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A METHOD FOR TESTING ANALGESICS IN MICE

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The methods available for testing the analgesic activity of a compound in animals can be divided into three groups, depending on the device used for producing pain. D'Amour and Smith (1941), Thorp (1946), Davies, Raventos, and Walpole (1946), and Woolfe and Macdonald (1944) used heat as the physical agent for producing pain. Eddy (1928, 1932), and Green, Young, and Godfrey (1950) used mechanical pressure; Macht and Macht (1938) and Dodds, Lawson, Simpson, and Williams (1945) used electric shocks for the same purpose. The methods of Thorp (1946) and of Davies, Raventos, and Walpole (1946) have both been carefully studied in this laboratory without much success, but a method devised by Reinhard and E. J. de Beer has given more satisfactory results. This method has not been published by the authors, but has been described with their permission by Burn (1950), who suggested that the estimation of the degree of analgesia in each mouse should be abandoned, and that the percentage of mice in which there was some analgesia should be recorded instead. This has now been done and a linear relation between the dose and the percentage of mice showing analgesia has been observed. The opportunity was taken to compare various analgesics by this method.

METHOD

An electric shock is applied to the tail of a mouse and the squeak is taken as a response to the stimulus. The mouse is placed in a holder which consists of three components made of plastic. The sides and the top are made of one piece, while the front piece is attached to a long base which can be moved in and out. The back piece can be moved in a vertical plane and has a groove which allows the tail to protrude when the mouse is in the holder. The tail is cleaned with ether and electrode jelly is rubbed on it. The jelly must be rubbed in and not just smeared, otherwise a good contact is not made with the electrodes. The electrodes are two pieces of clock spring 1 cm. wide and 1.5 cm. apart with weights on the ends to keep them pressed on the tail. The circuit used is shown in Fig. 1. Leads from A.C. mains go to a variac and then to a small 6/1 transformer. There is a circular contact which rotates once a second and completes the circuit for 1/26th sec. A voltmeter is in the circuit between the transformer and the stimulating electrodes and there is also a key in this circuit. When the key is closed the circuit is completed and current flows to the electrodes. Before starting the test the variac is so adjusted that it gives a voltage of 8.5 volts at the electrodes and this is read on the voltmeter. The electrodes are dropped on the tail of the mouse and the key is closed. The number of shocks required to cause a squeak is counted, each shock being indicated by a flick on the voltmeter.

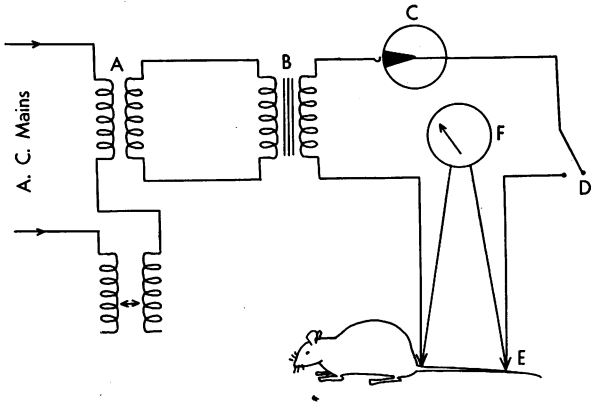

FIG. 1.—A diagram of the circuit used for applying stimuli to the mouse tail in test for analgesics. A, variac; B, 6/1 step down transformer. C, a rotary make-and-break contact which completes a revolution once every second and the contact is made for 1/26th of a second; D, key; E, stimulating electrodes; F, voltmeter which flicks every time a shock is applied to the tail.

Carrying out the test.—When a voltage of 8.5 volts was used for stimulation about 85 per cent of mice squeaked within five shocks and those which did not squeak within five shocks were not used for the experiment. As soon as the mouse squeaked the key was opened and the stimulation stopped. Groups of 10 mice weighing 20–25 g. were used for the test. Each mouse was examined before giving the analgesic and the number of shocks required to make it squeak was determined. The majority of mice squeaked after the same number of shocks when tested a second time after an interval, but a certain percentage varied within limits of two shocks. It was therefore decided that unless the difference between the number of shocks required to produce a squeak before and after an analgesic was greater than three it was not taken as evidence of analgesia. The drug was given by subcutaneous injection in the back, and 15 min. later the number of shocks required to cause a squeak was determined again. The same dose of analgesic was tested in at least six different groups of 10 mice each. Not less than 60 mice should be used for any one dose of analgesic. The results were expressed as the percentage of mice showing analgesia after a given dose of analgesic and a linear relation was observed between the dose and the percentage of mice showing analgesia. The same group of mice was not used more than twice a week.

RESULTS

The following compounds were tested for their analgesic activity: morphine hydrochloride, pethidine hydrochloride, amidone, phenadoxone (heptalgin), and phenazone.

Table I shows the results obtained with these five analgesics, the number of mice used for each dose, and the percentage of mice showing analgesia.

Fig. 2 shows the results obtained with morphine, pethidine, amidone, and phenadoxone; the percentage of mice showing analgesia has been plotted against the log of the dose. The data have been analysed statistically by Dr. D. J. Finney, and he reports that " the test of significance of deviations from linearity of probit regressions

$$\chi^2 = 2.17 \text{ with 6 degrees of freedom.}$$

As a test of deviation from parallelism

$$\chi^2 = 1.55 \text{ with 3 degrees of freedom.}$$

Neither is anywhere near to being significant."

TABLE I

PERCENTAGE ANALGESIA IN MICE AFTER SEVERAL ANALGESIC DRUGS

Morphine HCl			Amidone			Phenadoxone			Pethidine HCl			Phenazone		
Dose mg./kg.	No. of mice	% analgesia	Dose mg./kg.	No. of mice	% analgesia	Dose mg./kg.	No. of mice	% analgesia	Dose mg./kg.	No. of mice	% analgesia	Dose mg./kg.	No. of mice	% analgesia
1.5	103	18.4	1.5	60	23.3	0.75	90	34.4	5	60	21.7	200	32	25
3.0	120	44.2	3.0	110	49.1	1.5	80	67.5	7.5	85	31.8	400	78	33.5
6.0	123	67.5	6.0	100	81.0	3.0	90	88.9	10	60	53.3			
									15	90	61.1			
									20	60	73.3			

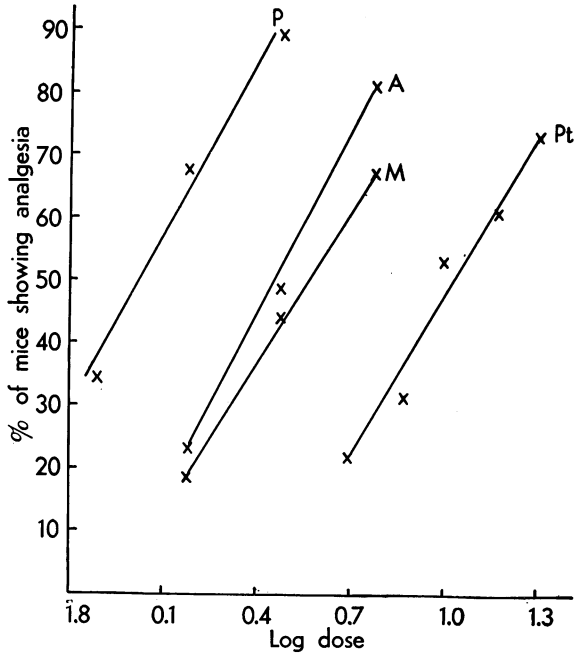

FIG. 2.—To show the relation between log dose of analgesic (abscissae) and the effect, measured as percentage of mice showing analgesia (ordinates). P=Phenadoxone. A=Amidone. M=Morphine. Pt.=Pethidine. Note the linearity of the regression lines and their parallelism.

It is convenient to express the activity of a new compound in terms of another compound of known activity and this has been done by comparing the doses required to produce 50 per cent analgesia. The potencies of each of the other three drugs relative to morphine have been calculated by Dr. Finney. The results are:

Amidone	1.25 (with	95%	fiducial	limits	at	1.03,	1.54)
Phenadoxone	3.56 („	95%	„	„	„	2.89,	4.44)
Pethidine	0.33 („	95%	„	„	„	0.277,	0.402).

Phenazone, even in big doses, when given by mouth did not show any marked analgesic activity. When 200 mg. phenazone per kg. mouse was given orally,

only 24 per cent of the mice showed analgesia and on doubling the dose to 400 mg./kg. the percentage of mice showing analgesia rose to 33.3; 800 mg./kg. of phenazone was a lethal dose for mice.

Discussion

Basil, Edge, and Somers (1950) while testing analgesics in rats obtained a value of 3.8 as the ratio of phenadoxone to morphine and 2.9 as the ratio of phenadoxone to amidone. The majority of workers using radiant heat stimulation methods have reported that amidone is 1 to 1.3 times as potent as morphine (Thorp, Walton, and Ofner, 1947; Cahen, Epstein, and Krementz, 1948; Thorp, 1949; Bonnycastle and Leonard, 1950). The figures obtained by the method described in this paper agree very well with those obtained by the heat method in rats.

Smith, D'Amour, and D'Amour (1943) by their method found that analgesics like amidopyrine did not confer analgesia unless very big doses were given, and the same was true with the Woolfe and Macdonald method (1944). The method described in this paper also did not show evidence of analgesia when small doses of phenazone were given. It seems that these compounds are too weak to raise the threshold of pain in an animal to a degree sufficient to prevent it from giving the response to the stimulus. In therapeutics the salicylate and antipyrine group of analgesics are useful in alleviating a certain type of pain originating from integumental structures only and not in other types of pain. Hardy, Wolff, and Goodell (1940) found that the threshold for cutaneous pain in human subjects, as determined by heat, was only increased to 35 per cent by 1.8 g. aspirin given orally, whereas an increase of 70 per cent was noted after injecting 15 mg. morphine sulphate intramuscularly.

" Squeak " as a response to painful stimulus is preferable to any other reflex mechanism involving only the spinal cord. Green, Young, and Godfrey (1950) in their comparison of pressure and heat analgesiometric method observed that the normal thresholds for the heat reaction time and " struggle " had a greater variance than the " squeak "; this they attributed to the greater degree of pain required to elicit a squeak. As evidence for this the authors quote unpublished observations in collaboration with White, that the variances decreased as the degree of pain increased. The use of a stimulus to produce a greater degree of pain would, therefore, make the method less suitable when testing substances with weak analgesic action, but the sharpness of the end-point as in a " squeak " is to be desired in a quantitative estimation. Moreover, a squeak involves the participation of the higher centres of the animal and, as analgesics are used in therapeutics for alleviating pain in man which is mediated through the thalamus and cortex, it seems desirable to have a method for testing analgesics which involves the participation of these centres and not merely a reflex of lower order, such as removal of the tail.

Summary

1. A method for testing analgesics in mice has been described, using the apparatus originally proposed by Reinhard and de Beer. An electric stimulus is applied to the tail of the mouse and the squeak is taken as a response to the stimulus. The result is calculated on the basis of percentage of mice showing analgesia after the injection of the analgesic. The essential modification in this method is that instead

of assessing the degree of analgesia in each mouse, the percentage of mice showing analgesia is ascertained.

2. Morphine, pethidine, amidone, and phenadoxone have been tested and their activity is expressed in terms of morphine. When the activity of morphine is taken as 1, that of pethidine is 0.33, that of amidone is 1.25, and that of phenadoxone is 3.56.

Phenazone when given by mouth in big doses did not show any marked analgesic activity.

It is a great pleasure to acknowledge my indebtedness to Professor J. H. Burn for his guidance throughout the course of this work. I wish to thank Dr. D. J. Finney for statistical analysis of the data.

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