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A METHOD FOR THE EVALUATION OF ANALGESIC ACTIVITY USING RATS

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Various methods for the experimental evaluation of analgesic activity have been described. They are all based upon the change which occurs in the response of the experimental subject to a painful stimulus after dosage with an active compound. Various animal species as well as man have been used as experimental subject and a variety of stimuli employed. In the examination of new compounds for analgesic activity it is undesirable to use the human subject, and if the number of compounds is large the smaller laboratory animals, rats or mice, become the animals of choice. We have tested a number of methods in which small animals were used, but none has proved entirely satisfactory.

In the ideal method the stimulus employed should provoke a characteristic and readily observed response and the intensity of the stimulus required to elicit it should vary little from animal to animal ; the method should yield reproducible results and these should be in agreement with clinical experience.

A method for analgesic assay using rats is described below which in our experience has been found to satisfy these criteria. It is a simple modification of the method of D'Amour and Smith (1941, 1943), which was itself adapted from the work of Hardy, Wolff, and Goodell (1940) in man.

Apparatus and Procedure

The method is based upon the reaction of the rat to a heat stimulus applied to a small area of the tail.

The apparatus can be constructed from material commonly available in the laboratory. It consists essentially of a sheet of asbestos board (e.g., uralite), 1/8 in. thick and about 4 in. square, supported horizontally and having on its upper surface two strips of the same material, 4 in. long, fixed in such a way as to leave a channel about 1/4 in. wide between them. At some point along this channel a hole 1/4 in. in diameter is drilled through the asbestos sheet and a small coil of resistance wire connected through a key to a 6-volt electrical supply fixed beneath it. The wire is of such a gauge and length that with the circuit closed it is raised to a bright red heat.

The rat under test is held in a cylindrical holder of perforated zinc, clamped horizontally ; its tail lies along the channel and over the hole, which must be not more than 1½ in. from its tip. When the animal has become quiet in this position the circuit is closed. After an interval the animal will withdraw its tail from the channel with a sudden and characteristic flick. This interval is timed with a stopwatch and is referred to as the *reaction time*.

Rats weighing between 120 and 160 g. with clean and healthy tails are used for experiment. The heating coil is adjusted initially to such a position that a majority of the animals react at about 5 seconds. The normal reaction time of a number of rats is determined precisely, the mean of three successive determinations at 2-minute intervals being taken, and those for which it is between 4 and 6 seconds are divided into groups of a convenient size. It has been found impracticable to deal with a group of more than six rats at a time. The compound under test is then given by the chosen route and at the desired dose level, and the reaction times of the rats are thereafter determined at 15-minute intervals. Analgesia is reflected in a prolongation of the reaction time; the increase over normal in the mean reaction time of the treated animals is taken as a measure of analgesic effect, and is hereafter referred to as *effect*.

When analgesia is marked, heating may be continued until the tails are severely burned without eliciting any reaction. To avoid unnecessary damage to the tails, heating is never continued for longer than 15 seconds, and if an animal has not reacted in that time analgesia is assumed to be "complete."

In preliminary experiments we were able by this technique to demonstrate analgesic activity for morphine, codeine, and pethidine, given intraperitoneally. Aspirin and phenazone, given by this route, appeared inactive in doses approaching the median lethal, but intravenously they produced readily measurable effects at much lower doses. In view of this result, intravenous administration seems preferable when new compounds are being examined for analgesic activity for the first time and was adopted for the remainder of our experiments. Injections were made into the tail vein in the third near to the root of the tail. Morphine, codeine, and pethidine were used as their soluble hydrochlorides, phenobarbitone as its soluble sodium salt, aspirin as its sodium salt by neutralizing the acid with N. NaOH to *p*H 6–6.5, phenazone in aqueous solution, and hashish as described below p. 262).

RESULTS

One drug examined fully by the procedure described above was pethidine hydrochloride. The mean analgesic effect at varying times after single intravenous doses of 1 to 15 mg./kg. is shown in Table I, where the number of rats used at each dose is also given, and plotted in Fig. 1. In computing mean values a reaction time of 15 seconds was assigned to animals showing "complete" analgesia.

TABLE I

ANALGESIC EFFECT OF PETHIDINE

Dose mg./kg. (i.v.)	No. of rats	Mean effect in seconds at the following times after injection					
		15 mins.	30 mins.	45 mins.	60 mins.	75 mins.	90 mins.
15	5	10·8	10·6	4·7	2·2	1·2	0·3
10	9	8·1	6·4	2·9	1·3		
7·5	10	6·8	2·5	0·9	0·2		
5	10	3·3	1·3	0·4	0		
2·5	9	3·3	1·7	0·4			
1	9	0·3	−0·1	−0·2			

It will be seen from Fig. 1 that the highest recorded effect was obtained 15 minutes after dosing. This was the case for other drugs examined in the same way.

In order to use this method for assay, the relationship between dose and response must be determined, and to do this it is necessary to decide on a measure of the response to any given dose. For this we could take either (a) the effect at a given time after dosing, or (b) the time taken to produce a given effect. The former is the easier to determine and the more reliable. The recorded effect at 15 or at 30 minutes after dosing or a value obtained by smoothing the curve might be used, but there is little to be gained by the latter procedure since by far the

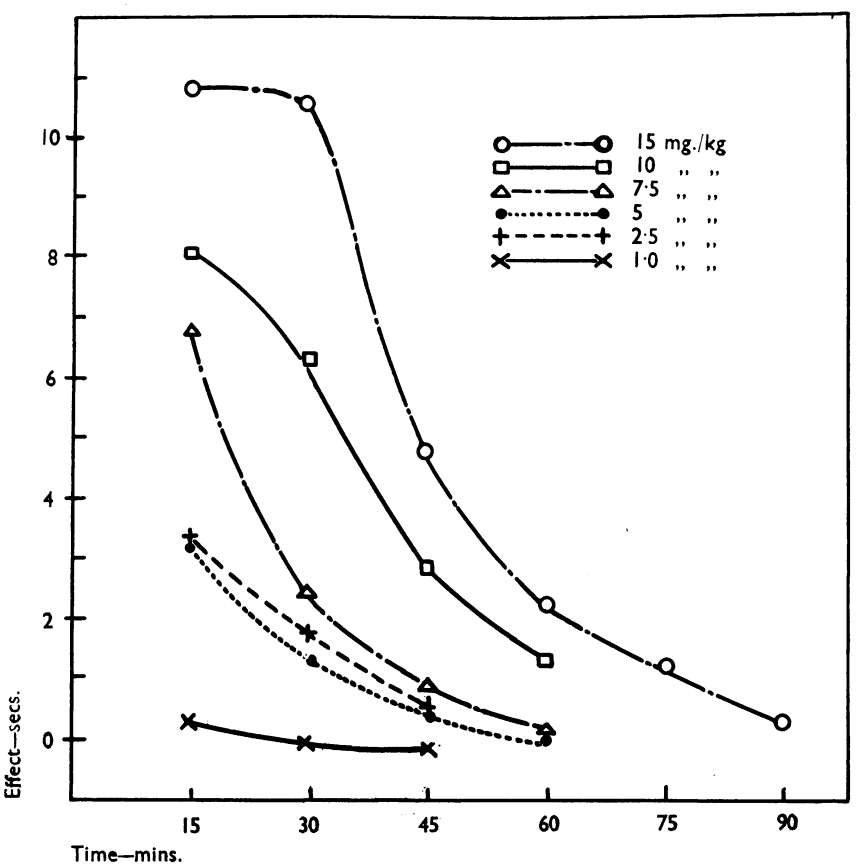

FIG. 1.—Analgesic action of pethidine. Effect plotted against time for doses from 1 to 15 mg./kg.

greatest source of error is the variation between the rats themselves. From an examination of the results for pethidine and other drugs, the recorded effect at 15 minutes was found to be a better measure than that at later times because a smoother and steeper curve was thereby obtained for the relationship between dose and response. It was therefore adopted as the basis of assay.

The reaction time at any given time after dosing for each rat is expressed as an average of three readings. The variation between repeat readings is considerably less than the variation between individual rats and therefore no worthwhile gain in accuracy can be obtained by taking more than three. A further appreciable increase in accuracy could only be obtained by increasing the number of rats used.

The mean effects 15 minutes after injection, the doses, and the number of rats per dose for a number of drugs are given in Table II. In Figs. 2 *et seq.* the mean effect at 15 minutes is plotted against log. dose.

TABLE II

Drug	Test								
Morphine	A	Dose mg./kg. ..	10	5	4	3	2·5	2	1·5	1
		No. rats	3	3	9	10	10	10	10	11
		Mean effect (secs.)	10·5	10·8	9·5	8·2	4·7	3·6	1·9	0·2
Codeine	A*	Dose mg./kg. ..	25	20	15	12·5	10	7·5	5	
		No. rats	4	5	9	10	10	—	4	
		Mean effect (secs.)	10	9·9	10·1	7·8	1·1	—	0·4	
	B	No. rats	—	—	10	10	10	10	10	
		Mean effect (secs.)	—	—	8·7	5	5·1	3·2	1·1	
Pethidine	A	Dose mg./kg. ..	15	10	7·5	5	2·5	1		
		No. rats	5	9	10	10	9	9		
		Mean effect (secs.)	10·8	8·1	6·8	3·3	3·3	0·3		
Hashish distillate	A	Dose mg. kg. ..	3	2	1	0·75	0·5	0·25	0·1	
		No. rats	10	10	10	10	10	10	10	
		Mean effect (secs.)	9·1	8·1	6·5	4	1·7	3·5	0·8	
Phenazone	A	Dose mg. kg. ..	500	350	250	150	100			
		No. rats	9	10	15	15	10			
		Mean effect (secs.)	9·8	8·3	6·2	3·8	1·8			
Aspirin	A	Dose mg. kg. ..	725	600	500	425	350			
		No. rats	10	9	10	10	10			
		Mean effect (secs.)	7·4	6·4	5·4	3·9	2·7			
	B	No. rats	8	9	10	10	10			
		Mean effect (secs.)	8·8	6·3	4·1	4·1	2·9			
Pheno-barbitone	A	Dose mg./kg. ..	100	75	50	37·5	25	17·5	10	
		No. rats	26	28	32	30	35	30	29	
		Mean effect (secs.)	0·4	1·3	1·8	2·1	3·9	1·8	0·5	

* The figures for Test A were obtained in preliminary experiments before the apparatus and technique were standardized.

The relationship is substantially linear over a wide range for all the drugs examined with the exception of phenobarbitone, which will be discussed below. For extreme maximal and minimal effects the curve flattens out as, of course, it must. No transformation of the effect variable is thus required provided reasonable care is exercised with extreme values. In certain cases it is well to omit

results obtained with doses at the extreme ends of the range—for example, of the figures given in Table II, that obtained with 10 mg./kg. morphine should be ignored. In an assay of the activity of a sample of a known drug, a range of doses which produces intermediate effects, e.g., 2 to 8 seconds, should be used.

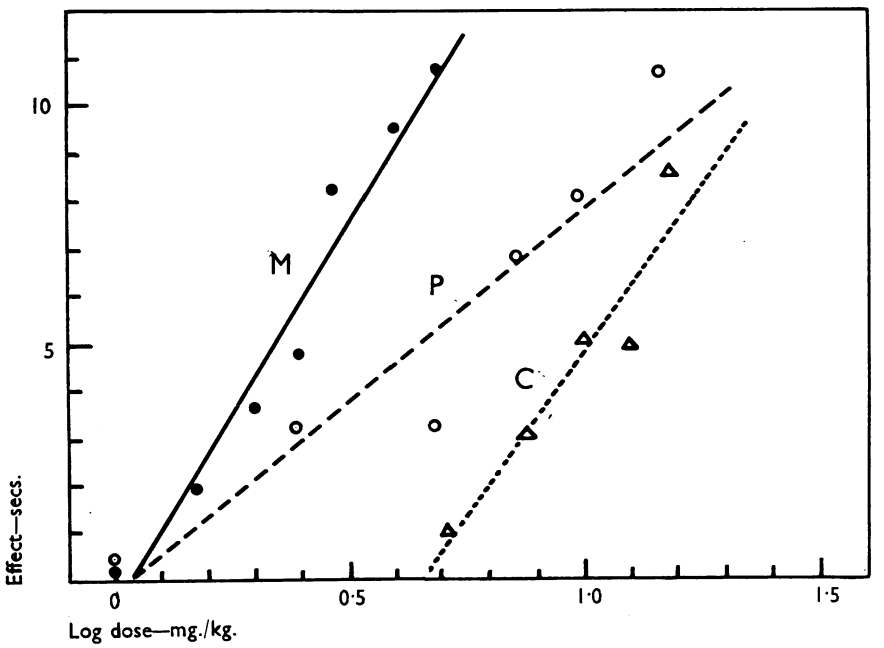

FIG. 2.—Analgesic action of morphine (M), codeine (C), and pethidine (P) administered intravenously. Effect at 15 minutes after injection plotted against log. dose.

Sources of error

The main source of error in determining the log. dose–response line is the variation between individual rats. In our experiments, this variation was related to the magnitude of the mean effect, being least for maximal and minimal effects. Except for these extreme values, however, the standard deviation was practically constant for different doses. Its average value was about 3 seconds for all the drugs examined.

Another source of error is that different results may be expected from experiments carried out on different days. When comparing the activity of two or more samples of any drug, the variation due to this factor may be eliminated by ensuring that in each group tested at one time all samples are equally represented. There is evidence that error has arisen from this source in the results reported here, but owing to the accidental arrangement of the treatments it has not been serious.

An overall measure of the error in these experiments may be obtained by calculating the variance about the best line of fit. Combining the results of all the experiments except those with phenobarbitone, we find that the variation about the linear regressions is no greater than expected from the standard deviation of 3 seconds for individual rats in each group. This indicates that the linear regressions give a satisfactory fit.

From the regressions we may calculate the doses required to produce an increase in reaction time of 5 seconds. These are shown in Table III together with the slopes of the regression lines for the various drugs examined (except phenobarbitone).

TABLE III

Drug	Slope	Standard error of slope	Dose to give mean response of 5 seconds	Standard error	Expected standard error of comparison of two samples with 100 rats each
			mg./kg.	%	%
Morphine	16·1	1·8	2·2	6	6·5
Codeine (Test B) ..	14	2·5	10	7·5	7·5
Pethidine	8·1	1·1	4·6	13	13
Hashish	5·7	0·8	0·72	16	19
Aspirin (Test A) ..	15	3·8	490	7	7
Aspirin (Test B) ..	17·4	4	490	7	
Phenazone ..	11·6	1·7	169	8·5	9

The slopes for morphine, codeine, aspirin, and phenazone do not differ significantly from one another. The slopes for pethidine and hashish are significantly lower than the remainder with the possible exception of phenazone.

The standard errors quoted above refer to the reproducibility within the conditions of the experiment. It is to be expected that larger variations would arise, especially in the dose required to give a mean response of 5 seconds, if a different batch of animals were used or if the experiment were repeated at a different time. Some measure of control is obtained by selecting rats with normal reaction times of 4 to 6 seconds, but we cannot say how effective this is in controlling the response at any given dose level.

The figure in the last column in Table III represents the standard error of a comparison of the activity of two samples of each drug when 100 rats are used for each sample and the following conditions are observed:

(a) That the two samples are equally represented in each group of rats treated and tested together.

(b) That the order in which the doses are given is randomized.

(c) That the dose range is so chosen that the mean effect in all cases falls on the linear portion of the log. dose–response curve. (Simplification of the analysis results if the doses are taken at equal logarithmic intervals.)

If the experiment continues over two days the whole test should be considered as two complete replicates, with 50 rats per sample in each. This idea can be carried further and the experiments regarded as 4 complete replicates with 25 rats per sample in each. Further subdivision is impracticable.

For comparing the analgesic potency of different drugs we adopt as a standard of comparison the dose of each required to produce an increase in mean reaction time of 5 seconds. This increase represents approximately 50 per cent of the maximum increase which can be measured.

The doses of various analgesics required to produce this effect are shown in Table IV.

TABLE IV

Drug	Dose required to produce an increase in mean reaction time of 5 seconds	
	Intravenous administration	Intraperitoneal administration
	mg./kg.	mg./kg.
Morphine	2·2	10
Codeine	10	30
Pethidine	4·6	30
Aspirin	490	Inactive
Phenazone	169	,,
Hashish distillate	0·72	,,
Phenobarbitone	*25	,,

* This dose produced an increase of only 4 seconds, the maximal obtainable with phenobarbitone.

Further observations upon the analgesic action of these drugs are given below.

1. *Morphine and codeine.*—Both these are active when given by intraperitoneal or intravenous injection. Rats dosed with them and showing pronounced analgesia as indicated by a marked increase in reaction time appeared normal in other respects. From Table IV it appears that morphine is about five times as active as codeine when both are given intravenously and three times as active intraperitoneally. This is in fair agreement with clinical experience and with the results obtained by Woolfe and MacDonald (1944), using mice.

2. *Pethidine.*—The response produced by increasing intravenous doses of pethidine is shown in Figs. 1 and 2. The slope of the log. dose–response curve is somewhat less than that for morphine or codeine, indicating that with increasing doses of this drug its analgesic action, relative to that of either of the others, becomes less.

This result confirms that of Woolfe and MacDonald (*loc. cit.*) and suggests that pethidine is relatively less active than morphine in the control of the more intense forms of pain.

3. *Aspirin and phenazone.*—As already mentioned, aspirin produces detectable effects after intravenous administration only. Even so the dose required to produce any effect is enormously greater than with pethidine or the opiates.

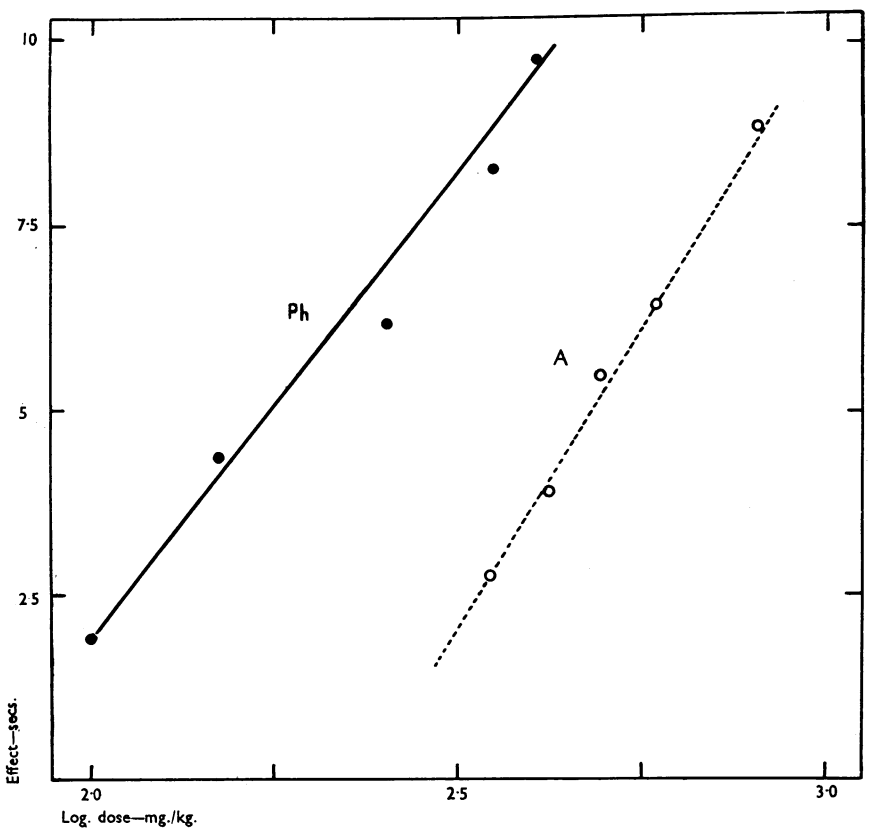

FIG. 3.—Analgesic effect of phenazone (Ph) and aspirin (A), administered intravenously. Effect at 15 minutes after injection plotted against log. dose.

Phenazone has a slight action when given intraperitoneally in large doses just below the median lethal. When given intravenously, it produces clearly demonstrable effects at lower doses (Fig. 3).

4. *Hashish.*—A sample of total distillate of hashish, for which we are indebted to Professor A. R. Todd, was examined by our technique. No analgesic effect was observed when a fine aqueous emulsion of the distillate was given intraperitoneally

or intravenously to rats. With the highest doses given, the animals died in convulsions shortly after being dosed.

Acetone solutions of the distillate, given intravenously, produced analgesic effects, but the results were vitiated by damage to the tails at the site of injection and the animals developed haematuria. After trials with various solvents we found that by diluting an acetone solution of the distillate with defribrinated rat blood a preparation suitable for assay by our technique was obtained. Great care had to be taken to keep the acetone content to a minimum, otherwise intense haematuria resulted. Not more than 0.1 ml. of acetone solution to every 1 ml. of blood was finally used in our experiments. Used in this way the distillate produced measurable effects in very small doses (0.1 mg./kg.). "Complete" analgesia was

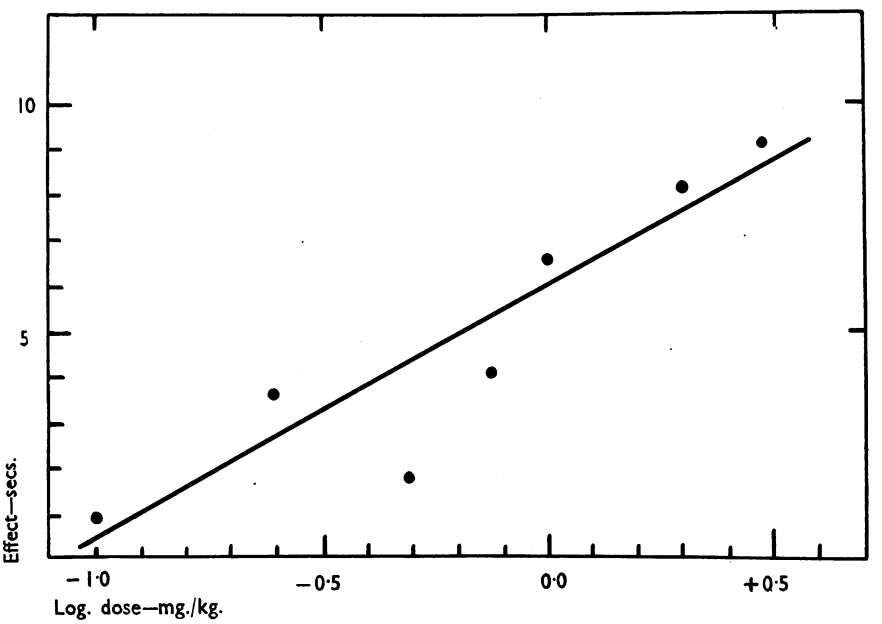

FIG. 4.—Analgesic action of hashish distillate administered intravenously. Effect at 15 minutes after injection plotted against log. dose.

only obtained with a dose fifty times as great as this, i.e., 5 mg./kg., which is very close to the median lethal. The slope of the log. dose–response curve (Fig. 4) was the lowest obtained with any drug examined. This is probably due to the fact that the sample tested was a mixture of the several active constituents of hashish.

5. *Phenobarbitone.*—Barbiturates are reputed to be mild analgesics. We were unable to demonstrate any analgesic effect with phenobarbitone given intraperitoneally in doses up to and including hypnotic doses. With still higher doses, near to the median lethal, an effect is obtained which we attribute to the general depressant action of the drug. On the other hand if phenobarbitone is given intravenously analgesia is produced by doses between 10 to 100 mg./kg. As the

dose is increased from 10 mg./kg. the analgesic effect increases to a maximum which is obtained with a dose of about 25 mg./kg. Further increase in the dose leads to a diminution of the effect until when hypnotic doses are attained analgesia

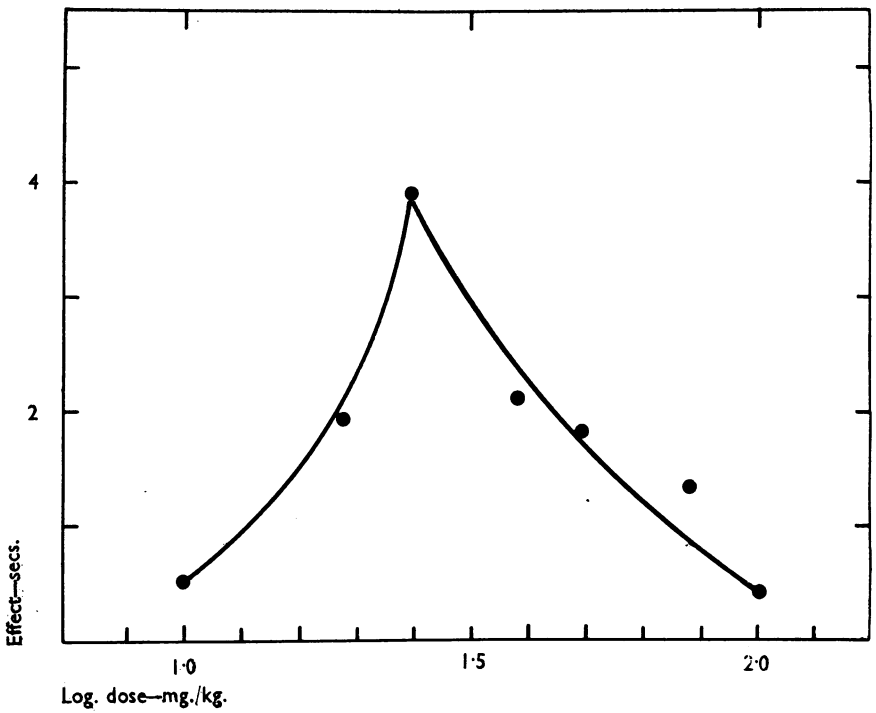

FIG. 5.—Analgesic effect of phenobarbitone administered intravenously. Effect at 15 minutes after injection plotted against log. dose.

is no longer observed. The results reproduced in Table II and Fig. 5 are the means of three different experiments using about 70 rats in each.

It is of interest to note that the analgesic effect of morphine can be demonstrated in rats fully anaesthetized with phenobarbitone. The results demonstrate the complexity of the action of analgesic and hypnotic drugs.

SUMMARY

A new method is described for the detection and evaluation of analgesic activity, making use of the response of rats to a heat stimulus applied to the tail.

By the use of this method the analgesic activity of morphine, codeine, pethidine, aspirin, phenazone, hashish, and phenobarbitone have been compared.

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