16294

A Biological Method for Determination of Curare and Erythroidine-Alkaloids.

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Recent studies[1] have shown that mice pretreated with morphine sulfate are especially sensitive to curare and erythrina-alkaloids. Since these alkaloids in microgram amounts antagonized both excitement and the characteristic Straub-phenomenon[2] on the tails of morphinized mice before any peripheral paralyzing action became evident, it was of interest to investigate whether this procedure could be employed as a sensitive biological method for the determination of low concentrations of these alkaloids.

Two methods which permit the estimation of curare *in vitro* have been developed which make use of isolated mammalian preparations: one method (Bülbring-Chou[3]) employs the phrenic nerve diaphragm preparation from the rat and tests the curare depressant effect of rapid motor nerve stimulation; the other (P. D. Garcia de Jalon[4]) is based on the well known antagonistic action of curare[5] on the acetylcholine contracture in *M. rectus abdominis* of the frog. These *in vitro* methods provide a rapid check on pure curare preparations, but are less useful for the exact measurement of impure preparations and tissue extracts. The rabbit head drop method of McIntyre and Holaday[6] provides an accurate *in vivo* assay for this type of preparation but requires the use of an appreciable amount of substance. Recently Skinner and Young[7] have proposed a mouse assay which is dependent on the peripheral paralyzing action of the alkaloids as determined in the sloping rotating cylinder apparatus of Young and Lewis.[8]

Method. Groups of 5 to 10 white mice, weighing 18-20 g each, are injected subcutaneously with 0.5 mg of morphine sulfate. Restlessness and the typical tail reflex occur generally within 5 to 10 minutes and persist for 2 hours or more. Doses smaller than 0.5 mg of morphine sulfate are not recommended because the characteristic morphine effect is either delayed or of not sufficient duration. Curare and erythrina preparations, dissolved in 0.85% sodium chloride, are injected intraperitoneally in a volume not exceeding 0.5 cc into animals exhibiting the typical morphine reaction. A positive effect appears within 5 to 10 minutes and is characterized by disappearance of the excitement phenomena and relaxation of the tails. This period lasts usually for 10-15-25 minutes and is followed by the gradual reappearance of restlessness and tail phenomenon. At this point, the mice may again be used for testing without danger of alteration of the alkaloid response since intraperitoneally injected curare is rapidly destroyed or excreted.

Table I shows the results obtained following the injection of graded doses of various curare and erythroidine preparations. From these data it can be determined that the median effective dose and its standard error of *d*-tubocurarine chloride is 2.8 ± 0.2 μg;

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2 Straub, W., *Deutsche med. Wchnschr.*, 1911, **37**, 1462; Straub, W., and Herrmann, O., *Biochem. Z.*, 1912, **39**, 216.

3 Bülbring, E., *Brit. J. Pharmacol. and Chemotherap.*, 1946, **1**, 38; Chou, T. C., *ibid.*, 1947, **2**, 1.

4 Garcia de Jalon, P. D., *Quar. J. Pharm. and Pharmacol.*, 1947, **20**, 28.

5 Riesser, O., and Neuschloss, S., *Arch. f. exp. Path. u. Pharmacol.*, 1922, **92**, 254.

6 McIntyre, A. R., and Holaday, H. A., quoted by Bennett, A. E., *Am. J. Psychiat.*, 1941, **97**, 1040.

7 Skinner, H. G., and Young, D. M., *J. Pharm. and Exp. Therap.*, 1947, **91**, 144.

8 Young, D. M., and Lewis, A. H., *Science*, 1947, **105**, 368.

TABLE I.
Graded Doses of Various Curare and Erythroidine Preparations Antagonizing the Effect of Morphine Poisoning on Mice.

Drug	Dose per 20 g mouse*	No. of animals	No. of animals responding with disappearance of morphine symptoms	Duration of curare effect in min.
Cryst. *d*-tubocurarine chloride	2.5 μg	10	5	5-10
	3.0 "	15	10	5-13
	5.0 "	10	10	25-50
Strychnos curare (Merck)	20.0 "	5	0	—
	30.0 "	5	5	12
	40.0 "	5	5	24
Cryst. dihydro-β-erythroidine-bromide	40.0 "	5	0	—
	50.0 "	10	10	18
	60.0 "	10	10	10
Intocostrin (Squibb)	15.0 milliunits	5	—	—
	20.0 "	10	5	26
	25.0 "	10	10	19
	30.0 "	10	10	17-35

* All animals were injected subcutaneously with 0.5 mg morphine sulfate 10 minutes before and showed typical symptoms of morphine poisoning.

TABLE II.
Doses of Various Alkaloids Influencing the Effect of Morphine Poisoning on Mice.

Drug	Dose in γ per 20 g mouse*	Effect on morphine excitement and tail phenomenon
Quinine methochloride	100	partial
" ethochloride	500	no effect
" sulfate	500	" "
Tetramethylammonium chloride	100	" "
	200	complete effect
Tetraethylammonium "	114	partial effect
Cryst. thiamine hydrochloride	500	no effect
	1000	complete effect
Acetylcholine "	50	no effect
Histamine "	300	" "
	400	complete toxic effect
Nicotine base	75	no effect
	100	partial toxic effect
Guanidine hydrochloride	1000	no effect
Bulbocapnine "	100	partial effect
	250	partial effect, quiet, tails erect
Scopolamine hydrobromide	100	no effect
	250	partial effect (excited)
	500	complete effect

* All animals were injected subcutaneously with 0.5 mg morphine sulfate 10 minutes before and showed typical symptoms of morphine poisoning.

for Strychnos Curare (Merck), 24.0 ± 2.0 μg; for dihydro-β-erythroidine bromide, 44.0 ± 3.0 μg; and for Intocostrin (Squibb), 20.0 ± 2.0 milliunits.

Several other drugs have been tested for their ability to antagonize morphine excitement and tail phenomenon. These results are shown in Table II. None of these drugs completely antagonize the effects of morphine poisoning in mice in doses smaller than 100 γ per 20 g mouse. Thiamine hydrochloride, quinine ethochloride, quinine sulfate, histamine hydrochloride, bulbocapnine hydrochloride, scopolamine hydrobromide, and guanidine

hydrochloride were without effect in doses of 400-1000 γ. Nicotine base exerted a partial effect in 100 γ dose; histamine was effective only in toxic amounts of 400 γ and acetylcholine chloride was ineffective in a dose of 50 γ. This analysis shows that although the several quarternary alkaloids and other drugs mentioned in Table II may influence the synaptic transmission or the myoneural junction, they do not compare with curare and erythroidine in their influence on the outward manifestion of morphine poisoning in mice. It may be noted here that Myanesin[9] [α,β-dihydroxy-γ-2-(methylphenoxy-propane)] in doses of 8 mg per mouse despite its curare-like action on the striated muscles, is not able to depress the tail reflex in morphinized mice. The data seem to indicate that because of the relatively small amounts of alkaloid required to produce a positive effect, this method may be useful for the determination of curare or erythroidine in tissue extracts or urine even though other active substances may be present.

Summary. A biological method for qualitative and quantitative determination of curare and erythroidine is described. The method depends upon the antagonistic action of these alkaloids on the excitement and tail phenomenon in morphine-poisoned mice. The median effective dose and standard error for crystalline *d*-tubocurarine chloride is 2.8 ± 0.2 μg; for Strychnos Curare (Merck), 24.0 ± 2.0 μg; for dihydro-β-erythroidine bromide, 44.0 ± 3.0 μg; and for Intocostrin (Squibb), 20.0 ± 2.0 milliunits. Far higher doses of other drugs, including quarternary alkaloids, are necessary to antagonize the effect of small amounts of morphine in mice.

9 Berger, F. M., and Bradley, W., *Brit. J. Pharmacol.*, 1946, **1**, 265.

16295

Effect of Anoxic Anoxia on Propulsive Motility of the Small Intestine.*

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It has been shown that although anoxic anoxia does not significantly affect the propulsive motility of the small intestine of the dog, it does so in the mouse.[1] Since there is this apparent specific difference it was deemed worthwhile to study the effect of anoxic anoxia on still a different species, namely, the rat.

Methods. Paired albino rats as nearly alike in weight and age as possible were fasted 24 hours. One served as a control and the other was subjected to anoxic anoxia. (In all, 42 control and 42 experimental animals were used.) The following partial pressures of oxygen were employed: 94 mm Hg, 80 mm Hg, 63 mm Hg, and 53 mm Hg; corresponding to simulated altitudes of 14,000, 18,000, 24,000, and 28,000 feet.

The experimental animal was given 2 cc of a charcoal-acacia mixture by stomach tube, and after allowing 10 minutes for some of this mixture to enter the small intestine, placed into a low-pressure chamber for 30 minutes. On removal from the chamber it was decapitated and the small intestine removed. The latter was slit open and the distance the charcoal-mixture had traversed the small intestine was measured. Control intubated animals were maintained at atmospheric pressure; in order to keep other experimental conditions as nearly alike as possible, they were placed in cages on top of the low-pressure chamber, so that they were exposed to the same noise and vibration as the experi-

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1 Van Liere, E. J., Northup, D. W., Stickney, J. C., and Emerson, G. A., *Am. J. Physiol.*, 1943, **140**, 119.