groups of ten guinea-pigs for each anaesthetic, together with the standard errors of these mean values. The two final means

TABLE II

MEAN DOSES OF ANAESTHETIC REQUIRED TO CAUSE PERMANENT RESPIRATORY ARREST IN GROUPS OF TEN GUINEA-PIGS. BOTH ANAESTHETICS WERE INJECTED AT 4·5 MG. PER MINUTE INTRAVENOUSLY

Thiopentone soluble			Pentobarbital soluble		
Group of 10 animals	Mean dose mg./kg.	Standard error	Group of 10 animals	Mean dose mg./kg.	Standard error
1	46·0	1·8	8	68·6	6·0
2	46·7	2·9	9	63·3	4·6
3	44·9	2·5	10	73·6	4·8
4	41·0	3·8	11	68·0	3·7
5	45·2	0·2	12	69·1	2·1
6	50·4	3·2	13	65·5	2·9
7	36·9	1·5	14	64·4	1·7
Mean	44·44	1·04	Mean	67·50	1·6

give an estimate of the LD50 of thiopentone soluble and pentobarbital soluble in guinea-pigs. Each group of ten guinea-pigs was treated on the same day, but the fourteen groups were used over a period of several weeks. It will be seen that the groups of animals give consistent results one with another and the standard errors within each group are small.

For the assessment of a respiratory stimulant drug two groups of ten animals were used, one with each of the anaesthetics, which were chosen to give both short and long durations of anaesthesia ; thiopentone soluble was chosen for short and pentobarbital for long durations. The former drug would be more readily antagonized and the latter, owing to its longer action, would provide a better test of the analeptic property.

The results obtained with picrotoxin are given *in extenso* in Table III and the corresponding mean results for leptazol and triazol 156 in Table IV. Triazol

TABLE III

TYPICAL SET OF RESULTS OBTAINED BY THE METHOD DESCRIBED SHOWING THE RELATIVE RESPIRATORY STIMULANT ACTIONS OF PICROTOXIN UPON THE RESPIRATION DEPRESSED WITH THIOPENTONE AND PENTOBARBITAL. *A* IS THE DOSE TO RESTART RESPIRATION AND *B* THE DOSE TO CAUSE OVER-STIMULATION

Thiopentone anaesthesia				Pentobarbital anaesthesia			
Animal No.	Picrotoxin dose mg./kg.		Result of observation for 2 hr.	Animal No.	Picrotoxin dose mg./kg.		Result of observation for 2 hr.
	A	*B*			*A*	*B*	
1	1·4	2·5	Lived	11	10·4	14·4	Lived
2	2·0	2·8	Lived	12	9·8	14·1	Lived
3	2·4	10·8	Lived	13	5·2	11·8	Lived
4	2·8	6·8	Lived	14	6·0	11·5	Lived
5	2·5	7·6	Lived	15	5·8	11·9	Lived
6	—	—	Failed to recover	16	1·6	17·4	Lived
7	6·0	13·7	Lived	17	5·5	15·0	Lived
8	6·3	14·1	Lived	18	7·4	17·7	Died
9	5·0	6·8	Lived	19	5·3	12·7	Lived
10	4·8	10·4	Lived	20	5·6	17·8	Died
Mean	3·5	8·3	9/10 lived	Mean	6·28	14·43	8/10 lived
Std. error	0·183	1·40		Std. error	0·92	0·79	

156 or *cyclo*hexyl-ethyl-triazol, also known as "azoman," was described by Behrens, Dinkler, and Woenckhaus (1937). This drug resembles leptazol in many respects, but is much more potent.

The results can also be represented graphically as shown in Figs. 1 and 2. These graphs were obtained by plotting the logarithm of the dose required by any one guinea-pig either to restart respiration (*A* curves) or to cause over-stimulation (*B* curves) against the probit value for the percentage of the total number of animals which responded to that dose of the stimulant drug or to smaller doses. The mean value corresponds statistically to the LD50 in a toxicity test and causes recovery in 50 per cent of the animals. The relative doses of the analeptics required can be seen from the graphs ; the steepness of the curves gives a measure of the efficiency with which the drugs antagonize the anaesthetics. In Table IV the ratios of the potencies of the three analeptics are given,

taking picrotoxin doses as 1.0. It will be seen that, with thiopentone anaesthesia, both the *A* and the *B* values for triazol 156 bear a constant ratio of 0.47 to the corresponding values for picrotoxin. This is because both drugs cause over-stimulation by the same proportional increase in the doses required to restart the respiration. This is not so with pentobarbital anaesthesia: picrotoxin causes over-stimulation quite readily, but triazol gives a very much smaller ratio for over-stimulation because it does not antagonize this long-acting barbiturate as readily as picrotoxin does.

TABLE IV

A COMPARISON OF THREE ACCEPTED ANALEPTICS. THE VALUES UNDER *A* GIVE THE MEAN DOSES REQUIRED TO RESTART RESPIRATION AND UNDER *B* TO CAUSE OVER-STIMULATION. THE RATIOS ARE THE INVERSE RATIOS OF THE DOSES, TAKING PICROTOXIN AS 1.0.

Analeptic drug	Thiopentone anaesthesia						Pentobarbital anaesthesia					
	A		*B*		No. alive	Notes	*A*		*B*		No. alive	Notes
	Dose mg./kg.	Ratio	Dose mg./kg.	Ratio			Dose mg./kg.	Ratio	Dose mg./kg.	Ratio		
Picrotoxin .. 2.0 mg./c.c. 0.6 c.c./min.	3.5	(1.0)	8.3	(1.0)	9/10	*a*	6.3	(1.0)	14.4	(1.0)	8/10	*b*
Triazol 156 .. 4.0 mg./c.c. 0.6 c.c./min.	7.5	0.47	17.8	0.47	10/10	*c*	6.0	1.05	46.4	0.31	4/10	*d*
Leptazol .. 100 mg./c.c. 0.6 c.c./min.	218.0	0.016	—	—	9/10	*e*	234.0	0.027	—	—	4/8	*f*

(*a*) Muscular twitching persisted for 5–10 min.
(*b*) Slight muscular twitching after injection.
(*c*) Very smooth respiration without twitching. Quick awakening.
(*d*) Difficult to produce over-stimulation.
(*e*) No over-stimulation possible. Awakening effect most marked.
(*f*) No over-stimulation possible.

It will be seen from Table IV and Figs. 1 and 2 that the most effective analeptic available to antagonize the barbiturate anaesthetics employed is picrotoxin, but that triazol 156 is not greatly inferior. Picrotoxin is effective against both the short- and long-acting barbiturates used, but leptazol is much less effective against the long-acting drug. Triazol 156 is intermediate between the two and antagonizes pentobarbital far less easily than it does thiopentone anaesthesia. There is much more variability in the results giving the *B* curve for triazol and pentobarbital anaesthesia, and hence the slope of the curve is lower.

Leptazol is the least effective of these stimulant drugs and it did not cause over-stimulation in these experiments. If the injection is continued for a prolonged period, however, secondary depression occurs; when this was observed the injections were stopped one minute after respiration had been re-established.

This secondary depression has also been observed by Das (1939). When leptazol is effective, however, it reduces the anaesthetic duration most markedly. Animals frequently appeared quite conscious within about ten minutes of injecting the drug, although a relapse into deep anaesthesia was common, especially when

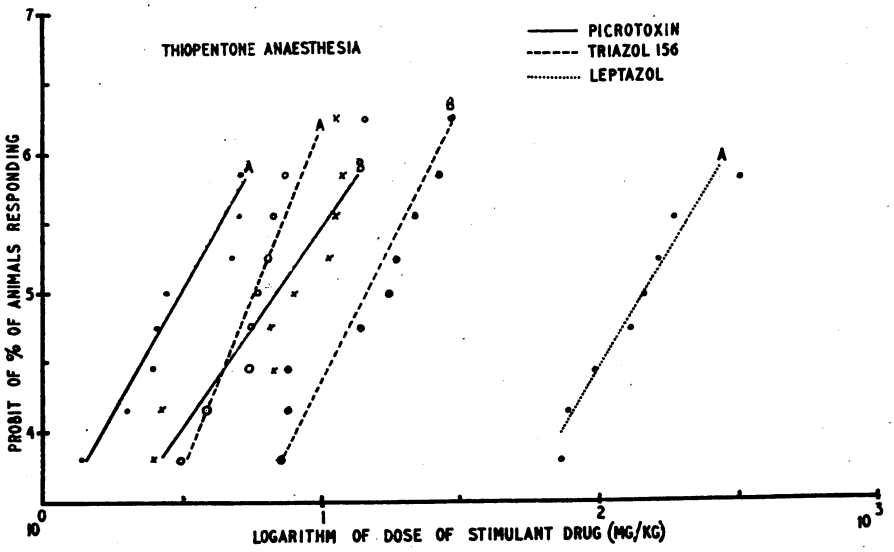

FIG. 1.

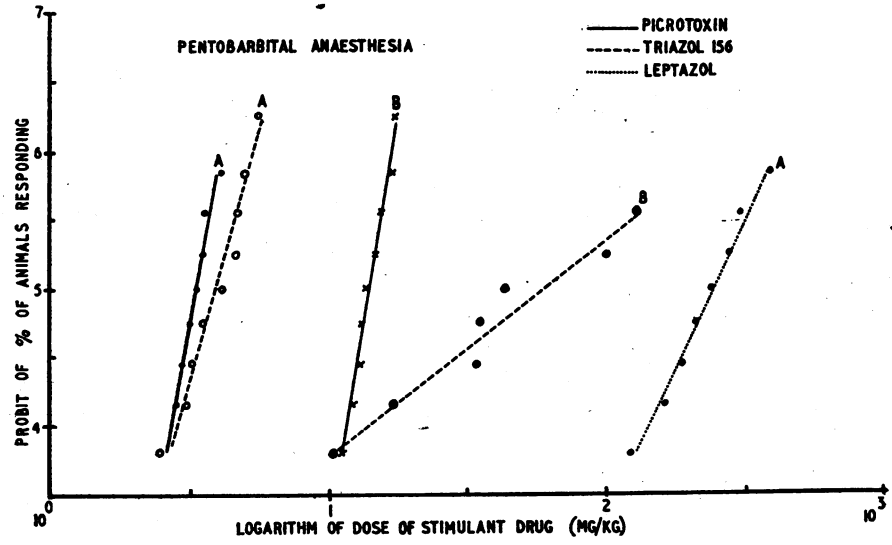

FIG. 2.

pentobarbital was used. This awakening effect was noted by Trevan, and can, in the mouse tests, give a false impression of the efficiency of this drug. Probably the best analeptic of the three tested is triazol 156, since it does not cause the muscular jerking shown by picrotoxin, although larger doses are required.

The method has been used to examine a series of synthetic compounds the results of which will be reported shortly. A compound can be examined in approximately three hours, and the method gives a result under conditions comparable with those of the projected therapeutic application. The method gives no numerical index of the duration of action of these drugs, but transient stimulation while injection is being performed has frequently been observed with new compounds. Whenever this test has indicated useful analeptic activity it has been our practice to use the method described by Das in order to assess the duration of action of the drug, given by various routes, but the method described here provides a valuable primary measure of stimulant activity.

Summary

A method of comparing the respiratory stimulant properties of analeptics is described. Guinea-pigs are anaesthetized by a continuous infusion of short- or long-acting barbiturates ; when respiration ceases for twenty seconds the analeptic is also infused. The doses of analeptic required both to restart respiration and to cause over-stimulation are measured.

A comparison of picrotoxin, leptazol, and triazol 156 by this method is described.

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