The Effects of Zinc on Contractility, Membrane Potentials, and Cation Content of Rat Atria

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ABSTRACT Zinc depresses the contractile force of electrically driven rat atria logarithmically with time. The threshold concentration is about 5×10^{-6} M zinc and the half-time for contractile depression at 10^{-4} M is about 25 minutes. Zinc also depresses spontaneous activity of atria and alters the transmembrane potential parameters in a manner similar to quinidine. Unlike quinidine, zinc causes an elevation of the resting potential and an elevation of cellular potassium which varies with time in the same way as the resting potential. Exposure to 10^{-4} M zinc for 60 minutes causes a statistically significant fall in atrial calcium content and an amount of radioactively labeled zinc is taken up which is quantitatively equal to the calcium lost. Zinc has no effect on rigor caused by iodoacetate but inhibits rigor caused by 1-fluoro-2,4 dinitrobenzene. It is postulated that zinc depression of contractile force is not due to metabolic inhibition, probably not due to quinidine-like action on the cell membrane, but may be due to an interference in the handling of calcium by the cell.

INTRODUCTION

The rather considerable amount of zinc present in muscle (Edman, 1960 *b*) and the interesting pharmacologic actions of this ion have resulted in much recent investigation of the effects of zinc on skeletal muscle. Thus zinc in the presence of ATP causes relaxation of glycerol-extracted rabbit psoas muscle fibers (Edman, 1960 *a*) but its effect on living frog sartorius muscle is to increase twitch tension, apparently by prolonging the duration of the active state (Sandow and Isaacson, 1960; Isaacson and Sandow, 1961, 1963). The latter effect seems to be due mainly to prolongation of the repolarization phase of the action potential (Edman and Grieve, 1961; Kobayashi, 1962; Sandow *et al.*, 1964; Mashima and Washio, 1964) although part of the potentiating effect of zinc may possibly be related to its ability to cause a transient displacement of bound calcium in the muscle fibers (Isaacson and Bianchi, 1962; Frank, 1962).

In view of the foregoing, it seemed to us that more information about the actions of zinc on heart muscle would be desirable. But, with the exception of the present investigation, a preliminary report of which has appeared (Ciofalo and Thomas, 1964) and one other investigation (Nayler, 1964) which came to our attention, there seems to have been no recent study of the effects of zinc on heart muscle. In her work, Nayler was able to show that zinc causes the release of a small amount of Ca^{45} from toad ventricle.

Earlier investigations (Harnack, 1875; Jaeger, 1931; Eichholtz and Birch-Hirschfeld, 1933; Mezey, 1937; Green *et al.*, 1952) have shown that zinc depresses contractility in heart muscle and that in frog heart this depression is reversible (Mezey, 1937). In preliminary experiments we confirmed these earlier findings and have made a few other observations on the frog heart which will be mentioned. However, our main study has been made on the rat atrium for the reason that much previous experience has been accumulated with this tissue in this laboratory particularly with respect to measurement of membrane potentials (Hollander and Webb, 1955; Webb and Hollander, 1959; Gimeno, Gimeno, and Webb, 1962).

METHODS

General

Intact isolated atria from young male albino rats (200 to 300 gm) were used in all experiments reported here. The bathing medium was a modified Krebs-Ringer's solution containing the following: 145 mM Na^+, 6 mM K^+, 1.2 mM Ca^{++}, 1.3 mM Mg^{++}, 147 mM Cl^-, 6.25 mM HCO_3^-, 1.3 mM SO_4^-, and 5.5 mM glucose. A gas mixture of 99 per cent O_2 and 1 per cent CO_2 was constantly bubbled through the medium, maintaining a pH of 7.4. A temperature of 30° C was maintained in all experiments.

In all experiments in which electrical potentials were not measured, atria were mounted vertically in 100 ml tissue baths with the pacemaker area of the right atrium held firmly against stimulating electrodes at the bottom of the bath. A silk thread attached to the left atrium was tied to a strain gauge which was connected into a 2-channel electronic ink recorder. In each experiment pairs of atria were allowed to equilibrate for 60 minutes before changing to a solution containing zinc or other test substances, and contractions were recorded continuously for 60 minutes after the introduction of the test solution. In a majority of the experiments the atria were stimulated electrically at a rate of 200 per minute. However, in a few experiments it was of interest to record the intrinsic rate of the atrial contraction, in which cases the stimulator was not used.

In a few experiments the effect of zinc on the development of rigor as induced by iodoacetate and 1-fluoro-2,4-dinitrobenzene (FDNB) was studied. After equilibration, zinc was added to the bath as $ZnCl_2$ to give a final concentration of 1×10^{-4} M, and after another 60 minutes one of the two contracture-inducing agents was introduced into the bath. Controls were recorded without the addition of zinc to the bath.

The rigor-inducing drug, either iodoacetate or 1-fluoro-2,4-dinitrobenzene, was added to the control bath at the same time as to the bath containing zinc. The rigor induced by the drug was followed for a period of 60 minutes in the case of 1-fluoro-2,4-dinitrobenzene and for 2 hours in the case of iodoacetate.
Rigor is expressed as:

$$\frac{\text{Diastolic tension} - \text{initial applied tension}}{\text{Initial applied tension}} \times 100.$$

In all experiments an initial tension of 750 mg was applied to the tissue by means of a micrometer attached to the strain gauge. This initial tension setting was then maintained throughout an experiment.

An attempt was also made to induce contracture with high concentrations of KCl and with ouabain, but the variation in the amount of contracture induced by constant amounts of each of these agents was so great in control preparations that it was impossible to study the effects of zinc on such contractures.

Ion Content Determination

The content of sodium, potassium, and calcium in atria and the uptake of radioactive zinc by atria were determined for preparations bathed as described above. In this group of experiments $ZnCl_2$ in Krebs-Ringer's solution was added to one bath to give a final concentration of 1×10^{-4} M and an equal volume of normal bathing medium was added to the control preparation. Sodium, potassium, and calcium were determined by flame photometry and because of the hazard of atmospheric contamination, the radioactive zinc determinations were done on a separate group of atria. The atria used for ion content or zinc uptake determinations were allowed to run for the desired period of time and then were quickly removed, lightly blotted, and weighed as rapidly as possible. They were dried overnight in an oven maintained at 110°C and reweighed. An atrium was then placed in a pyrex test tube containing 0.5 ml of concentrated HNO_3, and kept in a constant temperature hot block at 130°C for 12 to 24 hours until the acid became clear. The top of the tube was covered with a glass marble to aid refluxing of the acid and to prevent evaporation. The tissue digest was then diluted with water to a total of 5 ml. One half of a 5 ml sample was used to determine potassium. The other half of the sample was diluted 1 to 1 with isopropanol as recommended by Geyer and Bowie (1961) and was used to determine calcium and sodium. Flame photometry was carried out with a Beckman model DU spectrophotometer with a flame attachment and a spectral energy recording attachment connected to a recording potentiometer. The extracellular space was assumed to be 23 per cent of the wet tissue weight (Chin, 1963). Sodium and potassium standards were made from the chloride salts. The calcium standard was made from calcium carbonate which had been thoroughly dried in an oven at a temperature of 110°C.

The ion content of non-perfused atria was also determined. These atria were isolated as above, carefully cleaned of all extraneous tissue, and were immediately blotted and weighed for analysis.

Zinc Uptake Determinations

The tissue was prepared as for ion determination with special care being taken not to cut the tissue other than where necessary so as to avoid adsorption artifacts. The atria were placed in the tissue bath chamber containing 100 ml of modified Krebs-Ringer's solution for a control period of 60 minutes. After equilibration, $ZnCl_2$ was added to the tissue bath from a fresh solution containing Zn^{65} to make a final concentration of zinc in the bath of 1×10^{-4} M. Atria were labeled for periods of 15, 30, 45, and 60 minutes and when a labeling period was finished, an atrium was weighed and digested with HNO_3 just as for sodium, potassium, and calcium determinations. Aliquots of the digest were then spread on stainless steel planchets for counting. Small aliquots of the labeled bathing solution were also spread on planchets and counted along with the tissue samples. The zinc uptake was calculated as millimoles of perfusate zinc taken up by the tissue according to the following formula:

$$(\text{CPM}_t - 0.23\ \text{CPM}_t \times 10^{-4}\ \text{moles})/\text{CPM/liter of labeling solution} = \text{moles Zn/kg tissue}$$

where CPM_t is the number of counts per minute per kilogram wet tissue and 0.23 CPM_t is the fraction of CPM_t that would be in the extracellular spaces. All counts were corrected for background.

Radioactivity was determined with a GM tube and the Zn^{65} was obtained from the Oak Ridge National Laboratory as $ZnCl_2$ in solution.

Electrical Measurements

Transmembrane electrical potentials were measured by the method of Hollander and Webb (1955) and the various parameters of the action and resting potentials were measured as described by Gimeno, Gimeno, and Webb (1962). The only exception to this was that the duration of the action potential was measured at two voltages: 40 mv from zero potential designated as APD_2 and 20 mv below resting potential designated as APD_1. As in the case of the preparations described above, the atria used for electrical recordings were equilibrated *in vitro* for 1 hour prior to exposing them to zinc chloride and during this exposure an average of one penetration was made every 2 minutes for an hour. Control preparations were run alternately with experimental preparations in order to compare zinc-induced changes with those that might normally occur during the 60 minute experimental period.

RESULTS

1. *Effects of Zinc on the Contractile Amplitude of Electrically Driven Atria*

The lowest concentration of zinc which produced an observable decrease in contractile tension within a 60 minute test period was 5×10^{-6} M. Increasing the zinc concentration of the bathing medium caused an increased depression of contractile tension. The maximum concentration of zinc used was 5×10^{-4} M which caused a 100 per cent decrease in amplitude in less than the 60 minute experimental period. The results of these studies are shown in Fig. 1. Each point on this graph is the average of four or more experiments. The data obtained are plotted as the log of the per cent of the contractile

tension of control preparations *versus* time and when so plotted give a family of straight lines. The same result can also be obtained by plotting the log of the per cent contractile tension of control preparations at any given time *vs.* the concentration of zinc. At the highest concentration of zinc used, 5×10^{-4} M, the atria completely failed to respond to the stimulus or to beat spontaneously after 40 or 50 minutes of treatment, thus explaining the sharp break in that curve.

Repeated washings with normal Krebs-Ringer's solution did not reverse the zinc-induced depression nor did 2×10^{-3} M Ca-EDTA affect the depression. This latter substance was tried because it had previously been found that Ca-EDTA will rapidly reverse the depressant effect of zinc on the frog

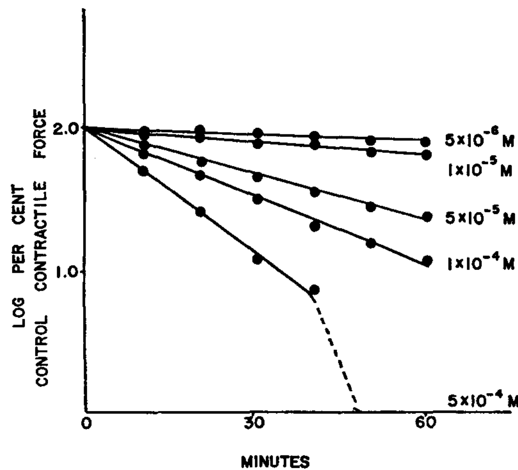

FIGURE 1. Effect of various concentrations of zinc on force of contraction of rat atria.

ventricle. An attempt was also made to alter the action of zinc on the contractile properties of the rat atrium by increasing the calcium concentration of the perfusion medium. Calcium concentrations of 1.2×10^{-3} M and 1.8×10^{-3} M were used with a zinc concentration of 5×10^{-5} M. At this zinc concentration, raising the calcium level by 50 per cent had no effect. However, when a threshold concentration of zinc (5×10^{-6} M) was used, a fourfold increase in calcium, from 1.2×10^{-3} M to 4.8×10^{-3} M, delayed the onset of the effects of zinc for an average of 20 minutes and reduced slightly the slope of the curve obtained for per cent depression *vs.* time.

Mezey (1937) found it possible to inhibit the action of zinc on the frog ventricle with calcium at 20 times the concentration of zinc, but it seems that the calcium to zinc ratio required for a detectable antagonism in the rat atrium is much greater (approximately 1000 to 1).

2. *Effects of Zinc on the Rate of Spontaneously Beating Atria*

The effects of zinc on spontaneous activity of the atria are not easily quantitated but nevertheless fall into two major categories. In some experiments

there was an early slight decrease in rate and 30 or 40 minutes later the beat became very erratic and then stopped altogether. In others there was no apparent effect on rate until 30 or 40 minutes after addition of zinc when again the beat became erratic and stopped. After spontaneous activity had completely ceased, all atria would nevertheless follow an electrical stimulus.

Repeated washings with normal Krebs-Ringer's solution for as long as 60 minutes were not effective in reversing the zinc action on rate. However, Ca-EDTA at a concentration of 1×10^{-3} M reversed the zinc effect on rate if it was added within 2 minutes after the atria had completely stopped or during the arrhythmic stage. If added after this 2 minute period had elapsed the atria would beat only when driven. In all cases, after Ca-EDTA reversal the atria responded by beating arrhythmically for a short while and then

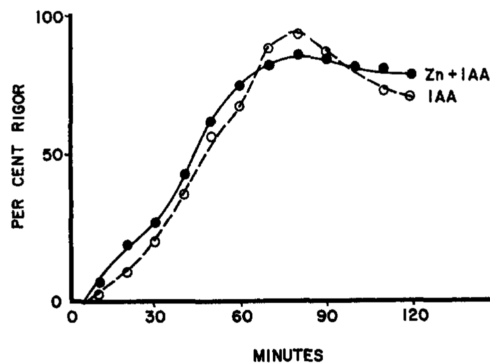

FIGURE 2. Effect of 10^{-4} M zinc on rigor of rat atria induced by 7.5×10^{-4} M iodoacetate.

became rhythmic. The time for rhythmicity to recover ranged from 10 minutes to 30 minutes. The final recovered rate of beating was less than 50 per cent of the previously observed control rate. Ca-EDTA, itself, had no effect on the rate of control atria over a 60 minute period.

3. *Effects of Zinc on Iodoacetate- and 1-Fluoro-2,4-Dinitrobenzene-Induced Rigor*

Rat atria were treated with 1×10^{-4} M zinc for a period of 60 minutes before the addition of 7.5×10^{-4} M iodoacetate to the bath. Control atria were not treated with zinc but were perfused with the normal medium for the same period of time before the addition of iodoacetate. Rigor which was induced by iodoacetate was measured for a period of 120 minutes. The mechanism by which iodoacetate induces rigor has not been fully elucidated as yet, but the bulk of experimental evidence points towards its reduction of the ATP level in the muscle as the probable reason (Bate-Smith and Bendall, 1947; Gallo, 1962). Assuming that iodoacetate causes rigor by blocking the Embden-Meyerhof pathway and reducing total ATP, then, if zinc has no effect in hastening the onset of rigor or in altering the magnitude of the rigor, it would

seem that the major action of zinc is not on a metabolic system. If zinc acted as an inhibitor of metabolism and thereby reduced ATP, then the rigor produced by iodoacetate would be expected to appear sooner than in non-zinc–treated muscle. The results are shown in Fig. 2, and it is apparent that zinc pretreatment had no effect on the rigor produced by this agent.

Another group of experiments was performed in which the pretreatment again was 1×10^{-4} M zinc for 60 minutes. In this series of hearts, 1-fluoro-2,4-dinitrobenzene (1×10^{-4} M) was used to induce the rigor. The rigor was followed for a period of 60 minutes. The mechanism by which this drug produces rigor is unknown. However, it has been used to inhibit creatine kinase in intact muscle (Cain and Davies, 1962) so that at least part of its rigor-producing effect could be as is assumed to be the case with iodoacetate,

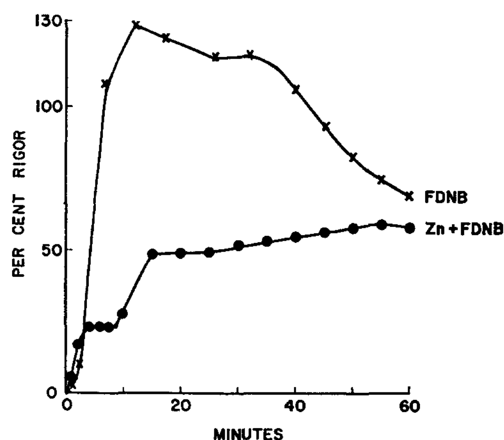

Figure 3. Inhibition by zinc on rigor of rat atria induced by 1×10^{-4} M 1-flouro-2,4-dinitrobenzene.

a reduction in cellular ATP content. Assuming this to be true, the effect of zinc on the rigor is in the opposite direction from that expected if zinc were acting as a metabolic inhibitor. As seen in Fig. 3, zinc largely inhibits the rigor. Particularly, it appears that zinc is most effective in inhibiting the early rapid onset of the rigor.

An attempt was made to treat atria with toxic doses of ouabain in order to observe the effect of zinc on this type of contracture. This was unsuccessful due to the large variations in the magnitude of contracture obtained with high concentrations of ouabain. However, some observations were made with isolated frog ventricles. These were treated with ouabain and allowed to go into contracture and then 1×10^{-3} M zinc was added to the perfusion medium. The ventricles were usually completely relaxed within 45 seconds after the addition of zinc but contractions did not return. When the perfusion medium was changed to Ringer's solution containing Ca-EDTA, the ventricles immediately went back into contracture. EDTA has much greater affinity for zinc than for calcium (Martell and Calvin, 1952) so it is presumed that this

effect of Ca-EDTA was due to binding of zinc in the extracellular spaces and at the membrane surfaces. The relaxation and contracture could be repeated several times by changing to the appropriate perfusion medium.

4. *Effect of Zinc on Total Ion Content*

Total potassium, sodium, and calcium content of rat atria were determined on tissue which had been treated for varying lengths of time with 1×10^{-4} M zinc. Control preparations consisted of atria taken directly from the animal and also preparations which were run in normal Krebs-Ringer's solution for the same length of time as the zinc-treated atria. The results are shown in Table I.

TABLE I

EFFECT OF ZINC ON TOTAL ION CONTENT OF RAT ATRIA

Solution bathing atria	Time in normal medium before zinc treatment	Zinc (1×10^{-4} M) treatment	No. of atria	Ion content* K^+	Na^+	Ca^{++}
	min.	*min.*		*mmoles/kg*	*mmoles/kg*	*mmoles/kg*
Not bathed	0	0	5	91.5±0.8	40.1±3.4	1.65±0.058
Normal medium	70	0	6	87.0±3.4	46.5±5.8	1.51±0.082
Normal medium + Zn	60	10	6	97.8±2.3	39.1±2.5	1.45±0.078
Normal medium	90	0	6	89.0±1.7	40.7±2.6	1.46±0.069
Normal medium + Zn	60	30	6	96.6±1.5	35.7±1.9	1.50±0.062
Normal medium	120	0	11	91.8±2.2	40.8±1.6	1.62±0.039
Normal medium + Zn	60	60	11	94.2±1.4	38.4±1.1	1.47±0.057

* Mean ± SE.

A. POTASSIUM The potassium content of control perfused rat atria slowly declined over the 60 minute equilibration period and then climbed during the following 60 minutes. When zinc was added to the medium, the potassium content within the atria rapidly rose to an average level about 7 millimoles/kg wet weight above the controls. The potassium remained at this concentration for 30 minutes and then declined over the last 30 minutes to the control level.

B. CALCIUM Calcium determinations on perfused atria yielded a wide range of values. No significant difference in the calcium content of control and zinc-treated atria was seen after 10 and 30 minutes perfusion, but after 60 minutes there was a statistically significant decrease in total calcium content of zinc-treated atria when compared to control atria ($p = 0.03$).

C. SODIUM The total sodium content of rat atria varied more widely than did the potassium content. All the zinc-treated atria had a lower content of intracellular sodium ions, the largest difference between control and zinc-

treated atria occurring at 30 minutes. In all cases, however, the decrease in sodium content was slight, and the standard deviation was at least 10 per cent (not significant).

5. *Zinc Uptake by Electrically Driven Atria as Measured with Zn^{65}*

Zn^{65} uptake by atria was determined at 15 minute intervals up to a total period of 60 minutes. The zinc concentration of the labeling solution was 1×10^{-4} M. The accumulation of zinc by the atria increased with time and reached a mean content of 1.22×10^{-4} moles/kg wet tissue weight after a period of 60 minutes (Table II). This very closely corresponded to the loss of calcium from atria treated with zinc for 60 minutes as seen in Table I.

TABLE II

ZINC UPTAKE (MEASURED WITH $ZINC^{65}$) BY ISOLATED RAT ATRIA

Perfusion time $zinc^{65}$ (1×10^{-4} M)	No. of hearts	Zinc uptake*
min.		*mmoles/kg wet wt.*
15	4	0.0215±0.00026
30	4	0.0713±0.0071
45	4	0.1044±0.019
60	4	0.1220±0.0072

* Mean ± SE.

6. *Effects of Zinc on Membrane Potentials*

Zinc appeared to change all parameters of the membrane potential. Data from measurements taken over a 60 minute period have been grouped into 10 minute intervals in Table III. These data were collected from ten zinc-treated atria and nine control atria. Fig. 4 is a diagram showing a typical atrial action potential and the early and late effects of zinc and Fig. 5 is a graphic plot of the per cent difference between experimental and control values from Table III.

The resting potential increased during the first 10 minutes and gradually decreased as the experiment progressed, approaching the control level at the end of the 60 minute period. The increase was statistically significant for the periods 0 to 10 minutes, 10 to 20 minutes, and 20 to 30 minutes ($p = 0.05$) but not for the remainder of the experimental period. An examination of the data in Table I will show that the changes in intracellular potassium content during zinc treatment ran a very similar time course. Thus there was also a rapid rise in cellular potassium followed by a gradual decline.

The action potential magnitude gradually decreased over the 60 minute

period and, although not shown in Table III, the overshoot was almost completely abolished during the first 10 minute period of zinc treatment and remained this way for the rest of the hour. The greatest change produced by zinc on any of the electrical parameters was on the depolarization rate. Thus during the first 10 minutes this was slowed by 32 per cent and by nearly 70 per cent at the end of an hour. We noted with interest that the rate at which the depolarization was depressed followed, for the first 15 minutes, a time course identical to the depression of contractile tension by 10^{-4} M zinc (see Fig. 1). The conduction rate was also rapidly depressed after the application of zinc (25 per cent during the first 10 minutes).

TABLE III

EFFECT OF 1×10^{-4} M ZINC ON PARAMETERS OF THE TRANSMEMBRANE POTENTIALS OF RAT ATRIA (TEN CONTROL AND NINE EXPERIMENTAL ATRIA)

Parameter	0–10 min.		10–20 min.		20–30 min.		30–40 min.		40–50 min.		50–60 min.	
	Control	Exper.	Control	Exper.	Control	Exper.	Control	Exper.	Control	Exper.	Control	Exper.
Action potential (AP), *mv*	84.2	80.5	82.4	71.9	84.4	73.4	81.6	68.1	84.0	70.2	83.4	66.3
Resting potential (RP), *mv*	67.2	71.6	67.3	69.7	68.5	71.3	66.8	66.3	68.5	69.9	68.2	69.9
Action potential duration (APD_2), *msec.*	34.2	29.7	32.5	28.7	35.0	30.9	35.5	39.6	33.9	38.2	31.9	39.3
Action potential duration (APD_1), *msec.*	41.2	40.6	39.5	39.3	43.5	44.1	42.5	48.7	42.5	52.9	40.1	53.0
Depolarization rate (RP), *volt/sec.*	78.6	53.4	74.8	41.1	76.8	39.9	79.5	32.9	76.7	30.2	78.8	26.5
Repolarization rate (RP), *volt/sec.*	1.8	1.8	1.8	1.7	1.7	1.6	1.6	1.2	1.8	1.2	1.9	1.1
Conduction rate (CR), *cm/sec.*	77.7	63.7	70.2	53.8	70.2	51.9	70.2	51.9	70.2	46.7	70.2	42.4
No. of penetrations	60	60	55	75	55	75	60	65	50	75	55	40

The change in the repolarization rate during the first 10 minutes was not statistically significant, but thereafter the decrease in rate caused by zinc was substantial. The effect was mainly on the last half of the repolarization. This unequal effect of zinc on the first and second halves of repolarization caused qualitative changes in the shape of the action potential. For this reason it was decided that the action potential duration should be measured at two different points. The duration measured at 40 mv above zero potential (APD_2) mainly reflected the early phase of repolarization. This showed a substantial decrease during the first 30 minutes and then a rapid increase during the final 30 minutes. The duration measured at 20 mv below the resting potential (APD_1) mainly reflected the last half of repolarization. This showed only a very slight decrease during the first 20 minutes followed by a very rapid in-

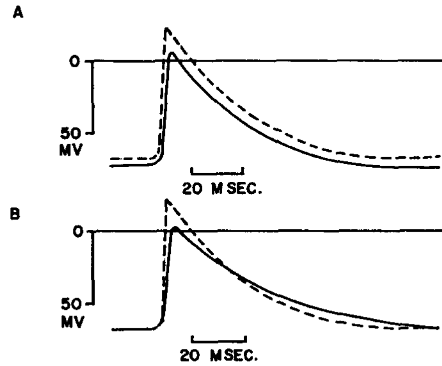

FIGURE 4. Typical effects of 10^{-4} M zinc on the shape of the rat atrial action potential after 15 minutes and after 45 minutes of zinc treatment. Each tracing made from at least ten photographs from three atria. Tracings show an average effect. Dotted curve is the normal action potential prior to zinc treatment. (*a*) Action potential after 15 minutes in zinc solution. (*b*) Action potential after 45 minutes in zinc solution.

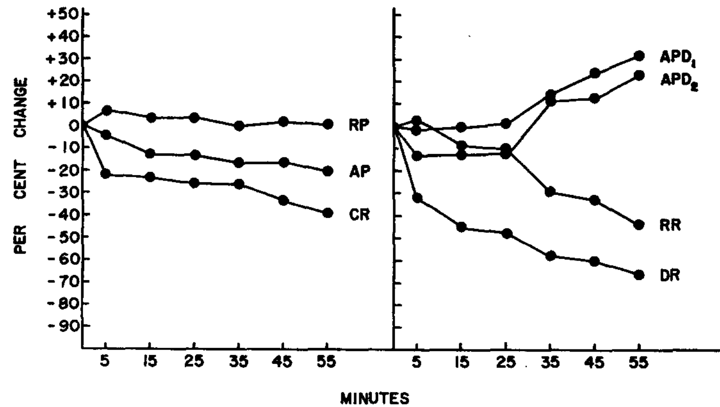

FIGURE 5. Changes on various parameters of the rat atrial membrane potential by 10^{-4} M zinc as a function of time. Zinc-treated preparations are compared to control preparations run for the same length of time. *RP*, resting potential magnitude; *AP*, action potential magnitude; *CR*, conduction rate; *RR*, repolarization rate; *DR*, depolarization rate; APD_1 and APD_2 (see text).

crease with time. These measurements indicate that the initial phase of repolarization became more concave during the first 30 minutes and then less concave than normal the remaining 30 minutes, while the last phase of repolarization was continually spreading out.

DISCUSSION

The effects of zinc on the membrane potential parameters of rat atria resemble the effects of quinidine and other antifibrillatory agents. According

to Weidmann (1955), Johnson (1956), and Vaughan-Williams (1958), the stabilizing action of these drugs on cardiac cell membranes is due primarily to a slowing of depolarization. Zinc caused a greater change in the depolarization rate (70 per cent reduction after 1 hour) than in any of the other parameters measured. Like quinidine, zinc also causes in the rat atrium a decrease in overshoot of the action potential, a decrease in conduction rate and, except for a transient initial increase in depolarization rate, this is also decreased. It is thus reasonable to assume that the loss of spontaneous rhythmicity during zinc treatment is due to the membrane-stabilizing action of this ion. The fact that Ca-EDTA will reverse this zinc effect also suggests the cell membrane as the site of this action. As Sandow *et al.* (1964) have pointed out, EDTA binds very tightly with zinc so that whenever a zinc ion is accessible to Ca-EDTA it will be bound and the calcium set free. They also state that Ca-EDTA penetrates into skeletal muscle very slowly or not at all and we presume that this is probably true for rat atria. As mentioned earlier Ca-EDTA has no pharmacological effect on normal atria. One effect of zinc apparently not characteristic of quinidine and the local anesthetics is the elevation of resting potential which we observed. Since there was a corresponding increase in cellular potassium content it seems reasonable (Page, 1962) to assume a causal relationship here. The mechanism by which zinc could bring this about is obscure. But it is perhaps worth noting here that zinc has been shown to react with sulfhydryl groups in muscle (Edman, 1960 *a*) and certain sulfhydryl-reactive enzyme inhibitors have also been found to raise the resting potential of rat atria (Webb and Hollander, 1959).

A major difference in the effect of zinc on the electrical parameters of rat atria as compared to those of frog skeletal muscle appears to be that in the latter, zinc in the concentration range used by us causes a profound slowing of repolarization but has little effect on depolarization rate, conduction rate, overshoot of the action potential, or resting potential. Higher concentrations (1 mM) of zinc apparently cause a significant slowing of repolarization rate in skeletal muscle (Kobayashi, 1962). The potentiation of the twitch by zinc in skeletal muscle correlates very well with the increase in action potential duration (Edman and Grieve, 1961; Sandow *et al.*, 1964; Mashima and Washio, 1964) and because of this we had expected at the outset that a correlation might be found in the rat atrium between depression of contractility and action potential duration. It has been found that a variety of agents affect contractility in this tissue, at least in part, by lengthening or shortening the action potential duration (Webb and Hollander, 1959). As it turns out only the time course of zinc depression of depolarization rate showed any correspondence with depression of contractility in rat atria. The rate at which these two processes were depressed by 10^{-4} M zinc was nearly identical for the first 25 minutes of zinc treatment but thereafter the depolarization rate

changed much less than did contractility. Probably this relationship between contracility and depolarization is fortuitous since quinidine can bring about similar changes in depolarization rate without any specific effect on contractility of heart muscle (Vaughan-Williams, 1958). Furthermore our finding that zinc depression of contractility is apparently irreversible sheds some doubt on a cell surface site of action for this effect. By the same reasoning as discussed above, Ca-EDTA should be able to inactivate any zinc ions on the cell surface but not in the cell interior. Nevertheless there may be an underlying similarity in the mechanism of action of zinc and quinidine on contractility. Contractility of heart muscle is also depressed by quinidine and the local anesthetics but like zinc these agents potentiate the twitch of skeletal muscle apparently by prolonging the action potential duration (Falk, 1961).

Since zinc has been shown to react with sulfhydryl groups (Edman, 1960 *a*) it could conceivably be exerting its cardiac contractile depression through metabolic inhibition. However, since no additive effect on rigor was found between zinc and iodoacetate or FDNB, we feel that gross metabolic inhibition can be excluded. As another possibility zinc may depress contractility of heart muscle by interfering with the excitation-contraction linkage or relaxation mechanisms. Recall that zinc has been shown to inhibit the contraction of glycerol-extracted muscle fibers (Edman, 1960 *b*). Zinc and manganese have also been shown to potentiate calcium uptake *in vitro* by sarcotubular relaxing factor granules (Martonosi and Feretos, 1964) and manganese injected *in vivo* in rats has been shown to increase the accumulation of Ca^{45} in microsomal fractions of the heart (Conrad and Baxter, 1963). Possibly our observation that an hour of zinc treatment significantly reduces the calcium content of rat atria is related to these observations. In any event more work will be required before a definite mechanism for zinc depression of cardiac contractility can be formulated.

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