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THE EFFECT OF ESTROGENIC HORMONES ON CARDIAC IRRITABILITY AND ELECTROLYTE EXCHANGE

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THE EFFECT OF ESTROGENIC HORMONES ON CARDIAC IRRITABILITY AND ELECTROLYTE EXCHANGE

CHAPTER I

INTRODUCTION

It has been known for many years that an increase of the calcium concentration in the environment of the heart augments the force of myocardial contraction, and that potassium has the opposite effect. The action of these ions is so uniform and invariable that it is made the basis of a laboratory exercise in most beginning courses in Physiology.

The cardiac glycosides also cause an increase in the force of myocardial contraction. Cattell and Goodell (1), showed that the digitalis glycosides cause a reduction in muscle potassium and advanced the theory that an increase in the calcium/potassium ratio might be responsible for the characteristic action of these drugs.

In toxic doses, the digitalis glycosides produce changes in myocardial excitability which pass progressively through varying degrees of nodal block, ventricular tachycardia, ventricular extrasystoles, atrio-ventricular dissociation and ultimately, ventricular fibrillation. If the theory of Cattell and Goodell is valid, it should be possible to reduce these toxic manifestations by reducing calcium and raising potas-

sium in the environment of the myocardium.

That this is possible, has recently been demonstrated by Smith and Grinnell (2), and by others (3, 4).

In the course of the experiments in which we used potassium-edethamil (ethylenediamine tetraacetic acid) to adjust the calcium/potassium ratio and reverse the digitalis intoxication, it was observed that some animals were unusually resistant to the toxic effects of the glycosides. In these experiments digoxin (0.15 milligram/kilogram), given intravenously, produced the characteristic arrhythmia within 15 to 25 minutes in all but the occasional resistant animal.

Reexamination of our records revealed that each of these resistant animals was a female, and in one instance where a second dose of digoxin was required to produce an arrhythmia, the animal was in a late stage of pregnancy.

These observations suggested that the apparent resistance to digitalis intoxication exhibited by these female dogs might be due to the influence of the sex hormones during estrus. Therefore, it seemed important to study the correlation between estrogenic activity and resistance to digitalis intoxication. It was also decided to study the serum electrolytes to see if there was any change in their concentration in relation to the estrogenic drugs administered.

CHAPTER II

HISTORY

For three-quarters of a century scientists have shown continued interest in the role played by potassium in myocardial function. It is known that profound alterations in the rhythmicity, conductivity and excitability of the myocardium frequently result from moderate alterations in the electrolyte content of the blood, and thus an understanding of the effect of inorganic ions on heart muscle is of major importance in any study of cardiac function. In view of the fact that there is no adequate explanation of the mechanism by which digitalis exerts its effects, it is hardly surprising that a possible relation between the action of that drug and potassium metabolism should be considered.

Ringer (5), demonstrated that three cations, sodium, potassium and calcium are necessary constituents of any perfusion fluid if it is to support contraction for more than a few moments. Early myographic studies of perfused frog hearts clearly demonstrated the major effects of the three essential cations on rate, rhythmicity, and contractility. A heart perfused with a normal saline solution soon becomes mechanically inactive. Addition of calcium to the perfusion fluid results in temporary restoration of contractions; however, diastole becomes less and less complete until the heart stops in systole. At this stage the

addition of potassium results in the resumption of regular contractions, and this activity is maintained for many hours (6).

The effect of increasing concentrations of serum potassium on the electrocardiogram was studied by Winkler and his colleagues in 1938 (7). Typically, these consist of an increase in the amplitude of the T wave, depression of the S-T segment, A-V block of varying degree and ultimate disappearance of the P wave followed by cardiac arrest. Irritability and contractility of the isolated heart are relatively unaffected by alterations in potassium concentration within a wide physiological range. At levels below 4 meq., myocardial irritability is depressed. Below 2 meq., contractile force is depressed. When the potassium concentration rises to 9 meq. or above, both of these functions are depressed. Apparently, low potassium does not affect conduction velocity; however, it does result in an increased automaticity, and the arrhythmia elicited by low potassium is superficially similar to that produced by toxic doses of the cardiac glycosides (8, 9).

Cattell and Goodell (1), showed that frogs' sartorius muscles soaked for 15 minutes in Ringer's solution containing 1:500,000 ouabain lose 23% of their intracellular potassium. Hagen (10), reported that toxic doses of digilanid C cause a distinct fall in heart muscle potassium. With the aid of the heart-lung preparation, Wood and Moe (11), extended this work and demonstrated that the loss of intracellular potassium caused by toxic doses of digitalis is apparently an exchange with an equivalent amount of sodium. However, Boyer and Poindexter (12), pointed out that therapeutic doses of digitalis produced a significant

increase in the intracellular potassium of the cat heart.

Using a radiopotassium technique combined with coronary sinus catheterization in dogs, Conn (13), reported a 15 per cent reduction in intracellular potassium of the myocardium after chronic administration of digitoxin at a dosage level of 0.2 to 0.4 milligram per day per animal until a positive inotropic effect appeared. O'Brien, et al., (14), showed that the mean arterial plasma level of potassium in 17 dogs rose from a basal value of 16 milligrams per cent to 28 milligrams per cent one hour after administration of 0.15 milligrams of digoxin per kilogram of body weight.

It is apparent that the actions of the digitalis glycosides are associated with changes in the potassium concentration within the myocardial cell and that the direction of the change depends on whether the dose is toxic or within the therapeutically effective range (10, 12, 15, 16). However, it has been shown that potassium in certain respects is antagonistic to the action of digitalis. It has been shown both in cats and dogs that the concomitant administration of potassium exerts a protective effect against ouabain intoxication (4, 17). Sampson (18), and his group abolished the symptoms of digitalis intoxication in human patients by oral administration of potassium acetate. Hajdu (19), expressed the opinion that the positive inotropic effect of digitalis glycosides, epinephrine, adrenal steroids and low extracellular potassium are all associated with an increase in the net outward flux of potassium.

When the calcium level is maintained constant, low extracellular potassium greatly augments the isometric tension of isolated papillary

muscle, and elevated potassium has the opposite effect. In either situation, if the calcium concentration in the perfusion fluid is then changed so that the calcium/potassium ratio returns to normal, the tension returns to control values (6).

The possible synergism of calcium and digitalis has been the subject of a considerable amount of research. Bower and Mengle (22), and Rogen (23), reported cases in which deaths from ventricular fibrillation have occurred after the intravenous administration of calcium gluconate to fully-digitalized patients. They also showed that intravenous calcium gluconate in the digitalized dog caused death from ventricular fibrillation. Blumenfeld and Loewi (24), state that digitalis changes the state of the heart in such a manner that it becomes hypersensitive to calcium and therefore reacts to the normal calcium concentration of the medium like a heart without digitalis reacts to high calcium concentrations. However, Wall (25), gave calcium gluconate intravenously to fifteen patients who were completely digitalized without any clinical evidence of untoward effect. Smith, et al., (26), administered calcium chloride by intravenous infusion to normal and digitalized dogs until death occurred. They concluded from their results that the lethal effects of calcium and digitalis are neither synergistic nor even completely additive. Recently, Barker and Barker (27), reported that in the ouabanized frog heart elevated calcium concentrations caused an increase in ventricular automatism characterized by paroxysmal tachycardia, flutter, and fibrillation.

However, it appears that calcium also has a direct action on the

contractile mechanism, since Edman (20), working with glycerinated muscle fibers, demonstrated that ouabain had no effect on the preparation in the absence of calcium, but in the presence of calcium was found to strengthen the force of contraction. He also reported that calcium by itself has an inhibitory effect on contraction. According to Hoff, et al., (21), the action of calcium on the heart appears in three different phases: a preliminary inhibitory phase, followed by a period of acceleration, sometimes terminating in ventricular fibrillation, and finally, a stage of slowing and arrest, occuring in all animals which escape ventricular fibrillation during the second phase.

CHAPTER III

METHODS

Healthy, adult, female dogs, of mixed breeds, weighing from seven to twenty kilograms, were used for these experiments.

The animals used in the experiments of Tables III and IV were spayed by a technique which included hysterectomy. A minimum of five weeks were permitted for post-operative recovery and for the elimination of residual natural estrogenic hormones. In most instances, three to four months were allowed to elapse before the acute experiments were performed.

The spayed animals were divided into two groups. The first group (Table III) served as the control. The animals of the second group (Table IV) were treated with one of three estrogenic substances according to the following dosage schedule:

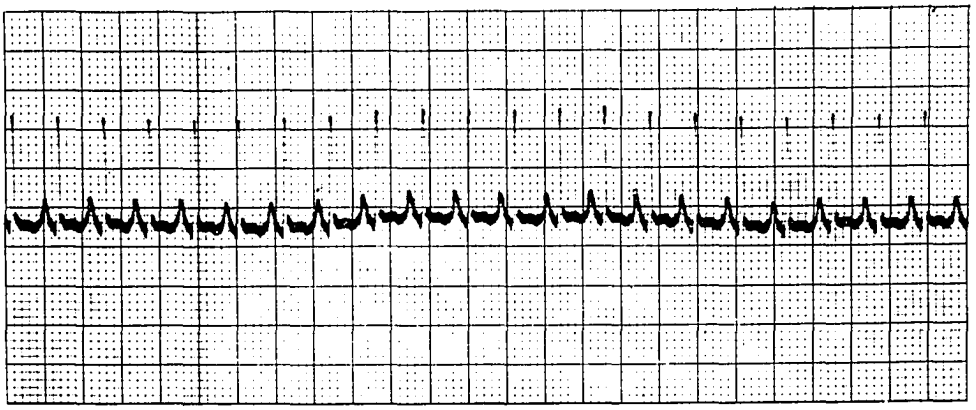
Estrone, 20,000 u./day injected intramuscularly.

Estradiol, 10 mgm/day injected intramuscularly.

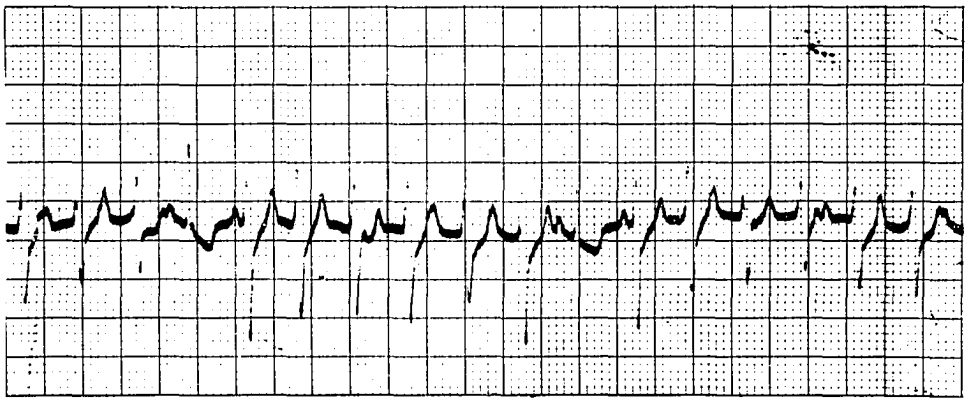
Diethyl stilbestrol, 10 mgm/day injected intramuscularly.

These animals were used for the acute experiments when they were proved to be in peak estrus by the vaginal smear technique.

The acute experiments were carried out in the same manner for both groups.



NORMAL ELECTROCARDIOGRAM (LEAD aVf)



TYPICAL ARRHYTHMIA AFTER DIGOXIN 0.15 mg/Kg (LEAD aVf)

FIGURE I

Food was withheld from the animals for 18 to 24 hours prior to the experiment. Water was allowed ad libitum during the fasting period. The animal was anesthetized with pentobarbital sodium, 30 mgm/kg. of body weight, and connected to a Cathode-Ray Electrocardiograph for continuous observation of the electrocardiogram. Lead aV_f was routinely employed.

After the normal electrocardiogram had been observed, digoxin was given intravenously at a dosage level of 0.15 mgm/kg. of body weight and the time of administration recorded. In over 75 experiments performed to date in this laboratory, this dose of digoxin has produced a typical arrhythmia in all but two animals. It will be referred to subsequently as the $E. D._{100}$. The animal's electrocardiogram was observed continuously, and the time of appearance of the arrhythmia was recorded. A record of a typical digoxin arrhythmia is shown in Figure I.

Tables V and VI represent those animals from Tables III and IV, respectively, on which electrolyte studies were made. Approximately ten milliliters of blood were taken from the femoral vein just prior to the administration of digoxin, then again at 15 minutes, 60 minutes, and 120 minutes. The blood was drawn into syringes which contained an amount of heparin sufficient to prevent coagulation, and centrifuged in tubes which were similarly prepared. The Baird Flame Photometer was used for the determination of plasma sodium and potassium. Calcium was determined according to the method of Natelson and Penniall (28).

CHAPTER IV

RESULTS

The results of the experiments are summarized in Tables I through VI. In Table I it can be seen that for eight normal female dogs, the average time required for the onset of the arrhythmia produced by digoxin was 33.1 minutes. It will be noted that one animal did not develope an arrhythmia even after administration of a second dose of digoxin. It should be explained that the dogs in this group were used in the experiments on the effect of potassium edethamil on digitalis toxicity to which previous reference has been made (2). These animals were subjected to a variety of drugs following the onset of the arrhythmia; therefore, the table contains no information as to the time and mode of death, since these were unquestionably modified by the experimental procedure. The time of onset of the arrhythmia in these animals ranged from 20 to 52 minutes.

Comparison of these normal females with the estrogen-treated castrate females of Table IV (average time of onset of arrhythmia 30.6 min.) shows that there was no significant difference in the average time of onset of the arrhythmia. However, when the untreated castrate females of Table III (average time of onset of arrhythmia 13.4 min.) are compared with the animals of Tables I and IV, it becomes obvious that there was a

very definite difference in the time of onset of the arrhythmia. Statistical analysis shows that the time of onset of the arrhythmia in the untreated castrate female is significantly different (to the 0.01 level) from the time of onset of the arrhythmia in normal or estrogen-treated castrate animals. Attention should also be directed to the fact that the mortality rate in the untreated castrate females was 90 per cent; the only survival was an edethamil-treated animal. On the other hand, females in natural estrus (Table II) or castrate females following estrogen treatment unquestionably exhibit a resistance to the lethal effects of digoxin.

Control determinations of plasma sodium and potassium on animals selected at random from the experimental groups during the week preceeding the acute experiment showed that these electrolytes did not vary significantly from animal to animal or in the same animal from day to day.

In most instances, the greatest change in potassium occurred during the first 60 minutes after the administration of digoxin, with only a slight further change during the next hour. In the column of Tables V and VI which is headed "Electrolytes after Digoxin", the values given for all electrolytes are those in the single sample which showed the greatest charge of potassium from the basal value; in most instances this was the 120 minute sample.

A comparison of the plasma electrolyte levels in Tables V and VI reveals that the only significant difference between the two groups of animals is the change in potassium. Statistical analysis shows that the average gain of 67.7% in the plasma potassium of the untreated castrate

TABLE I

INTERVAL BETWEEN ADMINISTRATION OF DIGOXIN (ED_{100}) AND THE ONSET OF CARDIAC ARRHYTHMIA

NORMAL FEMALES

EXP. NO.	TIME IN MIN.	REMARKS
9	30	
10	35	
13	32	
14	52	
28	20	
31	32	
38	31	
29	--	0.165 mg. Digoxin/kg; No arrhythmia.
MEAN---	33.1	

TABLE II

INTERVAL BETWEEN ADMINISTRATION OF DIGOXIN (ED_{100}) AND THE ONSET OF CARDIAC ARRHYTHMIA

NORMAL FEMALES--NATURAL ESTRUS

EXP. NO.	TIME IN MIN.	REMARKS
37	50	Survived--Edethamil-treated
69	120	Survived
71	45	Survived--sacrificed
MEAN---	71.6	

TABLE III

INTERVAL BETWEEN ADMINISTRATION OF DIGOXIN (ED_{100}) AND THE ONSET OF CARDIAC ARRHYTHMIA

CASTRATE FEMALES--UNTREATED

EXP. NO.	TIME IN MIN.	REMARKS
40	17	Death--ventricular fibrillation
41	16	Survived--Edethamil-treated
42	5	Death--ventricular fibrillation
43	11	" " "
44	20	" " "
48	5	" " "
61	14	" " "
62	14	" " "
73	15	" " "
74	17	" " "
	MEAN--- 13.4	

TABLE IV

INTERVAL BETWEEN ADMINISTRATION OF DIGOXIN (ED_{100}) AND THE ONSET OF CARDIAC ARRHYTHMIA

CASTRATE FEMALES--ESTROGEN-TREATED

EXP. NO.	ESTROGEN	TIME IN MIN.	REMARKS
46	Estrone	40	Survived--Edethamil-treated
47	Estrone	30	" " "
58	Stilbestrol	27	" " "
59	Stilbestrol	30	Survived
60	Stilbestrol	25	Survived
63	Estradiol	29	Survived--sacrificed
65	Estradiol	32	Survived--sacrificed
66	Estradiol	28	*see note
68	Estradiol	35	*see note
	MEAN---	30.6	

*Ventricular fibrillation occurred at 2 hours during withdrawal of a blood sample by direct heart puncture.

TABLE V

EFFECT OF DIGOXIN ON PLASMA ELECTROLYTES
IN CASTRATE FEMALE DOGS

UNTREATED CASTRATE FEMALES

EXP. NO.	ONSET OF ARRHYTHMIA Minutes	ELECTROLYTES BEFORE DIGOXIN Elect.	mgm %	ELECTROLYTES AFTER DIGOXIN Elect.	mgm %	DIFFERENCE PERCENT	
61	14	K	13.5	K	22.5	+	66.6
		Na	380.0	Na	362.5	-	4.6
62	14	K	14.5	K	28.0	+	93.0
		Na	372.0	Na	378.0	+	1.6
73	15	K	14.5	K	19.5	+	34.5
		Na	347.0	Na	341.0	-	1.7
		Ca	8.7	Ca	9.5	+	9.6
74	17	K	14.5	K	24.5	+	69.0
		Na	362.0	Na	351.0	-	3.4
		Ca	8.8	Ca	9.0	+	0.2

Mean Percentage Difference

Potassium = + 67.7
Sodium = - 2.0
Calcium = + 5.7

TABLE VI

EFFECT OF DIGOXIN ON PLASMA ELECTROLYTES
IN CASTRATE FEMALE DOGS

CASTRATE FEMALES--ESTROGEN-TREATED

EXP. NO.	ONSET OF ARRHYTHMIA Minutes	ELECTROLYTES BEFORE DIGOXIN Elect.	mgm %	ELECTROLYTES AFTER DIGOXIN Elect.	mgm %	DIFFERENCE PERCENT	
63	29	K	15.3	K	20.0	+	31.0
		Na	350.0	Na	367.5	-	5.0
		Ca	8.9	Ca	10.5	+	18.0
65	32	K	15.3	K	16.5	+	8.0
		Na	353.0	Na	335.0	-	7.9
		Ca	9.7	Ca	8.1	-	16.6
66	28	K	15.6	K	17.5	+	12.0
		Na	352.0	Na	392.0	+	11.3
		Ca	7.9	Ca	8.8	+	11.4
68	35	K	12.0	K	12.0		0.0
		Na	346.0	Na	347.5	+	0.1
		Ca	8.8	Ca	8.8	-	0.7

Mean Percentage Difference

Potassium = + 13.0
Sodium = - 0.4
Calcium = + 3.3

animals was significantly different from the average potassium gain of 13% in the estrogen-treated castrate animals, to the 0.01 level. Changes in plasma sodium and calcium proved to be satistically insignificant.

CHAPTER V

DISCUSSION

Digitalis is known to have more than one effect on cardiac muscle. In effective doses it enhances contractility, and it is primarily for this action that digitalis is used therapeutically. As the dose is increased, alterations in conduction and rhythmicity become increasingly important. The evidence available at this time supports the view that the influence of digitalis on contractility is exerted directly upon the actin-myosin-adenosine triphosphate system, and that the effects on conduction and rhythmicity take place at the cell membrane.

The first observations in this series, in which the time of onset of arrhythmia was used as an index of sensitivity to digitalis, showed that estrogenic substances exert a definite protective influence against these toxic effects. It has been pointed out (14), that digitalis in toxic doses causes a loss of intracellular potassium from the myocardium. Consequently in the later experiments of this series, plasma levels of sodium and potassium were determined. In a few instances, calcium was also determined. The total plasma calcium is not considered important since it gives no information concerning the active, diffusable portion.

It is well known that the ionic effects of digitalis are not

confined to heart muscle. It has been established that the cardiac glycosides cause potassium loss from the saitorius muscle of the frog (1), thigh muscle and the liver of rats (32), adrenal gland, abdominal muscle, liver, spleen, kidney, lung, skin, and subcutaneous tissue of the rabbit (33), and the human erythrocyte (34). Therefore only a part of the changes in plasma electrolytes can be attributed to the heart. In this regard, the dog is a fortunate experimental animal since the dog erythrocyte contains sodium as the predominant intracellular cation, and the large potassium pool in the red cells of other species is unavailable.

The magnitude of the sodium and potassium changes in the plasma proved to be greater in the untreated castrate female than in castrate females brought into artificial estrus with estrogenic substances.

Higher animals typically maintain a high-potassium, low-sodium concentration of the intracellular fluids, with the opposite situation existing in the extracellular fluids. Certain drugs or pathological conditions which cause a fall in intracellular potassium have been shown to increase the myocardial sensitivity to digitalis (29, 32).

The exact mechanism by which cells maintain this intracellular sodium/potassium ratio is not known; however, three general theories have been advanced: 1) selective impermeability, 2) ion binding, and 3) active sodium transport. Of the three, active transport seems to be the most acceptable.

Steinbach (3), has evaluated the evidence for each of the above theories. He concludes that ion-binding can account for only a small

part of the ionic distribution but that active extrusion of sodium by a metabolic process within the cell is potentially capable of maintaining the observed intra-cellular/extra-cellular ionic gradient. He points out that whenever a physiological event occurs with great rapidity, ionic permeability, expressed as the potential rate of diffusion, becomes of prime importance. Reversible changes during excitation, occurring in milliseconds, might well be controlled by relative rates of penetration of specific ions. Even the slower changes during growth and differentiation might depend in their details upon permeability rates. However, the steady-state changes which are reflected in the normal maintenance of the internal sodium and potassium concentration of muscle cells are probably best regarded as relatively time-independent; thus only incidentally will permeabilities, as rates, be involved.

Steinbach caused frogs' sartorius muscles to become sodium-rich, potassium-poor by overnight immersion in potassium-free Ringer's solution at 2°C. When these muscles were subsequently exposed to potassium-Ringer's solution, the major fraction of the excess sodium was extruded during the first 30 minutes. He also showed that the extrusion rate became independent of the internal sodium concentration when the latter rose above 60 mMol/Kg. This implies that sodium is being extruded by a specific chemical carrier system, presumably located near the external limiting boundary of the cell, and present in a concentration which can become the limiting factor in sodium extrusion at high internal sodium concentrations.

If active sodium transport is the mechanism which operates to

maintain the correct ionic distribution in cardiac tissue, then it is reasonable to assume that digitalis at toxic dosage levels in some way affects the carrier system, thus allowing sodium to enter the cell at the expense of intracellular potassium. The result is an increase in plasma potassium with an equivalent fall in plasma sodium. The latter change is difficult to detect with accuracy because of the high normal level of plasma sodium.

The synergism between calcium and digitalis has been mentioned previously. The mechanism by which calcium ions augment the effects of digitalis is not known. However, one of the most important effects of calcium on the function of myocardial fibers is to change the level of membrane potential and sodium permeability. It has been shown that an elevation of calcium enhances the increased sodium permeability of the fiber membrane which occurs during depolarization. Conversely low calcium decreases the change in sodium permeability accompanying excitation (6). Thus the ability of the heart muscle cell to respond and conduct abnormally early during repolarization is augmented by high serum calcium and diminished by low. It is, therefore, logical to conclude that calcium must augment the action of digitalis, since digitalis at toxic levels is known to increase cardiac automaticity and excitability.

The mechanism by which calcium influences the resting membrane potential is by sensitizing the membrane to changes in potassium concentration. An increase in serum potassium causes membrane depolarization in the presence of normal calcium levels. When the fiber membrane is depolarized to a level near the threshold potential it becomes less stable

and repetitive firing of multiple areas of the myocardium is likely to ensue. During this period of partial depolarization, fibrillation can occur (6). With further increase in potassium concentration, this situation undergoes a reversal and the threshold is increased until the heart becomes completely inexcitable.

Weidmann (31), showed that the capacity of the fiber to undergo an increase in sodium permeability is related to the level of the membrane potential prior to stimulation. Thus, if the resting potential is low, the maximum sodium current elicited by activity is less than normal; therefore, the rate of rise and the magnitude of the action potential of the cardiac fiber are both diminished. It is very probable that changes in the action potentials caused by changes in potassium are a result of the depolarizing effect of that ion. Finally, just as a change in calcium influences the effect of potassium on the resting potential, a rise in potassium antagonizes the effect of high calcium on the duration of the action potential. This might explain the value of potassium salts in the treatment of digitalis intoxication, and the potential danger of administering intravenous calcium salts in the digitalized subject.

The report of Smith, et al., (26), has frequently been quoted in support of the view that the administration of calcium after digitalis is not hazardous. It is now apparent that their results are subject to a quite different interpretation. As calcium is administered by slow intravenous infusion to the digitalized animal, a critical concentration is reached at which the myocardium is hyperexcitable. At this time ventricular fibrillation is likely to occur, due to the spontaneous

firing of multiple, hyperexcitable foci.

If by accident, fibrillation does not occur at this critical calcium concentration and the infusion is continued, a calcium concentration is reached which tends to stabilize the membrane and make excitation difficult. A heart may fibrillate at this time due to the establishment of randomly distributed areas of complete non-irritability. The excitation wave by-passes these areas, and re-entry and repetitive activity result. However, complete cardiac arrest is much more likely, and it should be pointed out that in the experiments quoted above, 9 of 12 animals which received calcium after digitalis died in cardiac arrest. These deaths occurred at plasma calcium levels lower than those which caused death, preponderantly from ventricular fibrillation, of the non-digitalized animals.

It seems reasonable from the foregoing discussion to speculate that digitalis exerts its effects by attracting calcium to the cell surface, making it available for the metabolic processes that bring about these alterations in membrane permeability, resting potential, threshold potential, and action potential. Furthermore, it is perhaps through the alteration of these factors that the toxic effects of digitalis result.

It is not possible from these experiments to explain the mechanism by which the estrogens exert their protective action.

CHAPTER VI

CONCLUSIONS

1. Castrate female dogs have been found to be highly sensitive to the toxic effect os a cardiac glycoside in comparison with normal females or castrate females treated with an estrogenic substance.
2. The rise of plasma potassium which follows the administration of a toxic dose of digoxin is significantly higher in the sensitive, castrate animal than in the normal or estrogen-treated castrate animal.
3. It is a reasonable conclusion that loss of potassium from the myocardium contributes significantly to the rise in plasma potassium.
4. The mechanism by which tissues maintain a high intracellular potassium is believed to involve an active process of sodium extrusion. It is, therefore, concluded that this mechanism is impaired in the absence of the animal's normal estrogenic hormones, and as a consequence the heart of the castrate animal loses potassium excessively under the influence of a cardiac glycoside at a toxic dosage level.
5. It is concluded that the excessive potassium loss from the heart of the castrate female animal contributes to its increased sensitivity to digitalis.

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