Action of Aconitine on the Cold-Blooded Heart.* (20589)

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Early studies on the cardiac action of aconite dealt largely with its use as a "sedative". A vagal action, supposedly central and antagonized by atropine, and a direct muscle action were recognized. Renewed interest has been concerned with those abnormal rhythms that may occur spontaneously or be induced by a single properly timed electric shock in a heart under the influence of that drug. Cushny (1) early held that the arrhythmias he observed resulted from 2 factors: 1) retarded recuperation of contractile and conductive power, and 2) an increased tendency to spontaneous movements.

The purpose of the present study has been to reexamine the action of aconitine on the primary properties of heart muscle. The heart of the turtle *Pseudemys elegans* was used as isolated whole heart, isolated auricles and ventricular strips, and a few observations were made on isolated frog hearts. Preparations were immersed in oxygenated phosphate buffered Ringer solution and temperature maintained at approximately 16°C. To follow mechanical activity whole auricles or ventricular strips were suspended in a muscle bath and the beat recorded on a smoked drum. Electrical activity was recorded by a Grass 2 channel amplifier with ink writer. Aconitine nitrate in concentrations ranging from 1:1 million to 1:250 was added to the bath.

Contractility. The auricular beat was early reduced by vagal action and could be promptly restored by atropine. This negative inotropic effect was independent of rate change. Later, contraction decreased either because of rate increase or direct muscle action. Contractility of ventricular strips was usually depressed, though at times the amplitude was maintained or temporarily increased, in spite of a higher rate of beating. Alternation, an effect emphasized by Cushny, rarely appeared in these preparations. Late muscle relaxation was the rule, and even with high concentrations diastolic shortening, a non-specific toxic effect, rarely occurred.

Rhythmicity. The rate response of the spontaneously beating auricle was variable and not always dependent on drug concentration. Usually, slowing and often standstill occurred within 2 or 3 minutes, or up to 12 minutes after adding the drug. From 10 to 20 minutes after that effect progressive quickening began and a high rate was maintained. The maximum rate recorded was 90 per minute. Early slowing or standstill was promptly relieved by atropine. In a few auricles early and progressive quickening occurred without previous slowing. Both early and late quickening could be slowed by acetylcholine, at times to standstill. In the initially atropinized auricle (atropine 1:10,000) brief slowing sometimes followed aconitine, but rate increase soon occurred. Extrasystoles were infrequent. In one frog preparation but in none of the turtle auricles did spontaneous fibrillation appear. In 2 spontaneously beating ventricular strips rate increase began 15 minutes after aconitine was added; in a third slowing with periods of standstill appeared after 3 minutes, with recovery 40 minutes later. In rhythmically stimulated ventricular strips spontaneous beating began from 3 to 90 minutes after aconitine, earlier with the higher concentrations. The maximum rate of spontaneous beating for these strips was 40. The appearance of spontaneous beating was independent of the effect of the drug on the threshold for electrical stimulation and could occur when threshold had been raised. With high concentrations extrasystoles and bigeminy appeared late and 1:500 caused late slowing. Ventricular fibrillation did not occur in any of the turtle preparations but appeared in one

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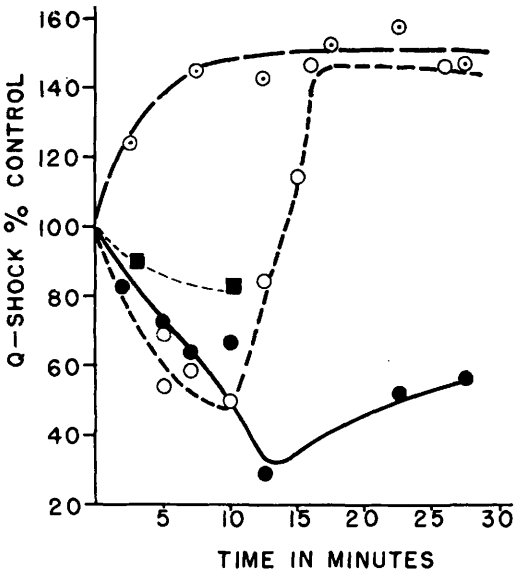

FIG. 1. Aconitine on auricular refractory period, measured as minimal shock to Q interval and expressed as % of control. Solid circles, aconitine alone (see text). Open circles, aconitine 1:50000 at zero time; atropine added after 10 min.; mean of 2 experiments. Dotted circles, aconitine 1:50000 added 12 min. after atropine at zero time; mean of 5 experiments. Solid squares, aconitine 1:500, 20 min. after atropine; 2 experiments. Atropine concentration 1:10000 in each instance.

of 5 frog hearts studied, and at a time of normal auricular activity. This sequence in the frog heart under aconitine was also observed by Cash and Dunstan(2) and suggests the importance of the vagus for the development of fibrillation. Rate increase in the spontaneously beating auricle and the initiation of spontaneous beating in ventricular preparations constitute the outstanding action of aconitine. The fact that in both preparations initial electrical wave forms were almost always maintained suggests that the effect is due to enhancement by the drug of the intrinsic rhythmic mechanism of the tissues.

Refractory period. In the spontaneously beating auricle aconitine greatly shortened the refractory period, measured as the minimal interval from a normal beat to the earliest shock followed by response. Shock strength and duration were kept constant throughout each experiment. Results for 4 groups of experiments, expressed as % of the control period, are shown in Fig. 1. Because of negligible differences with concentrations of 1:50,000 and 1:100,000 the mean value for those 2 in 8 experiments was used (solid circles). The apparent rise which began 15 minutes after the drug may be explained by threshold increase due to the drug or to increased rate of beating while shock strength remained unchanged. For drug concentrations up to 1:1000 the shortening was entirely vagal and promptly terminated by atropine (open circles). It has been shown that high concentrations of atropine affect the refractory period of the turtle heart only by vagal antagonism(3). In the initially atropinized auricle with aconitine concentrations less than 1:1000 the refractory period was not shortened. With concentrations of 1:500, shortening was produced by direct muscle action in a few experiments, but usually the early threshold rise and rate increase prevented refractory period measurement. In the forecited observations no distinction was made between single and multiple responses to a single shock. In certain auricles response followed shocks that arrived on the downstroke of the depolarization wave. The interval between that early point and the peak of the repolarization wave usually represented the time during which multiple response might follow single shocks. The period which separated single and multiple responses was highly critical, ranging from 0.05 to 0.10 sec. During

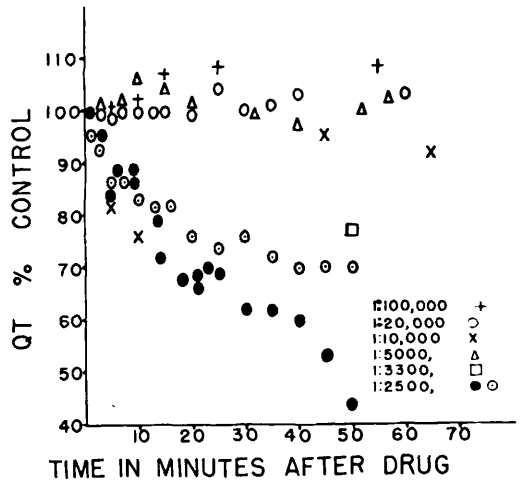

FIG. 2. Aconitine on ventricular refractory period, expressed as % change of Q-T interval of electrogram.

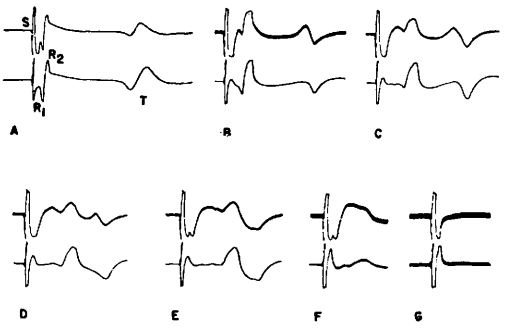

FIG. 3. Effects of aconitine 1:2500 on electrograms of a driven ventricular strip. A. Control. After aconitine: B. 11 min.; C. 25 min.; D. 40 min.; E. 45 min.; F. 50 min.; G. 60 min., no response.

auricular standstill due to aconitine the tissue responded regularly to a rapid series of shocks, and properly placed double shocks could evoke multiple response. Observations on the multiple response reaction seen in these experiments and its possible relation to the genesis of spontaneous arrhythmias will be published separately(4). Because of the tendency of aconitine to cause early spontaneous beating in driven ventricular strips, examination of its effect on ventricular refractory period was limited to a few observations on the Q-T interval of the electrogram. Although response to shocks arriving before T waves in the aconitine treated auricle suggests that the Q-T interval may not always be equivalent to the absolute refractory period, as was found in the normal and digoxin treated turtle ventricle(5), they may be expected to vary in the same direction. Only with concentrations greater than 1:5000 did significant Q-T shortening occur, Fig. 2. Marked Q-T shortening by 1:2500, followed by the appearance of diphasic and finally monophasic complexes is illustrated in Fig. 3. Such conversion to monophasic complexes by aconitine was early noted in the frog heart by Hermanns(6). Multiple response was not obtained in ventricular strips.

Threshold and conduction. The effect on threshold for electrical stimulation was observed in the ventricular strips from which the beat was mechanically recorded. Again, the early appearance of spontaneous beating limited the study. With aconitine concentrations up to 1:32,000 either slight increases or decreases occurred but in only one of 12 experiments was there a significant and sustained rise, Fig. 4. Rate increase alone will raise the threshold for stimulation. With a concentration of 1:500 the threshold for single extra shocks placed at the end of the recovery period rose promptly and even doubled before the

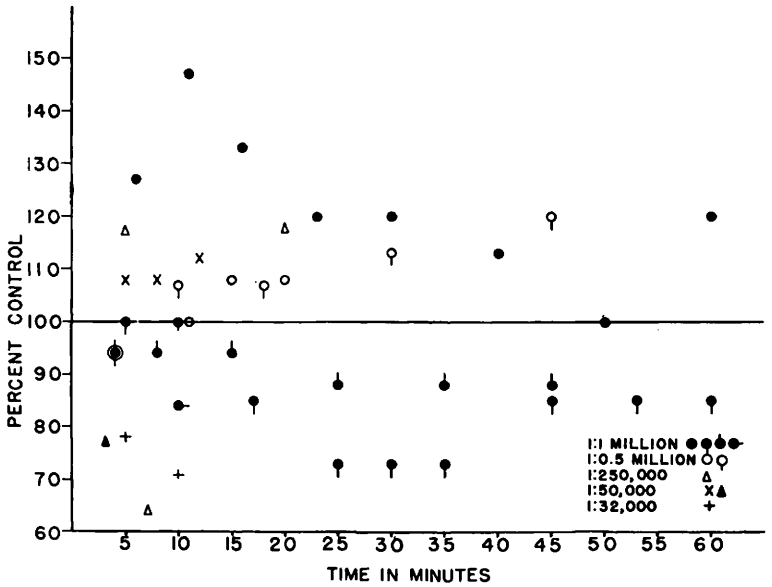

FIG. 4. Aconitine on resting threshold for electrical stimulation of turtle ventricle, expressed as % of control.

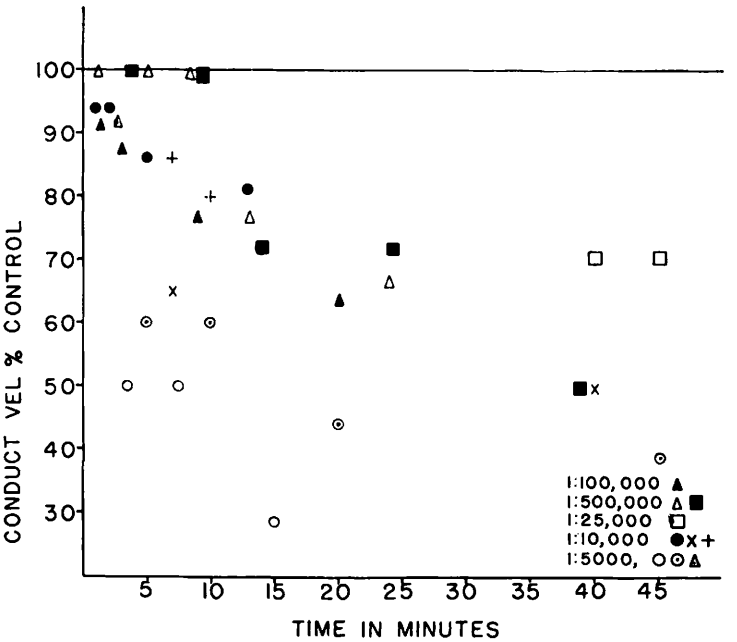

FIG. 5. Aconitine on conduction velocity in strips of turtle ventricle driven at 15 beats/min., expressed as % of control.

rate rise due to spontaneous beating. Subsequent threshold rise exceeded that anticipated for rate increase. Resting excitability was further studied in 5 preparations by determining strength-duration curves for threshold rectangular shocks that would drive ventricular strips at 15 per minute. With aconitine concentrations of 1:25,000 and lower minor variations in the rheobase were seen but calculation of chronaxie revealed no significant change. The early onset of spontaneous beating prevented detailed excitability studies with higher concentrations. As far as could be determined the appearance of a spontaneous rhythm was independent of any excitability change. *Conduction velocity* in driven ventricular strips was estimated from the R-R peaks of the electrograms with unipolar electrodes separated by about 15 mm. For the experiments recorded in Fig. 5 decreased velocity is shown. It is recognized that these may represent selected experiments since in others the distal intrinsic R deflection could not be identified. Moreover, in tissues with relatively poor initial conduction further depression will be more readily produced. There seemed no doubt, however, that aconitine was able to depress conductivity, at times by comparatively low concentrations.

In driven ventricular strips the electrical latent period, the interval from the shock to the depolarization wave, was prolonged only by aconitine concentrations greater than 1:20,000 (Fig. 3).

The QRS complex of certain ventricular electrograms was appreciably prolonged by aconitine, an effect not always related to concentration. In Fig. 3, showing the response to 1:2500, QRS after 60 minutes was 120% of the control. In another experiment the QRS interval was 150% of the control 30 minutes after 1:100,000. In many experiments the magnitude of the complexes was increased. The reason for those changes is not apparent. Since the electrodes were small wicks and the leads practically unipolar, such alteration may not be due solely to conduction effects but may represent some slowing by the drug of the depolarization process itself.

Summary. The effect of aconitine on the primary properties of heart muscle has been studied in the cold-blooded heart. Its constant action was to increase the rate of spontaneous beating or to initiate a spontaneous

rhythm in driven ventricular strips. This action appeared to be independent of change in resting excitability and to result from enhancement of intrinsic rhythmicity. Vagal action on the auricle depressed the pacemaker, decreased contraction amplitude and shortened refractory period. Contractility and refractory period changes were independent of rate change. In the atropinized auricle high concentrations shortened refractory period by direct muscle action. During the stage of shortened refractory period there was a critical interval when a single extra shock might elicit multiple responses. In the turtle ventricle, which has no vagal endings, high concentrations shortened refractory period, decreased conduction velocity and raised threshold for electrical stimulation.

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Effects of N-Methylformamide and Related Compounds in Mouse Sarcoma 180.* (20590)

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Recently a series of formamides was evaluated as possible solvents for parenteral administration of organic chemicals in the sarcoma 180 screening program of this Institute(1). The compounds proved too toxic for this purpose. However, 2 of the agents, formamide and N-methylformamide (Fig. 1), were found to inhibit the growth of the assay tumor. A third, N,N-diethylformamide, had questionable inhibitory activity whereas other members of the series were without effect. The present report is a study of the inhibitory actions of the formamides in sarcoma 180 (S-180). Special emphasis is given to the N-methyl derivative since it appears to be the most effective of the series. The inhibitory action of urethane against S-180, which has been mentioned previously(1), is also reviewed since the chemical configuration of this agent resembles that of the formamides (Fig. 1) and since it has been found effective against a variety of experimental and clinical neoplasms(2-5).

Tumor inhibition test. Female mice of the Swiss-Webster (CFW) strain weighing 18-22 g were employed. Small, uniform cubes (*ca*

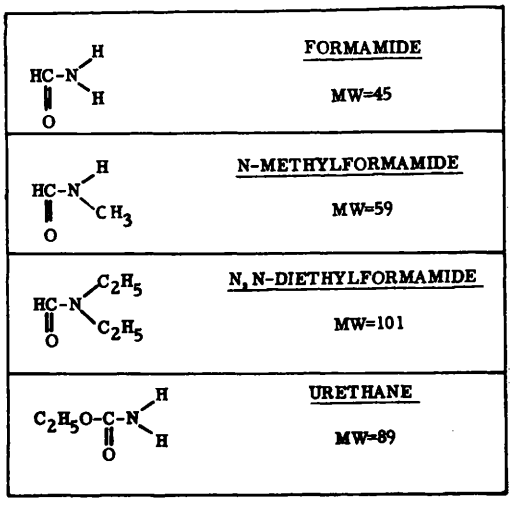

FIG. 1.

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