E L E C T R O L Y T E S

I N

H E A R T D I S E A S E

by

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T h e s i s

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TABLE OF CONTENTS.

INTRODUCTORY SECTION

The title of this thesis is a broad one and purposely so, for the problem was first conceived in such general terms. But it soon became obvious, that the role of many electrolytes in many different types of heart disease could not possibly be investigated by one person in a short time. Therefore, in approaching such a study, the problem had immediately to be limited. Because potassium is such an important electrolyte in cellular metabolism and because variations in potassium concentrations have such a marked effect upon the functions of the heart, it was decided that this ion should be the principal electrolyte involved in the investigation. Because changes in the potassium concentration frequently involve reciprocal changes in sodium concentration, sodium, to an extent, also enters this study.

The term heart disease also needed closer definition. It was planned to include two general types of heart disease in the investigation, acute and chronic heart disease. This acute and chronic myocardial damage would also have to be of a type easily induced by experimental procedures.

Such a problem as the roles of these electrolytes in experimentally induced acute and chronic heart disease could be approached in different ways. For instance, the heart disease could be induced and then the level of serum and tissue electrolyte concentrations determined. On the other hand, the effect of variations of serum sodium and potassium concentration on the acutely and chronically damaged heart could be investigated. Because this last approach seemed to offer the greatest therepeutic opportunity, it was the principal one adopted.

As an introduction to this problem, several aspects of sodium and potassium physiology must be considered. Particular attention will be given to what is known of the effects of varying the concentration of these ions on the heart. Then, the relevant work which has been done on the changes in the concentration of these ions which occur in heart disease will be summarized. And finally, the effects which have already been noted on administering these salts to patients with heart disease will be reviewed. At the end of the introductory section, certain possibilities will be suggested, which the experimental work will then attempt to establish.

Both sodium and potassium are present in liberal amounts, in ingested food. The average dietary intake on this continent is probably about 4 - 6 grams of sodium and 2 - 4 grams of potassium per person per day. (1). However, the amount of sodium and potassium contained in the diet may vary considerably and these variations are well tolerated by the healthy person. (2). On the other hand, there seems little doubt that in certain disease states the intake of these ions may become important. For instance, in hypertension, a low intake of sodium has become one of the prime considerations in the management of the disease. (3) (4). A high intake of potassium has frequently been used therapeutically as will be seen later. Diets very deficient in sodium may be very prostrating. (3), while those deficient in potassium may cause other severe deficiency states in experimental animals. (5).

In the gastro-intestinal, tract the sodium and potassium salts ingested in the food and excreted in the intestinal juices are very largely absorbed so that very little is found in the faeces. (1)(2), except in severe diarrhea. (1) (6). In adrenal insufficiency the absorption of electrolytes seem abnormal (7)(8)

With absorption into the body complicated mechanisms operate not only to keep the serum concentrations (Na = approx't 140 m e/l; K = 4 - 5 m e/l) within physiological limits but also to control distribution throughout the body. These complex mechanisms can not possibly be discussed in detail here but certain facts (and opinions) concerning the renal excretion of sodium and potassium, the role of the adrenal cortex in the excretion and distribution of these ions and the dispersement of these ions between the intra- and extra-cellular compartments must be stated.

The chief avenue of excretion of sodium and potassium is the kidney. Both ions pass through the glomerular filter and both appear to be reabsorbed to some extent in the convoluted tubule. Winkler and Smith (9) state that "within the range of potassium concentration which can be tolerated physiologically, the greater part of the filtered potassium is still being reabsorbed". They point out that potassium excretion is directly correlated with the serum potassium concentration and that the clearance of potassium therefore varies with the glomerular filtration rate. Lately, Berliner and Kennedy (10) have suggested that there may be a tubular secretory mechanism presumable in the distal tubules, on the basis of experiments where hypertonic potassium chloride solutions were injected and yielded rates of potassium excretion above the rate of filtration of potassium at the glomeruli. In regard to sodium, in a recent paper, Smith and his group (11) point out that reabsorption of sodium in the proximal tubules is an active process and that the fraction of filtered sodium reabsorbed proximally is fairly constant so that the load of sodium delivered to the distal tubules will increase or decrease with the filtration rate. As the distal reabsorption of sodium is probably limited by the maximal rate of tubular transport, increasing the filtration rate will exceed the capacity of these cells and sodium will escape while decreasing the filtration rate will

reduce the distal load of sodium well below this capacity so that sodium reabsorption will be complete. This work is in agreement with Merrill (11A) who found that the retention of sodium in chronic congestive heart failure was due to a decrease in the filtration rate, the result of the diminished renal plasma flow.

Though there is no doubt that the kidney is the principal avenue of excretion of these bases and though the serum concentration, glomerular filtration rate and tubular reabsorption are the important factors in this renal control, there seems equally no doubt that the adrenal cortex exerts a tremendous influence on the kidney in regard to the quantity of sodium and potassium excreted. In 1932, Loeb (12) demonstrated, in Addison's disease, the striking decrease in sodium concentration in the serum and pointed out that the potassium was at a high normal level or else elevated. The following year his group (13) published observations on the electrolyte concentrations in the urine in Addison's disease which quite conclusively demonstrated the relation between the adrenal cortex and renal function in that the urinary sodium was shown to be increased and the potassium decreased. However, they attributed the decreased potassium to a decreased dietary intake. Harrop (14) in 1936 withdrew the maintenance dosage of cortical hormones from adrenalectomized dogs and demonstrated that they consistently showed, on withdrawal, an increased urinary loss of sodium and chloride and retention of potassium. In 1939, Harrison and Darrow (15) wrote that the marked disturbance of renal function following adrenalectomy was of a specific type. The renal tubules failed to reabsorb sodium adequately from the glomerular filtrate at a time when the serum concentration was low and

did not allow the excretion of potassium when the serum concentration was high. Just how the adrenal cortical hormones cause this change in tubular function is not known.

The question now arises as to which compounds of the adrenal cortex are responsible for the control of electrolyte excretion. Engstrom, (16) in a recent review, lists some twenty-eight steroids thus far isolated from the adrenal cortex. If we disregard for our purposes, the estrogenic, androgenic and biologically inactive compounds we may divide the remaining isolated substances into those having a marked effect on salt and water metabolism and those having a marked effect on carbohydrate metabolism. (7). In the former group fall compounds without an oxygen at C11, such as 11 desoxycorticosterone, an extremely active compound in causing retention of sodium and excretion of potassium (16). In the latter group, having either a hydroxyl or a ketone group at C11, is 17 hydroxy corticosterone which is very active in carbohydrate metabolism and may besides cause a temporary increase in sodium excretion, (16) and 11 dehydrocosterone and corticosterone both of which have a less intense action on carbohydrate metabolism but which will both cause a moderate retention of salt and water (16). The acetate of 11 desoxycorticosterone, (DCA), the highly salt active compound, was synthesized in 1937 by Steiger and Reichstein (17).

DCA and adrenal cortical extract have both been shown to cause, in adrenal insufficiency, a retention of sodium and water and an excretion of potassium (7)(18)(19). Even with physiological adrenal function, DCA in high doses increases the excretion of potassium and decreases that of sodium so that, in the serum, the potassium concentration is lowered and sodium raised. (20)(21). In the Addisonian, overdoses of this drug, especially if there is added salt, may cause edema, congestive heart failure, and cardiac dilatation, each of these probably

being to a variable extent dependant upon an excessive salt and water retention (18) In addition, overdosage in the rat has caused patchy necrotic lesions in the myocardium (22). Because these lesions are similar to those seen following a diet low in potassium (5) and because they are exaggerated by a low potassium diet (22A), they have been attributed to the lowered serum and cellular potassium (22A). The lesions were not seen in several species Selye reported (23) but they have been seen in an Addisonian dying of DCA overdosage with a low potassium intake (24). Selye (23) in the experimental animal, has produced vascular and renal disease (23) and hypertension (25) employing large doses of DCA and saline. These effects which he describes can be markedly reduced by the use of a low sodium diet (26) but with an ordinary sodium intake the hypertensive and electrolyte changes are still seen though they are less intense (27). The administration of potassium together with the DCA and saline will prevent the fall in the potassium level (21) but does not prevent the development of the kidney lesions or of hypertension (26)(28). Selye, (29), therefore, believes that the low potassium of DCA overdosage is not responsible for cardio-vascular renal changes which he reported. To him, it seems probable that the organ changes with overdosage are due rather to the sodium retaining properties and supports this by emphasizing the role of administered sodium in their development.

Probably because of its potassium lowering effect, DCA definitely increases the tolerance of experimental animals to large doses of potassium (30) (31). There are many other effects of DCA which will be discussed as we go on to describe the relation between extra and intra cellular electrolytes.

The extracellular fluid is as ultra-filtrate of the plasma and differs from it in containing less protein and lipid (32). The principal cation of the extracellular fluid is sodium as it is in the plasma. In contrast, in the intracellular fluid about 70% of which is in muscle, (33), the principal cation is potassium. The cell membrane appears to be freely permeable to the potassium

ion but allows the sodium ion to pass in small quantities only, except under unusual circumstances. (32). Peters (32) and others (34) believe this disparate distribution of electrolytes - the almost complete exclusion of sodium from cells and accumulation of potassium within them - could not be achieved by diffusion alone. It would require a considerable amount of energy to maintain such a distribution. They believe that direction of movement of the potassium ion across the membrane depends on the nature of the metabolic activity. It used to be thought that the energy for the movement of potassium was gained from the small fraction of radio active potassium in the cells but Glasko and Greenberg (35) have thrown in doubt the hypothesis that the radio-activity of natural potassium is responsible for its "unique physiological properties". In contrast to these theories, Conway (36) suggests that while the potassium ion is allowed to pass the cellular barrier freely, the larger sodium ion is to a great extent excluded and, while, the chloride and the bicarbonate anions may pass, the large anions such as sulphate and phosphate in the cell may not. Therefore potassium accumulates in the cells to satisfy the surplus negative charges. Thus, this theory of passive transfer based on differential permeability is at variance with the energy transfer theory.

All the sodium in the extracellular fluid appears to be osmotically active (32) while about 20 - 25% of the intracellular potassium is bound to protein and being undissociated is osmotically inactive and non-diffusible (37). The two compartments are in osmotic equilibrium which is brought about by shifts of water into and out of the cell (38). The sodium concentration in the extracellular fluid appears to be most important in controlling the distribution of water between the intra and extra cellular compartments (32). Thus, if there is a decrease in extracellular sodium, water enters the cell to reduce the osmotic

differences. (38). If the extracellular concentration is increased, as in fluid loss, there is a movement of water out of the cell giving cellular dehydration. (38)

The cell gains potassium during anabolism and loses it during catabolism (2). Because cells lose potassium in protein breakdown, studies on the excretion of potassium must consider the urinary potassium in relation to the nitrogen output (6)(38).

In certain conditions, where either the intake of potassium is very low, as in experimental deprivation (6)(39)(40), or where the excretion is very high as in severe diarrhea (38), or as in DCA therapy (22)(21), the potassium content of the heart and skeletal muscle cell falls and sodium enters the cell (32)(38)(2)(41) to replace it. Where there is a potassium deficit in the cell, an abundant intake of sodium chloride tends to aggravate the loss of potassium (33). In contrast, this tendancy of the cell to accumulate sodium and lose potassium is prevented by increasing the potassium intake (42). On the other hand, in adrenal insufficiency with retention of potassium (43)(34) or in the parenteral administration of potassium (38) there is an increase in the amount of potassium in the skeletal and cardiac muscle cell and a decrease in the sodium. Thus, there is often a reciprocal relationship between the sodium concentration and the potassium concentration in the muscle cell but this relationship is not always present (44)(45)(33).

The serum potassium concentration fairly well reflects the level of potassium in the muscle cell. Thus high concentrations of serum potassium are usually associated with high concentrations in heart and skeletal muscle while low concentrations are usually associated with low concentrations in the muscle. But again, this relationship does not always hold (38)(34)(44)(45). Large amounts of

potassium may enter or leave the cell with relatively small increments or decrements of the extracellular fraction (45). Although an abnormally low serum potassium is always associated with a cellular deficit, the converse is not true for, on occasion, the only way a cellular deficit can be detected clinically is by equaling potassium retention with pre-existing deficit since potassium given to normal individuals is not retained (45) and is excreted as the concentration rises (46). On the other hand, occasionally there is a high concentration of potassium in muscle with only a slight elevation of the serum level (34). Thus, we see that the potassium level in the serum does not always represent the depletion or repletion of the cellular potassium.

There are someauthorities (32)(47) who maintain that cortical hormones are necessary to maintain the integrity of the cell membrane in addition to their effect on renal excretion. They feel that the salt active corticoids may shift the equilibrium between cellular and extracellular potassium. An overdosage of DCA leads to a very low potassium level and an absence of the hormone results in high levels. That the effect is not entirely renal is supported by those experiments showing a life prolonging action of DCA given to adrenalectonized nephrectomized animals, in comparison to nephrectomized animals with intact adrenals (48).

A few words must be said about the fate of injected sodium and potassium salts. If sodium chloride is injected intra-venously, it rapidly diffuses into the extracellular fluid. If the potassium content of the cell is intact the sodium ion is largely excluded from the intracellular fluid but if the potassium is depleted, it may pass the cellular barrier (32). Ordinarily, however, as its concentration rises in the extracellular fluid, water passes from the intra-to the extra-cellular compartment (38) to maintain the osmotic equilibrium and prevent the concentration from rising markedly. If this process exceeds certain variable bounds

edema results. The excess sodium is finally excreted by the kidney but not as rapidly as injected potassium is (32).

The fate of injected potassium is different. Winkler, Hoff, and Smith (46) found that the increase in serum concentration following the injection of potassium, was about 1/3 of the expected level and showed that the rest was taken up by all the body water. However, Darrow and his workers (34) showed that potassium was not distributed equally throughout the body water. He (49) agrees with Fenn (2) that injected potassium rapidly disappears from the blood and that liver and muscle absorb relatively more than any other tissue as concentration increases. Crismon et al (50) showed that muscle takes up potassium quite rapidly and that heart muscle accumulates more than akeletal muscle. In addition to this cellular storage, in normal subjects as the serum concentration of potassium rises the electrolyte is rapidly excreted by the kidney (46). Subsequently, the excess potassium is released from the cells in which it has accumulated so that the extra potassium is not retained (32). Low potassium concentration in the cell probably increases the extent of the entery into the cells and, in contrast, this accumulation is retained by the cells. (45). Thus, large movements of potassium into the cell can occur with a large exogenous supply whether or not there is a cellular deficit. (45).

There is another influence upon the potassium level in the serum and cells which should be mentioned briefly. There seems to be considerable evidence that potassium follows the carbohydrate cycle from muscle to liver and back again (2). Glycogen storage particularly in the liver is connected with potassium, withdrawing it from the serum (51)(52). Thus, the administration of glucose (2) (53) or of insulin (54)(55) will cause a fall in plasma potassium. With the administration of adrenalin there is a transient rise in potassium probably coincident with deglycogenation in the liver (56) and then a prolonged fall (56)(59) coincident with the delayed, prolonged, liver glycogenic reaction which follows adrenalin administration. Just in connection with carbohydrate metabolism

it should be pointed out that, because some corticoids are concerned with glycogen metabolism, these sugar active compounds may exert an indirect influence on the potassium level (51).

To date some of the factors concerned with the control and distribution of sodium and potassium in the body have been reviewed. The importance of sodium in the distribution of water has already been noted. The importance of potassium in muscle physiology and in neuro-muscular and neural mechanisms will now receive some attention. Then sodium and potassium in relation to cardiac function will be discussed.

Concerning neuro-muscular and neural mechanisms the remarks will be particularly confined. To quote Donnegan (51), "there seems to be little doubt that potassium is important for the neuro-muscular mechanism - not the absolute potassium concentration, evidently, inside or outside the cell, but something more elusive in the shape of intracellular distribution." There is some evidence that small variations in the potassium concentration produce a marked variation in the parasympathetic response. Thus, a slight increase in concentration will increase the sensitivity of the heart tissue to vagal stimulation. (58). These findings by Hoff confirm those of Howel (59). Moore (60) points out that in large concentrations of potassium such as occur in inflammatory exudates, according to the German literature, the ion is capable of penetrating nerve structures and giving rise to stimulation of pain sensitive endings. Little more will be said about this phase of potassium activity.

In muscular physiology, there is some question about the role of potassium. Fenn (2) and others (61)(62) state that muscular activity causes a loss of potassium and a gain in sodium and, further (63), that this loss is proportional to the intensity of contraction and is favoured particularly by rhythmical variations in length and tension. Hoagland (64) has confirmed this loss of muscle

potassium following exercise. It might be said here that heart muscle also seems to lose potassium during activity (2) and this loss is increased in ventricular fibrillation. (65). However, Mitchel, (66) although confirming the potassium loss in a muscle stimulated to exhaustion, could not confirm it under more physiological circumstances. In addition, Miller & Darrow (67) conclude that within wide limits the amount of potassium in the muscle does not limit its perfomance. But they attributed poor performance following DCA with resultant lowering of potassium to heart lesions rather than to skeletal muscle debility. Walker (68)(69) on the other hand showed a marked decrease in muscle responsiveness with a low potassium following DCA but could not rule out changes at the myo-neural junction. This worker and others (2) have found also that increasing potassium to a certain degree seems to increase muscle performance but too great an increase (62) causes inexcitability. In man, extremes of potassium concentration may cause marked weakness, this being associated occasionally with marked elevations of potassium as in potassium therapy (70) or with marked depressions as in DCA overdosage (20) (71) as in familial periodic puralysis (47) and as in diabetic acidosis in the recovery phase. (72) It is, however, impossible to state whether this disability is due to a purely muscular disability or whether other factors enter into italso.

Thus, potassium may be important in normal muscular physiology and muscular perfomance may, to a degree, be dependent on the potassium content of the cells. Without going further into general muscle physiology, consideration will now be given to the effect of varying the potassium and sodium concentration on the heart.

It was James Blake (73), in 1839, who first investigated the effects of intravenously injected potassium. He noted, in the dog, that cardiac arrest was the cause of death and that the rapidity of death depended upon the strength

of the injected potassium. He also showed that different kinds of potassium salts all caused the same type of cardiac death. On the other hand, with "subcarbonate of soda" he showed that "the heart retained a remarkable degree of its irritability after death" when the lungs were very ecchymotic and heavy. In 1881, Feltz & Ritter (74) injected intravenously, into a dog, the same doses of potassium as Blake had used but showed that the salt was not toxic if injected slowly. Shortly thereafter, Ringer (75) published a paper showing the effect of various inorganic ions on the heart (he did not specify the species used). He found that perfect contractions could be maintained by a perfusing solution the basis of which was physiological saline with traces of calcium and potassium added. He demonstrated the tetany of the heart with excess calcium and the diastolic arrest of excess potassium, besides showing the necessity of a physiological concentration of sodium. In America, in 1899, this work was confirmed by Howel (76) and Greene (77) who emphasized the importance of physiological saline osmotically, in keeping the ventricular muscle of a terrapin in condition for contraction and the necessity of adding calcium and potassium in a certain balance to produce and sustain contractions. Howel, (59) as mentioned before, showed that increases in the potassium content of the perfusing fluid increased the vagal response of the heart while decreases diminished it. Mathison (78) in 1911 working on the mammalian heart injected various quantities of potassium chloride and observed the disturbances in rhythm produced by it, namely, sinus slowing, heart block, and ventricular fibrillation. This last he found to be a common irregularity before death but also stated that the heart may come to rest in diastole.

It was in 1930 that the electrocardiograph was used to demonstrate some of the effects of potassium on the heart. Wiggers (79) applied a 5% solution of potassium chloride to the surface of the dog heart and obtained a deflection in which the descending limb of the R wave is extended by a rounded hump or merges into

a declining plateau and pointed out the similarity of this pattern to that seen in acute coronary occlusion. In the same year, he showed (80) that potassium would stop electrically induced ventricular fibrillation in dogs. In another paper on fibrillation, he (81) noted further that potassium adminstration could also produce fibrillation. In addition, he (81) showed that this fibrillation was regularly preceded by a diminuition in the R deflection, a deepening of the S deflection, a prolongation of the entire QRS complex, depressed ST segment, and a pronounced increase in the T wave. In 1933, McLean, Bay, and Hastings (82) reported on the cardiographic effects of varying the potassium concentration of the perfusing fluid in the rabbit heart. They showed that increasing the potassium concentration to 9 m e/l or above was regularly followed by prolongation of the PR interval, deepening of the S wave, marked broadening of the QRS and a marked increase in the height of the T wave. If higher concentrations were used (12 m e/l), these charges were accentuated and ventricular fibrillation was seen. In higher concentration still (18 m e/l), there was cardiac standstill in diastole. All changes were found to be reversible when perfused with a physiological solution. These workers also demonstrated, for the first time, the cardiographic changes caused by a decrease in potassium. At a concentration of 3 m e/l, a reduction in the size of the T wave was seen, and at lower concentrations still, an inversion of the T wave sometimes appeared. These changes were also reversible.

A more comprehensive study of the relation between serum potassium levels and changes in the electrocardiogram (ECG) was undertaken in 1938 by Winkler, Hoff and Smith, (83). Using the dog, they were able to demonstrate a sequence of cardiographic changes which occurred coincident with increases in serum potassium concentration. They injected intravenously an isotonic solution of potassium chloride (1.2%) slowly. At levels of 5 - 7.8 m e/l, there was an

increased amplitude of the T wave accompanied by a shortened duration of the T wave. This progressed until the T wave equalled the QRS in amplitude and brevity. At concentrations of 7.8 - 9 m e/l the ST segment often sank below the isoelectric line. The P wave lost amplitude and disappeared at 9.4 - 11 m e/l. The QRS became widened at 10 - 12 m e/l until the whole QRS and T was a broadened biphasic complex. Finally the heart stopped in diastole. Figure 1 is a diagramatic representation of these changes in the dogs taken from these authors(84).These workers (83) felt that the rate at which the serum concentration rises might be an important factor in the toxicity of potassium but postulated that there was a critical level of potassium concentration at which the heart stopped. Nahum and Hoff (85) confirmed these findings in the rabbit and went on to show that a rapid injection of potassium produced fibrillation while slow injection resulted in cardiac standstill. Chamberlain et al (86) working on the cat, agreed with this work also, but they found less constant changes in the T waves, flattening, inversion, or coving, being seen. They felt that, while there was a general relationship between the sequence of ECG changes and the serum potassium level, there was no way of predicting the level of serum potassium at which the cardiographic changes would take place. In 1943, Crismon, (50) and his workers confirmed, in the cat, the results obtained by Winkler, Hoff and Smith, in the dog. They examined the electrolyte level in the cardiac tissue and serum at a time when the toxic effects of potassium, as measured by the cardiogram, were critical. They could find no support for the concept of a critical level of potassium concentration in the serum. They emphasized the importance of the rate of rise of potassium in the serum and the rate of penetration into the cell. However, although the cellular potassium was found to be uniformally elevated by the injection, it was no more so in the group of animals developing block than it was in the group not developing it, so that there was no necessary relationship between the absolute cellular potassium and the signs of potassium poisoning.

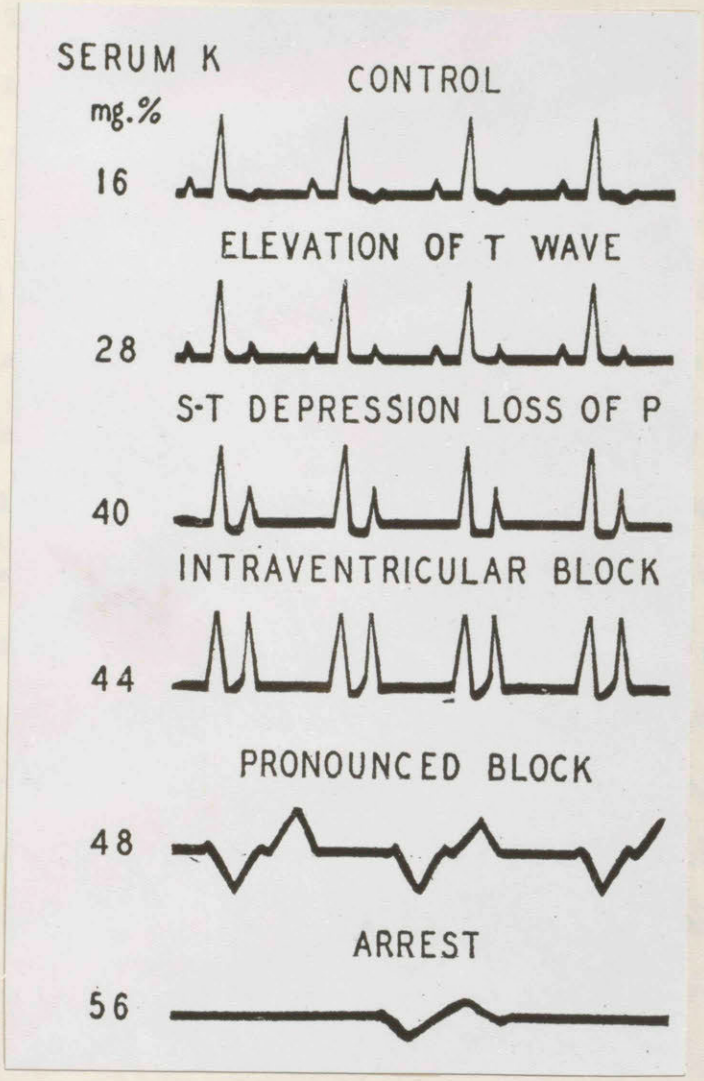

Figure 1

Diagram showing the relation between serum potassium concentration and changes in the ECG in lead 2 in the dog from Winkler, Hoff, and Smith (84) Divide mgm% by 4 to obtain m e/l.

Winkler, Hoff and Smith (87) have stated that potassium has a highly specific effect on the heart muscle which is not shared by skeletal muscle generally. Crismon (50) and his group go further and suggest in addition a highly specific effect on the purkinge cells.

Thus far, then we have shown that sodium is very important osmotically to the heart and that potassium and calcium are antagonistic in their action, a correct ratio being needed for contraction. An excess of potassium produces a functional alteration in cardiac tissue characterized mainly by a diffuse interference with conduction which may finally result in fibrillation or arrest in diastole. As the serum concentration of potassium rises and these functional alterations occur, there is a typical sequence of changes seen in the ECG. On the other hand, diminishing the serum potassium concentration also seems to have an effect on the function of the heart which is reflected in the ECG in addition to the structural effect of a lowered potassium discussed earlier in this paper in connection with low potassium diet and prolonged DCA administration. It has not yet been definitely established whether the effects of variations in potassium concentration on the myocardial fibre are due entirely to an alteration in extracellular concentration or whether changes in the intracellular concentration also play a part. Certainly there is a rough correlation between the ECG changes and serum potassium concentration. But changes in the serum potassium often reflect corresponding changes in cellular potassium. However, it is probably safe to say that the gradient between the intra- and extra-cellular compartment is disturbed. Possibly, this disturbance of gradient interferes with the potential difference developed in relation to the cell membrane and in this way interferes with cardiac function (88).

At this point, it is appropriate to describe some conditions in man in which the potassium level is altered with particular reference to the effect on the heart as gaged by the ECG. In the human, it is very difficult to study

the effect of one variable such as potassium or sodium because there are often so many other variables operative. Certain changes in the ECG, however, which are described as occurring shortly after the administration of a potassium salt, coincident with a rise in serum potassium concentrations are very probably pure potassium effects. Similarily, it would seem that the cardiographic pattern, seen in a patient in an attack of periodic paralysis, is probably a pure pattern of hypopotassemia for the picture disappears when potassium is administered. In other conditions, where the potassium balance is disturbed, there are so many other factors which may be contributing to the cardiographic pattern that the ECG may not be indicative of a potassium excess or lack even if one or the other condition prevailed.

The incidence of potassium intoxication in man seems to have been greatly enhanced by the use of potassium salts therapeutically. These salts have long been used extensively as diuretics, as expectorants, and in the treatment of cardio-vascular syphilis. Smillie (89) warned against their use in nephritis on the basis of experiments where animals with experimental nephritis were given potassium salts. On the other hand, in 1925, Rabinovitch (90) in a paper on electrolytes in uremia, reported at least three cases with very high potassium all of whom had normal cardiograms. In 1935, Keith (91) was able to recommend doses of potassium nitrate of 12.5 grams daily as a diuretic in renal disease, cautioning only against its use in anuria. Finch and Marchand (70) report that their nephritics received as much as 25 grams daily of potassium chloride.

But what of the changes in the ECG associated with an increased serum potassium. In 1937, Harris and Levin (92) injected 5 cc of 5% potassium chloride into normal subjects and showed that sometimes the T waves were elevated by the

administration. The year following the publication of Winkler, Hoff, and Smith's paper (83), Thompson (93) reported on the electrocardiographic abnormalities resulting from the therapeutic use of potassium salts in 24 cases of heart and kidney disease. In 15/24 cases, there was an increase in the height of the T wave and in these patients, the average increase in potassium concentration was 8 mgm%. A few patients showed sino-auricular slowing, a prolonged PR interval, and heart block. In 1941, Stewart and Smith (94) reported on 5 non-renal cases who had received potassium therapeutically. They did not do potassium concentrations but noted certain cardiographic abnormalities that appeared with potassium administration and disappeared with potassium withdrawal. These changes included prolongation of the PR interval, auriculo-ventricular block, auricular standstill, and intraventricular block. There were changes in the ST and T waves which resembled changes seen in coronary occlusion. In 1943, Finch and Marchant (70) described the ECGs taken during the terminal stages of uremia with oliguria in two patients receiving potassium as a diuretic, and in one of these cases the potassium concentration was determined and found to be 8.85 m e/l and terminally 10.5 m e/l. The ECG changes described were practically indistinguishable from those seen in experimental animals with marked potassium intoxication. No P wave was discernible. The QRS was very broad and rounded, the S wave deep, and the T wave round and high. Both patients showed such terminal arrhythmias as slow coarse fibrillation and ventricular asyptole and both died of cardiac arrest. Keith (88) has found 13 uremic patients with hyperpotassemia in 7 years. Three of these were receiving potassium a diuretic and, in two others, a test dose was given. The serum potassium concentration varied from 7.7 m e/l to 10.5 m e/l. They all had typical ECG findings such as those discussed above. These findings were often terminal. He found a rough correlation between the serum potassium

level and the ECG changes but pointed out that a definite picture could not be predicted from the cardiographic picture. He also correlated the pathological findings with the ECG and found that potassium can exert its action on a normal or diseased heart supporting the concept of a functional alteration rather than structural. Tarail (95) has also noted the peaked T wave, prolongation of the QRS, and increased PR interval, in 4 patients suffering from renal insufficiency and oliguria. Stewart et al (96) have demonstrated similar but less extensive cardiographic findings in addition to high serum potassium in 2 patients with unimpaired renal functions receiving potassium therapeutically. Byswater (97) has shown these concommitant ECG and serum potassium changes occurring after crush injuries with anuria. Thus, we see that the toxic effects of potassium upon the heart are seen not only in severe renal disease spontaneously or following the ingestion of potassium therapeutically, but also, in patients with unimpaired kidney function receiving potassium salts medicinally.

So far as Addison's disease is concerned, Thompson (98) presented a case showing high peaked T waves which regressed under treatment as the serum potassium fell until they were flat when the serum potassium concentration was low. However, in 1942, Thorn et al (99) stated that there was no pattern of the ECG which was specific for Addisons disease in a large number of cases. At this point it is well to emphasize two points. The first is that potassium toxicity can not be diagnosed from a single ECG for the pattern is not diagnostic (100)(88). Serially however, certain patterns emerge either progressing or regressing and it is this sequence of changes following the serum potassium levels that may be important. The second point is that a "pure" potassium effect should not be expected in a complicated condition like Addisons disease. But if, in an Addisonian, following DCA administration serial cardiograms are taken the T waves may become flat or even small inverted (101) and the

PR and QT prolonged (99) as the potassium level falls. Raab (102) has shown flattening of the T wave in normal men receiving large doses of DCA but Loeb (103) was not able to demonstrate this change after the subjects had received the drug for 2 weeks.

Further to the ECG findings with a low serum potassium, Stewart, Smith and Milhorat (135) first correlated the findings of the low serum potassium in an attack of periodic paralysis with changes in the ECG. They observed a prolonged PR, QRS, and QT, with a flattening of the T wave. Other workers (104) have found this lowering of the T wave and prolonged QT interval. Danowski et al (105) have presented evidence attempting to show that during an attack of paralysis the potassium moves from the extracellular phase to the intracellular and that this shift is the cause of the decline in serum potassium. If this is so, it means that the changes in the ECG are due to the lowering of the extracellular potassium concentration. Once again, however, it may be the disturbance of the normal gradient that is truly important.

In addition to these situations where the potassium is lowered, it may be decreased in chronic nephritis with a lowering of the T wave. (106). Also, patients recovering from diabetic acidosis were noted by Billet and Dyer (107) to have prolonged QT and flattened T. In 1946, Holler (108) associated these changes with the fall in potassium coincident with glucose administration, insulin administration, diuresis, and dehydration. He showed that the cardiographic abnormalities were abolished by the administration of potassium. Danowski and his group (109) recall that a very low cellular potassium in DCA overdosage or in low potassium diet will produce, experimentally, degenerative changes in the myocardium and report that a 26 year old boy with the lowest serum potassium they have encountered in this condition developed inverted T waves that did not return to normal for 2 months. Although this change may easily be due to

other factors, it seems possible that here is an example of a structural defect resulting from the potassium depletion. These workers (109) and others (110) note the rapidity with which administered potassium leaves the serum without appearing in the urine or faeces indicating that it is taken up by the depleted cells. Thus, it is possible that the increase in intracellular potassium is the factor improving the cardiogram following potassium administration in this condition. But against this is the fact that at the height of coma, the cellular potassium may be severely diminished yet the serum potassium and the ECG may be within the range of normal (109). It is only after the serum potassium falls in the recovery phase that this change in the ECG developes. Although it seems highly likely that a disturbance of extracellular potassium concentrations is responsible for the electrocardiographic changes, it can not be definitely stated that the intracellular changes do not have a role.

It is time now to consider the changes which occur in the potassium content of the diseased heart. In view of the possible role of potassium in muscle physiology, Harrison, Picher, and Ewing, (72) in 1930, approached the problem. Taking specimens for analysis from recently deceased patients, they found that the potassium content of skeletal and cardiac muscle of individuals dying of congestive heart failure was less in dry weight than that of subjects dying from other causes. They wondered if this loss of potassium connected with heart failure might be an important factor in causing subsequent breaks in compensation. They raised the question as to whether functional changes resulting from a loss in potassium might not be reversed by administering potassium. This same group (111) carried out analysis on skeletal muscle of living patients with congestive heart failure in an attempt to rule out post mortem changes as the cause of the low potassiums found in their previous report. In the patient with congestive heart failure and edema, the potassium content of the wet gastrocnemius

was invariably low and the sodium and water content high. The amount of potassium in the dry muscle was usually, but not always, diminshed. As the edema decreased, the potassium content of the dry muscle sometimes rose. They demonstrated that administered potassium increased the potassium content of the muscle. More recently Myers, (112) and Decherd and his group, (113) have confirmed the loss of potassium and, in addition, indicated a loss of creatine per unit weight in chronic hypertrophy of the heart in experimental animals, the loss being greatest in the affected ventricular muscle. Thus, in some types of chronic heart disease, the myocardium loses potassium. The question is whether this further impairs the function of the muscle.

The problem in acute myocardial infarction is somewhat more difficult. Baetzer (114) in 1935, summarized the various conditions under which skeletal muscle loses excessive potassium. He listed injury and death; perfusion or emersion in solutions low in potassium; severe fatigue; increased acidity. He amplified this list by showing that haemorrhage, vasoconstriction or temporary block of arterial supply caused a marked and rapid loss of muscle potassium as measured by a marked increase in the potassium concentration in the femoral venous blood if the venous blood flow was reduced. Now the point is could these changes in potassium concentration be occurring in the myocardium following acute coronary occlusion? Dennis and Moore (115) tried to answer this question in 1938. Working on cats and ligating the coronary artery, they showed that samples taken from the venous blood in the coronary sinus, which was also ligated to fascilitate the procedure, showed a marked increase in potassium in comparison to a sample withdrawn simultaneously from the vena cava. They showed that neither congestion from the coronary sinus ligation nor the operation itself significantly increased the potassium in the sample so that they felt justified in attributing the increase in the potassium in the outflow blood to ischaemia.

Hermann & Erhard (116), considering this indirect evidence, worked on dogs ligating a branch of the anterior descending branch of the left coronary artery. They took tissue samples 1 to 22 hours after ligation inside and outside the infarcted area. They found a loss of creatine but not of inorganic phosphate and potassium. It should be noted here that the area infarcted was small and it would be inevitable that they would include normal with asphyxiated tissue and again the smallness of the branch ligated would make it impossible to be sure that the area actually was infarcted and no attempt was made to prove it. The creatine (117) which was lost almost certainly came from the damaged muscle so that probably potassium and phosphate would be released too but if this were the case these electrolytes must have been held in the damaged area. At any rate, Hermann & Erhard could not agree with Dennis & Moore.

In 1939, Dr. Blumgart and his group (118) approached the problem with a view to finding why electrocardiographic evidences of infarction were often present before there was any morphological change in the tissue. After occluding the left circumflex branch in dogs for 45 minutes the animals were sacrificed in 24 hours and a tissue sample taken inside and outside the infarcted area. They found on tissue analysis a marked increase in extracellular fluid, but no increase in intracellular fluid. The potassium showed no change when expressed per unit cell water. Once again we do not know for sure whether the area is infarcted, though previously this procedure had produced diagnostic ECG findings. And most important we do not know the size of the tissue sample. This is very important for it is known that in infarction the subepicardial layer is most frequently spared (119). How much ischemic tissue was in the sample?

This work all seems very incomplete and conflicting as yet. It seems very probable theoretically, at least, that with the sudden catastrophe of occlusion, the degenerating muscle would release considerable quantities of

creatine, phosphate, and potassium. If potassium accumulated around the ischemic muscle the present methods of estimation might not show such an important change in distribution. The increase in extracellular fluid seems reasonable in view of the exudation that probably occurs.

There is yet another facet of this problem of potassium change in heart disease. Wood and Moe (120) state that "it has been conclusively demonstrated that digitalis has a direct effect on the contractile power of cardiac musculature". In view of this fact and in view of their work on the loss of potassium in cardiac failure, it was logical for Calhoun and Harrison (121) to investigate the relation digitalis bore to potassium. They found that digitalis in therapeutic doses either did not change the potassium content of the heart muscle of dogs or only slightly diminished it. On the other hand, in toxic doses, digitalis caused a diminuition of the potassium content. Wood and Moe (120) showed that digitalis in both toxic and therapeutic doses caused a loss of potassium from the myocardium and after reviewing the physiological evidence that potassium loss is proportional to tension developed, postulated that potassium loss from cardiac muscle during digitalis action was the result of increased cardiac activity. Wedd, (122) however, could not agree fully and showed that when potassium loss occurred, it was a late toxic manifestation. Friedman and Bine (123) have recently confirmed this. Hazen (124), and Boyer and Poindeter (125), agreed that potassium was lost with toxic doses of digitalis but they, on the other hand, present evidence to show that digitalis in therapeutic doses causes a slight increase in cardiac potassium.

Zwemar and Lowenstein (126)(127) point out that strophanthin and DCA are both protective against lethal doses of potassium chloride and that adrenalectomized and potassium chloride treated animals are particularly resistant to strophanthin. Kinsell (128) and his workers point out that excessive doses of

DCA or of strophanthin produce a loss of potassium from the cell and replacements by sodium. They believe that both DCA and strophanthin in physiological doses tend to maintain the balance between intracellular and extracellular potassium as they both antagonize the action of thyroxin in causing a potassium loss from the cells.

Even though some of this evidence is conflicting, on the whole, it seems to indicate that the functional integrity of the heart muscle cell is partially dependant on its potassium content and that digitalis acts at least in part by preventing or restoring the loss of potassium from the myocardium with damage.

Another matter that should be considered is the role of the general adaptation syndrome (29) in acute coronary occlusion. This chain of reactions must surely be brought into play in some such cases for the stimulus is not only severe but may be quite prolonged. The electrolyte changes which occur during this syndrome may be important to this problem. Selye (29) states that in the shock phase of the alarm reaction, after exposure to many different stimuli, there is an increase in serum potassium and an increased urinary potassium excretion. He feels this is due to a discharge of potassium from the tissues into the blood. In addition, in this phase, there is a diminuition of urinary sodium and chloride but these substances are also reduced in the blood which Selye (29) suggests is due to leakage into interstitial spaces. These sodium and chloride values return to normal in the resistant phase but may fall again with stage of exhaustion. The potassium changes past the shock phase have not yet been conclusively demonstrated. (56).

Thus, the general blood picture following stress is a high serum potassium and low sodium. These changes in the blood are similar to those found in adrenal insufficiency but the urinary picture is different. At any rate, compounds of the adrenal cortex have been widely used to combat shock. In animals with intact adrenals, Selye (29) found "under certain conditions these hormones

can prevent shock and fascilitate the development of counter-shock phenomena. It is possible that some types of damage are better combatted with salt active and others with sugar active curticoids." However, he found in general that cortical extracts and carbohydrate active corticoids are more efficient than salt active ones. These last he found of greatest value in dehydration and potassium poisoning. It should be pointed out here that sugar active compounds have some salt action (16) directly and some action on potassium levels indirectly because of their carbohydrate effect. The zona fasciculata of the cortex has been shown to be the zone of production of these sugar active corticoids while the glomerulosa produces the salt active (129)(130). In stress, the fasciculata appears to be the zone principally affected., (129)(130) so that it seems possible that some of the electrolyte changes in stress are due to over production of sugar active compounds.

Certain workers point out in the shock following severe muscle injury, the injured muscle loses potassium and gains sodium (131)(132 and coincident with the alterations in the serum concentrations, the uninjured muscle gains potassium and loses sodium (132). Several authors (132)(133)(134)(135) have suggested that the increased serum potassium in injury and stress might be harmful. However, Winkler, and Hoff (136) point out that the increased potassium concentrations encountered in experimental traumatic shock, although they may cause an elevation of the T wave, are only rarely of a sufficient degree to cause a cardiac death. In addition, if potassium is so important in injury and stress, it is difficult to see why DCA has not been the most effective counter-shock hormone. On the other hand, if the heart itself were the acutely injured organ and if the uninjured tissue gained potassium and the injured tissue lost it, and if the alarm reaction of the general adaption syndrome is associated with a high serum potassium and a drop of cellular potassium, it is possible

that these disturbances of the normal extracellular-intracellular gradient might be an additional factor disturbing the functional integrity of some of the myocardial tissue.

The time has now come to consider what work has already been done on the effect of varying the potassium concentration on certain cardiac disorders. Thompson (93) had pointed out the elevation of the T wave after administration of potassium to certain cardiac and mephritic patients. Keith (137) and Sharpey-Shafer (138) have shown that the inverted T wave which is often seen in the ECG of hypertension in a pattern commonly referred to as ventricular strain became upright after administration of potassium salts and then gradually became inverted again as the potassium concentration fell. Bryant (139) from F.N. Wilson's laboratory reported in 1948 that potassium besides elevating the T waves reduced the voltage and the length of the QRS. These improvements she said were of the same order as those found after sodium restriction and after sympathectomy. Sharpey-Shafer (140) has also reported that the flat T wave of the myxedematous heart were elevated by potassium. These results seem to indicate a definite action of potassium on the T wave of these chronically diseased hearts. Whether the elevation of the T wave also indicates better myocardial function in these circumstances is not known - all that can be said is that such increases in amplitude of the T wave in other conditions often parrellels clinical improvement. But in this particular case, the T wave change may be only a toxic one indicating further danger to the heart rather than a sign of improvement. As far as can be found, no one has carried out the suggestion made by Pilcher and his worker (111) that potassium be used in the treatment of a chronically damage heart by publishing a controlled study having potassium as the only variable in the treatment of chronic heart disease.

In acute myocardial infarctions, Sharpey - Shafer (138) administered potassium salts, and in these cases, in contrast to the changes seen in chronic heart disease, the pattern of infarction in the ECG became markedly accentuated - that is to say T1 and T4 became deeply inverted in the case of anterior infarction and T2 and T3 more inverted in the case of posterior infarction while in both types the upright T waves in the other leads became more upright. Once again it is impossible to interpret this finding except to say that it seems evident that in acute infarction potassium administration seem to exaggerate those myocardial changes which are responsible for the inverted T wave. However, the basic change in the myocardium responsible for T wave changes is still not known. One clue, however, to this potassium action might be found in the work of Linder & Katz (141) who showed that in a denervated living coronary vessel increasing the potassium concentration from $1\frac{1}{2}$ to $2\frac{1}{2}$ times the normal concentration produces a severe and long lasting constriction of the vessels even to the point of occlusion. The vagal effect of potassium in intact animals may even exaggerate this.

It has been noted previously that toxic doses of digitalis cause a loss of potassium. Sampon and his group (142) have shown that the ectopic beats of digitalis intoxication can be abolished in every instance by potassium salts. Previous to therapy, there was no abnormality in the serum potassium levels and with ingestion there was no disturbance of the serum potassium absorption curve. Not only were the ectopic beats abolished by potassium administration, but also they failed to return in many instances after the potassium concentration has fallen to normal levels. They felt that this indicated that the potassium had become fixed to, or had altered the state of, the cardiac muscle. They also felt that digitalis toxicity was definitely due a disturbance in potassium balance. Here, then, is an instance where a disturbance in potassium balance

has a detrimental cardiac effect which is corrected by potassium administration. Here, also, is an instance of a low cardiac potassium which is not reflected in the blood potassium level.

Having to date considered many aspects of sodium and potassium physiology, and having taken note of some of the cardiac effects of these ions, I would like now to pose the problem as it suggests itself to me.

Because of the loss of potassium which has been shown to occur in some types of chronic heart diesase (72), because the potassium content of the muscle cell may be important to its functional integrity (68,69), and because some of the beneficial effects of digitalis may be due to its power to maintain or increase cellular potassium (124), it seemed that there might be some value in the treatment of chronic heart disease with potassium. The effect of potassium on the T wave of the ventricular strain pattern in the ECG of hypertensive heart disease is most interesting for a similar change in the T wave after sympathectomy and after sodium restriction has been taken as evidence of a patients improvement (139). Improvement in myocardial function following sodium restriction may be due to a number of factors, among them a reduction of extracellular fluid volume, cellular hydration, or a reversal of the tendancy of high sodium to increase potassium loss. But whatever the role of potassium in some types of chronic heart disease, in view of the low glomerular filtration rate of many patients with congestive heart failure because of the diminished cardiac output (11A) and, possible associated kidney disease, and in view of the fact that potassium excretion depends upon glomerular filtration rate. (9), the administration of potassium in these conditions seemed to be fraught with danger. At any event, it was not possible to carry this work beyond its preliminary stages.

The field which seemed most promising was the field of acute myocardial damage. By varying the electrolyte concentration in an infarcted area, the blood and oxygen supply are certainly not going to be restored. But what was

of particular concern was the "twilight" zone surrounding the central, severely damaged area. In this irregular surrounding zone of relative ischaemia and disturbed metabolism, there is an increase in extracellular fluid, an exudate which contains many red and white blood corpuscles. Now, could a decrease or an increase of the potassium in the myocardial fibres or extracellular fluid in this zone further impede the function of the myocardial fibres in the region? If either of these propositions is true, then raising or lowering the potassium concentration therapeutically might be expected to have an effect on the myocardial function. Let us make out a case for the need to increase the potassium concentration in that region. It seems probable that the necrotic cells would lose potassium. Now is it possible that the myocardial cells in the "twilight" zone would also lose potassium? This has not been shown experimentally but, if it were a fact, certain findings should be recalled. Muscle cells which exhibit a potassium deficiency probably have a diminished functional capacity (68)(69). Certainly, myocardial function suffers with the loss of potassium occurring in digitalis intoxication. (121). In chronic heart disease with a diminished cardiac function a loss of potassium is associated (72). Although it is probably that different factors acting upon the myocardium may cause similar changes in the ECG, it is notable in respect to this problem that an inverted T wave is seen in both coronary insufficiency and potassium deficiency. In view of these facts, if there is a potassium deficiency in the surrounding zone, it seemed possible that increasing the potassium concentration might improve the cardiac function.

On the other hand, is it possible that the potassium diffusing out of the necrotic muscle cell and red blood cells raises the extracellular concentration around the necrotic zone to high levels? Do the potassium ions accumulate in the surrounding zone further impairing the function of the myocardium?.

Dennis & Moore (115) point out the very high potassium concentration in inflammatory fluid recorded in the German literature. The possible vaso-constrictor action of such an accumulation has been mentioned (141). It has also been noted that a potassium loss can often not be demonstrated in an infarcted area but yet creatine is lost. (116). Does this indicate that the potassium lost from the degenerating cells accumulates in the region attracted by some metabolite for potassium might well be released from the cell if creatine were being released? Sharpey-Shafer's (138) work showing that potassium administration accentuated the coronary pattern of the ECG might suggest that there already is an accumulation of potassium disturbing myocardial function so that the administration of further potassium exaggerates the disturbance. Although the ECG picture in acute infarction is not that of potassium excess, the picture of excess as we know it is one of a generalized excess. The picture of a localized potassium excess is not known except for the pattern produced by localized surface applications of potassium which Wiggers (79) demonstrated to yeild a pattern similar to a coronary pattern. In the early stages of the general adaptation syndrome, there is frequently an increase in serum potassium. Thus, is it possible that lowering the serum potassium would have a beneficial effect of the acutely injured heart?

It is realized that both these propositions are extremely hypothetical. However, it seemed just possible that this type of approach in the treatment of acute coronary occlusion might be of some value. Thus, this problem of the effect of varying the electrolyte concentration on an acutely damaged heart is the most important part of the experimental work in this paper.

Experimental Section.

A. Choice of Animals. - Albino rats were chosen for this work for several reasons. First, hardy imbred strains of Wistar and Sherman animals were available and facilities were at hand to manage these animals in large numbers. It was felt that numbers were extremely important in this type of study. At McGill, there is often considerable difficulty in obtaining large numbers of larger animals, such as cats and dogs which are so frequently used in experimental cardiac investigation. Even if they were obtainable, there are so many uncontrolled variables present when these animals are used experimentally. Just to mention a few of these, there is age, breed and strain dietary and environmental background, and the condition of the coronary circulation and myocardium. In fact, there are so many variables that a group to be reliable would have to be extremely large. In the rat used here, breed, strain, age, diet, environment, are all known. None of the animals in our experiments was more than 4 months of age, and in the rat coronary disease is rarely if ever seen at this age (143) so that this factor is controlled also.

B. Electrocardiography. 1. Apparatus Technique. - The logical way to record the effect of varying the electrolyte concentration in these animals is by electrocardiography, particularly in view of the effect of potassium on electrocardiogram (ECG). Accordingly, a Sanborn Cordiette being available, tracings were taken on normal anesthetized animals placed face down on a board with the appropriate limbs stretched onto three electrodes fixed unto the board. The board and contact plates are shown in figure 2 while the typical tracing obtained with this set up are shown in fig. 3. Very quickly certain

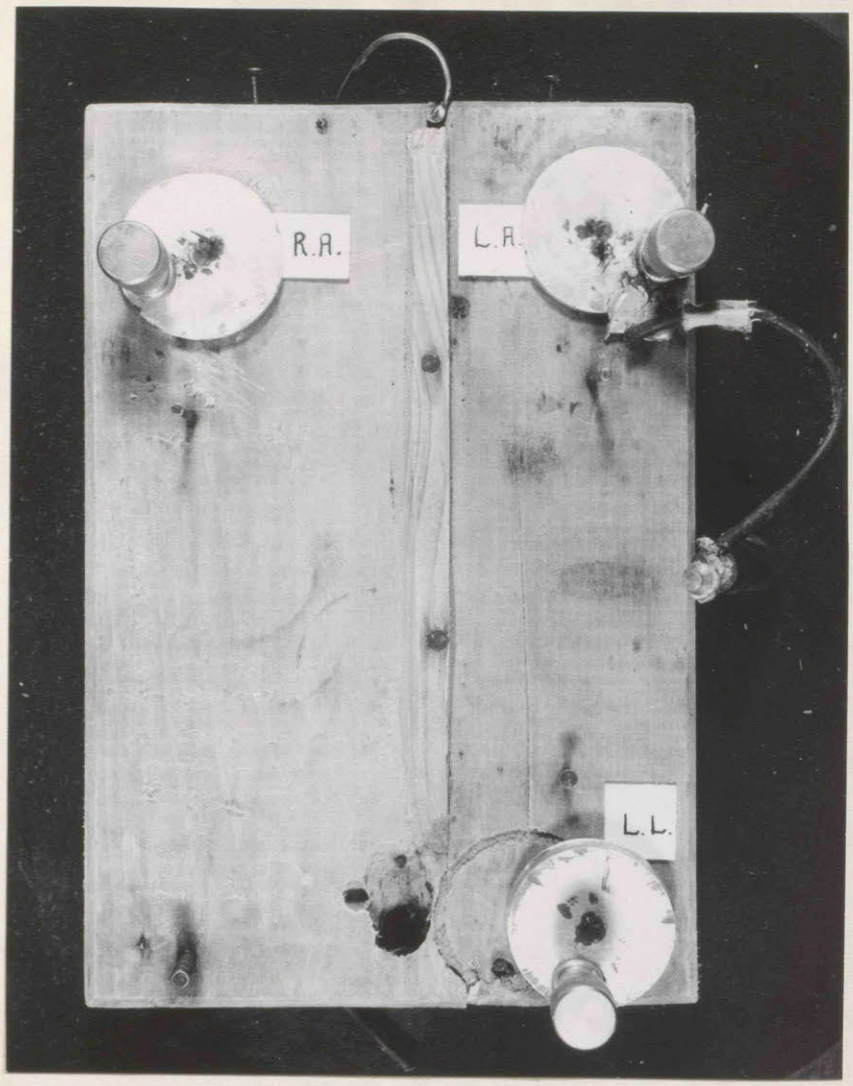

Figure 2.

The posturing board. Note the 3 electrode plates, the small manual chest electrode, the metal arc. at head of board, the drilled tail hole and the central ridge pole.

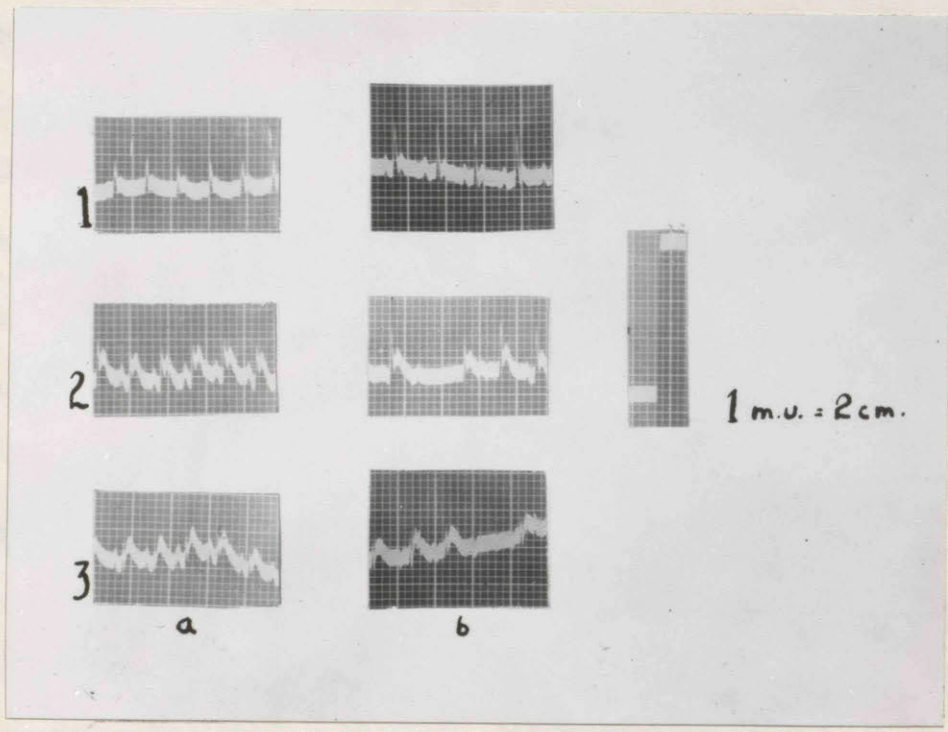

Figure 3.

Tracings with ordinary commercial electrocardiograph. (a) Low amplitude, ST takeoff elevated, no S wave. Note especially lead 1. (b) Better tracing but notice ST takeoff elevated lead 2. Note skipped beats.

facts became obvious. The complexes in lead 1 were often of a low amplitude. The S wave was often absent in all leads. The ST segments were frequently well above the isoelectric line. These tracings were definitely unsatisfactory. On consulting the literature, an excellent paper by Rappaport and Rappaport (144) on the ECG in small laboratory animals soon showed why this was so. Evidently, in ordinary commercial cardiographic apparatus, which is designed primarily for human use, the speed with which the string responds to electrical stimulation is usually about 0.01 seconds. This is perfectly satisfactory for most heart rates encountered in the human heart but in small laboratory animals the heart is often more rapid and these higher speeds require a higher string speed for in such animals the QR interval is often less than 0.01 seconds. This means that the galvometric speed is slower than the electrical phenomena being recorded. Thus when using a slow string speed to take a tracing on these animals, the cardiac action potential which stimulates the string is terminated before the recording beam has traversed the true distance so that there is an alteration in the amplitude of the QRS. It means also that the T wave commences to register before the R deflection has had time to return to the isoelectric line. Thus, the S wave is not shown and the ST segment appears well above the isoelectric line. The string speed of a machine can be gaged by the standardization tracing. The string speed can be increased to 0.0015 seconds by tightening the string to 1/10 normal sensitivity (1 millivolt gives a deflection of 1mm instead of 1 cm) so that now the string speed is considerably faster than the QR interval in small animals. This string speed will accurately record electrical events in animals with heart rates up to 750 beats per min. With this reduction in string sensitivity, an amplifier is needed, however, so the deflections will be large enough to read. With the string sensitivity set at 1/10 normal, the amplifier is now adjusted so the 1 millivolt through the amplifier deflects the tense string 1 cm. A dampening device is also inserted into the circuit to prevent over shooting and

distoration from the high string tension. Using this equipment a standardization curve need only be done once for each tracing, it not being required for each lead because the amplifying device automatically compensates for patient resistance in different leads. In consultation with M.B.Rappaport (145), a string galvanometer machine (a Sanborn Electric - Porte Electrocardiograph) was obtained along with a dampening device and an amplifier of the correct type (Sanborn Amplifier). The string tension was set to 1/10 normal sensitivity and the amplifier adjusted so that 1 mv gave a string deflection of 1 cm. The paper speed was increased to 50 cms. per second to spread the complexes out. Thus, the time lines on the abscissa were separated by 2 mm instead of 1 mm but the time between these lines is still 0.04 seconds. Figure 4 shows this equipment, the standardization curves, and a typical tracing.

The animal was spread-eagled on its back on the board shown in fig. 2 with the limbs well coated in electrode jelly and held firmly to the electrodes with elastic bands. This position was felt to be more standard with respect to the electrical axis of the heart than the one previously used. Electrical interference at first was easily overcome by simple grounding of the apparatus, but later, changes in the electrical set up in the building introduced a tremendous amount of alternating current interference. Rappaport (144) points out that these little animals have a high resistance and that in subjects of high resistance alternating interference may cause considerable distortion. He advises taking every step to reduce the subject resistance. After trying all kinds of grounding combinations to reduce the interference, the problem was probably solved by piecing the skin, a maneuver which would effectively reduce subject resistance and maintain a constant contact. Thus, later in this work, the sharp curved point of a surgical cutting needle was set into each electrode plate and the animal's paws were transfixed in the web between the digits.

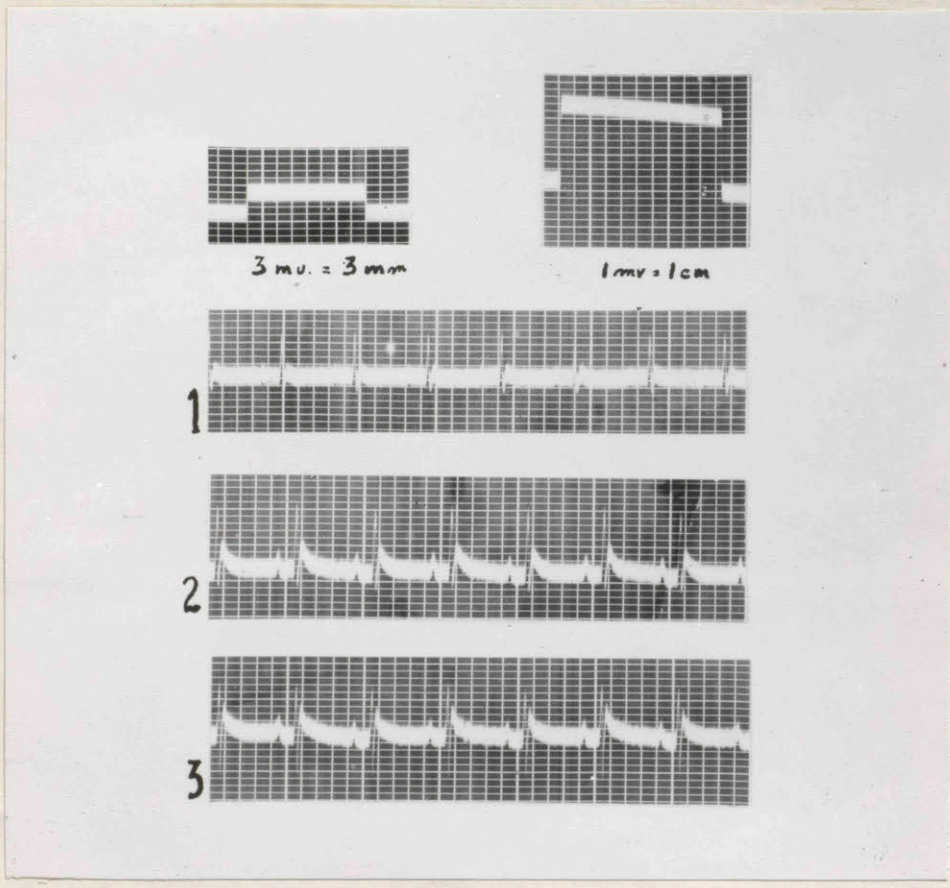

Figure 4.

Above is the cardiographic apparatus, including the string galvanometer, the amplifier and dampening device. Below, note the 1/10 normal standardization on main machine as string is tightened so that 3 mv. deflects string 3 mm. Standardization through amplifier 1 mv. deflects string 1 cm. Note also rapidity with which string responds to stimulation. A typical tracing is also shown.

2. Anesthesia. The question arose as to whether the animals should be anesthetized. In this work, the size of the animals would make restraint of the unanesthetized animal extremely difficult. It seemed probable that the anger, struggling and fighting encountered in pinning down an unanesthetized animal would introduce a tremendous variable into the tracings and this might be particularly troublesome in the comparison of repeat tracings. A suitable anesthetic would not only make the animals much easier to handle but also would impose on them a relatively constant condition under which the tracings would be taken and thus fascilitate the comparison of repeat tracings. The problem was to find the suitable anesthetic which would be uniform in action and non-toxic to the heart in the doses used.

Ether was the first anesthetic tried. Cushny (146) states that in the concentration used in anesthesia, ether does not damage the heart but Bennet & Fisher (147), on the basis of a review of the literature and some experimental work, point out that, at the second plane of anesthesia and at greater concentrations, the dynamic changes produced are those of a failing heart. Other workers (148) had shown that inhaled ether caused a transient but precipitant drop in serum potassium - a most undesirable variable in this type of work. Thus, on these bases, ether seemed unsatisfactory. In addition, after using ether before its disadvantages were fully realized, it proved unsatisfactory. The desirable level of anesthesia was very difficult to maintain because so much attention had to be devoted to the operation of the apparatus. The respirations often became heaving and troublesome to the tracing. In 60 odd tracings taken under ether in this initial stage of the work, there were five deaths. This mortality of 8.3% might be very awkward in experimental work on a limited number of animals. As far as the tracings obtained were concerned, skipped beats (fig. 3b) were seen occaisionally and in one animal a a series of tracings taken on successive days revealed the gradual development of an inverted T1. (fig. 5). Thus after using ether for a brief period it was dis-

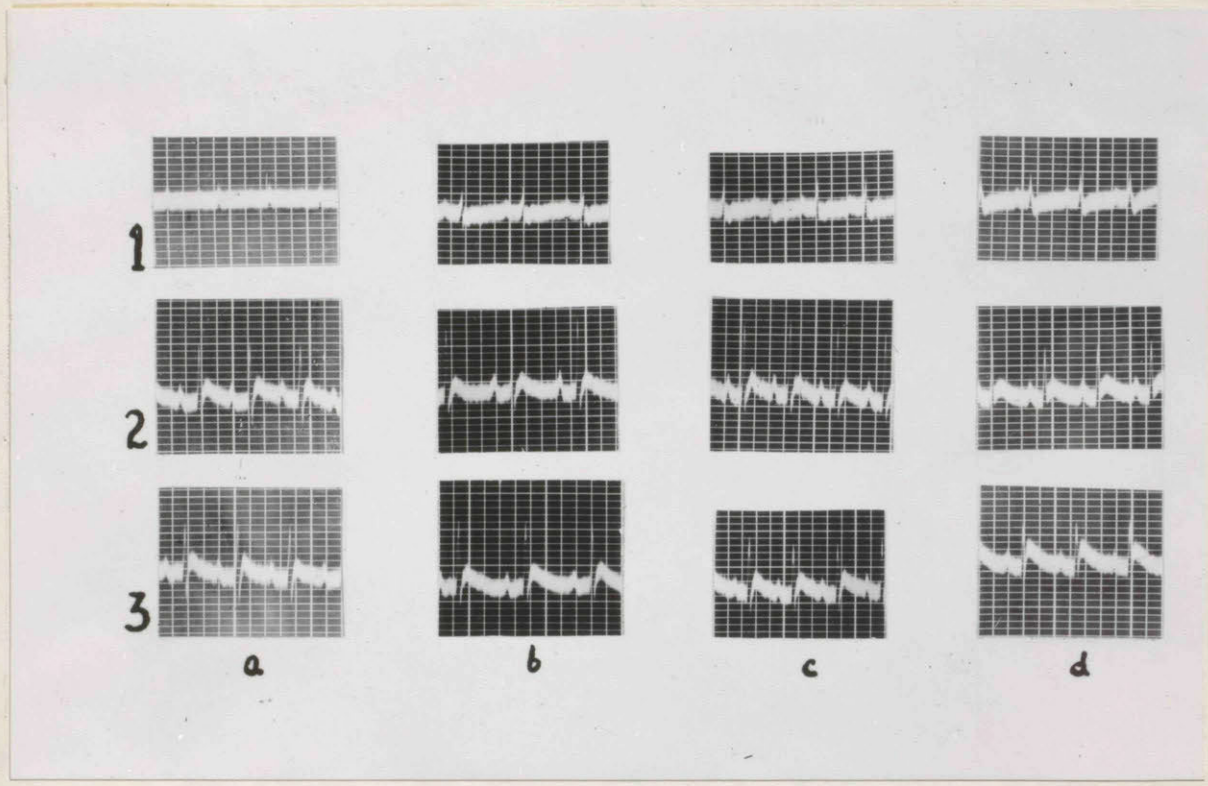

Figure 5.

Inversion of T1 with repeated ether anesthetics. (a) original tracing. (b) 3 days later. ST & T segment slightly depressed. (c) Following day T1 small inverted (d) Following day. Evipal. T1 still small inverted.

carded.

Nembuttal was given a trial. Waller and Charipper (149) had used this anesthetic in the dose of 30 mgm per kilogram given intraperitonially. This barbiturate is almost completely destroyed in the liver so that induced kidney damage was not likely to increase its toxicity. (146). However, in this work, it was found that either the animal was not deeply enough anesthetized or else was too deeply so. At a satisfactory level of anesthesia the animal was often unconscious for four hours or more, its respirations being very shallow and its colour bluish. On one animal, the tracing was repeated after a four hour period of unconsciousness and depressed respiration (fig. 6) and this showed a marked increase in the height of the T waves which had returned to normal the following day. This increase in the height of the T wave after this prolonged period of depressed respiration might well have been due to the increase in serum potassium concentration which occurs with anoxia, (150), and indeed this increase in the height of T waves has been reported following anoxia (98). Here again is a variation in potassium which is undesirable in this type of work. In 10 animals receiving a dose of 35 mgm per kilogram, which was the dose found to be uniformly effective, there were two deaths. This drug was rather slow in taking effect also, but had the advantage that all animals could be anesthetized at one time and the tracings taken in rapid succession.

By far the most satisfactory drug was found to be evipal. Initially, it was decided to try this drug because it is very fast in action and the anesthesia induced is very short in duration (146). In addition, because it is destroyed in the liver (146) its toxicity would not be influenced by induced kidney disease. It is not supposed to have any detrimental effect on the heart, its toxic effects being due to respiratory depression (146). The soluble evipal sodium was diluted with distilled water so that a concentration of 10 mgm /cc. was obtained. The intraperitonial dosage needed for this electrocardiographic procedure, especially when the paws were transfixed on the electrode plate was for the male about 10 mgm/100

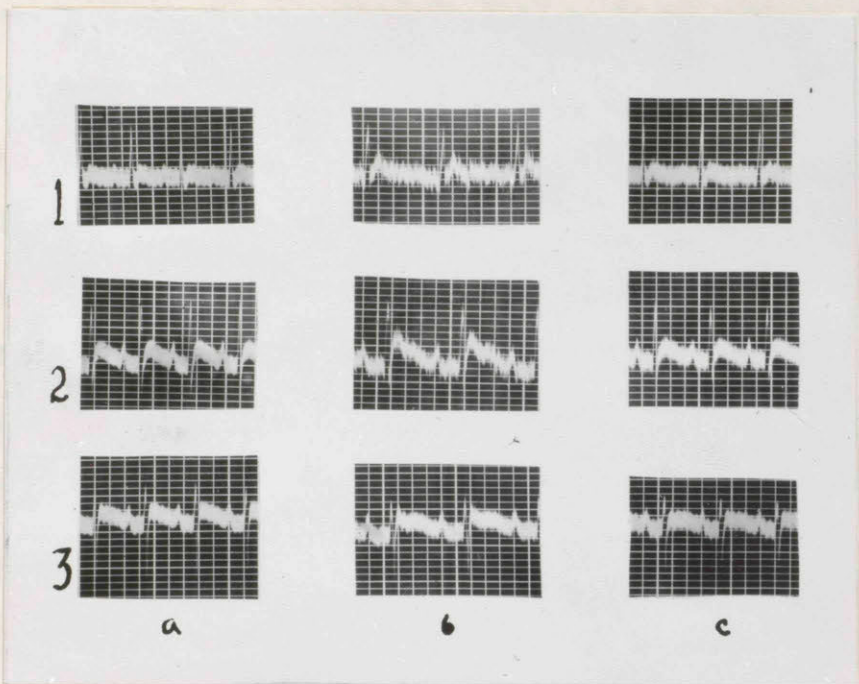

Figure 6.

Change in T waves with nembuttal. (a) original tracing (b) tracing after 4 hours of respiratory depression. Note increase height of T waves (c) Following day. Similar to (a)

grams and for the female about 9 mgm/100 grams. In this dilution, these dosages were very easy to handle. Thus, a 125 gram male received 12.5 mgm of evipal delivered by injecting 1.25 cc of the solution. These dosages were subject to slight variations according to strain, general physical condition of the animal, and size. In a small animal under 100 grams, doses just under those prescribed above seemed effective - that is a male of 80 grams could be managed usually on 7 mgm (.7cc). Large animals over about 250 grams on the other hand were more uniformly handled with slightly increased doses - for instance a 300 gram male would be given 31 or 32 mgm rather than the 30 mgm prescribed above. If one is careful with this dosage, evipal sodium is very safe. In over 600 anesthetics given, there were only three normal animals dying for a percentage of about 0.5%. An additional three animals in very poor condition following an experimental procedure died following the anesthetic but it was felt that this was a very special circumstance. However, even, if these deaths are counted, the total would be 6 deaths in 600 anesthetics for a mortality of 1%. In two other animals, by mistake, dosages well above those prescribed were used. These mortalities were not counted.

This anesthetic, injected intraperitonially, takes only 3 - 5 minutes to be effective. If one rat were injected just before another had a tracing taken, the animal was well anesthetized by the time the tracing was finished. If the animal hangs limply on its back in your hand, breathing quietly and yet has a slight reaction to pain when it is pinned down, the anesthetic level is just right. If, as occasionally happens, because of some variation in animal susceptibility, the rat does not hang back limply, an additional dosage can with safety be given. This additional dosage is in the order of 3 - 6 mgm (.3 - .6cc) depending on size. During the anesthetic the animal breathes quietly and regularly and has a good colour. Within 5 - 15 minutes after the anesthetic level has been reached, they are usually becoming

active again. They may appear dopey and sleepy for half an hour or so but respond well to stimulation. As will be seen later, in the ECGs obtained under this anesthetic, there were only slight variations seen in repeat tracings. There was nothing suggestive of a potassium effect in the tracings taken under this anesthetic but then hypoxemia was not apparent. I can find no evidence in the literature to suggest an effect of barbiturates on the potassium levels. Potassium concentrations under dial (150) anesthesia are well within a normal range. Under amytal (133), 8 blood samples failed to reveal any change. In the absence of any indication in the literature of an effect of barbiturate on potassium levels, in the absence of signs of hypoxemia, and in the absence of any suggestive alteration in the tracings, a special study on the effect of evipal on the serum potassium concentration was not undertaken.

3. Posture. - Before going on to the normal ECG findings, it should be pointed out that with the animal anesthetized with evipal and spread-eagled on the board and with the use of the cardiographic set up described, lead 1 was sometimes of a very low amplitude and uninterpretable. Waller and Charipper (149) also found this. Since lead 1 is so important in detecting changes on the lateral wall of the heart (119) and since this lead was expected to be the most valuable in showing the cardiac changes produced by hypertension or experimental heart injury, it was particularly important that this lead be improved. Innumerable authorities have pointed out the effect of the position of the heart on the electrical axis. In the rat, the heart is centrally placed and vertical and this position would tend to produce a small deflection in lead 1. It was hoped that if the animal were tilted well to the left, this would throw the heart into a more horizontal position and thus make lead 1 more prominent. This proved to be a useful device in those animals with a small lead 1 deflection. In fig. 7, a tracing taken in the horizontal position is compared to that taken with the board at an angle of about 70 degrees. Such a poor lead 1 was occaisionally seen with the board horizontal but never with it tilted.

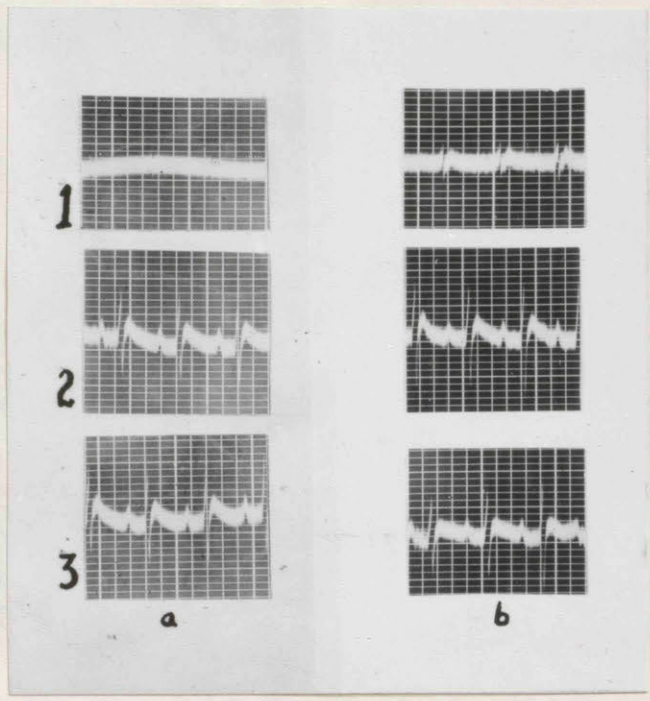

Figure 7.

(a) This flat lead 1 seen occasionally when tracing taken with the animal in the horizontal position (b) Same animal with board tilted to left.

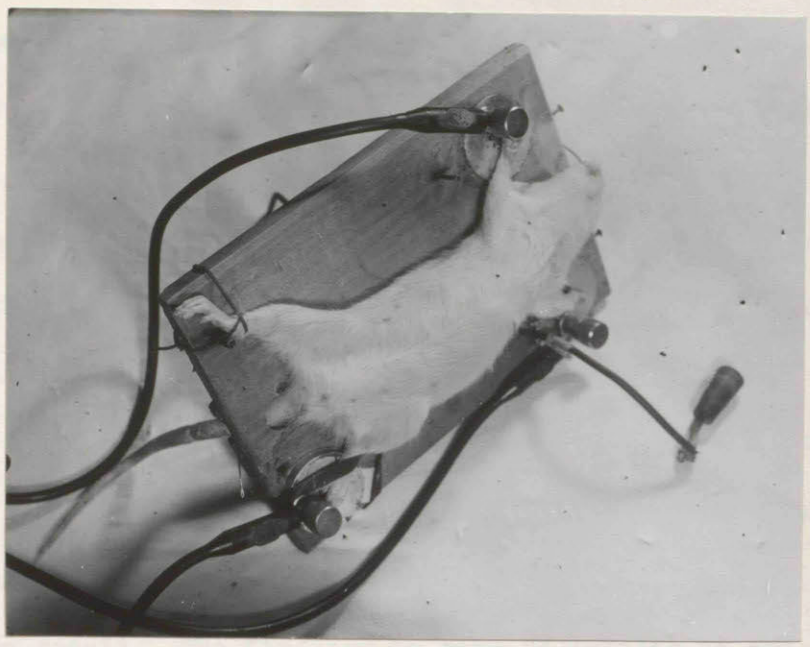

Figure 8.

The rat fixed onto the tilted board.

This device proved to be particularly useful in young lean animals but in larger plump animals the hearts natural position is probably more horizontal and in these animals the tilting sometimes seemed to produce a left axis deviation. However, because many of the animals to be used would be between 100 and 200 grams, this tilting was standard procedure in all animals.

Because of this tilting, the animals had to be fixed more firmly on the board to maintain the straight line of the back. Thus, a metal arc was placed at the top of the board under which the animals incissors were hooked. A hole was drilled in the lower part of the board through which the animals' tail was placed and pulled tight. The tail was then transfixed with a needle. A ridge was tacked onto the board between the arc and the tail hole (fig. 2). These maneuvers ensured a rigid position with the tilting of the board. Fig. 8 shows an animal fixed unto the tilted board.

4. Normal Ranges. Since the new cardiographic set up was obtained and since evipal was chosen as the anesthetic, there have been tracings taken on some 130 male and female albino rats which can be considered as normal. All of these will not be reported in detail. The findings in some 21 females of the Wistar strain are summarized in Table 1. Certain facts should be emphasized. The heart rate is usually between 400 and 460 beats per minute with 315 being the slowest rate found and 500 being the fastest. The rhythm was in all cases regular. The PR interval is remarkably constant. An interval longer than 0.05 seconds was not seen in these tracings so that it should be safe to consider 0.06 the upper limit of normal. Similarly, the duration of the QRS complex was not, in these tracings, over 0.02 seconds and only reached that value occasionally. Thus, intervals much above this time should probably be considered abnormal. Observing the amplitude table, one is struck with the range of some of the deflections but this is comparable with the wide variations in amplitude observed in a large number of humans. It should be noted that

TABLE 1
RANGE OF NORMAL
21 Female Albino Rats - Wistar.

	Average	Range.
Weight (grams)	132	105 - 185
Rate (per min)	420 (400-460)	315 - 500
PR interval (sec)	0.045	0.04 - 0.05
QRS interval (sec)	0.015	0.01 - 0.02

Amplitude in mm.
Standardization 1 millivolt = 1 cm.

	Deflection	Usual	Full Range
Lead 1	P	+ $\frac{1}{2}$	0 - 1
	R	+ 3 - 5	2 - 8
	S	$\frac{1}{2}$	0 - 1
	T	+ $\frac{1}{2}$	0 - 1
Lead ii	P	+ $1\frac{1}{2}$ - 2	$1\frac{1}{2}$ - $2\frac{1}{2}$
	R	+ 7 - 10	5 - 12
	S	0 - 3	0 - 3
	T	+ 2 - 3	$1\frac{1}{2}$ - 4
Lead iii	P	+ 1 - 2	1 - 2
	R	+ 3 - 6	2 - 7
	S	0 - 3	0 - 7
	T	+ 2 - 3	$1\frac{1}{2}$ - 4

sometimes a respiratory variation in the amplitude of a deflection is seen particularly in lead 3 and particularly in the S wave of lead 3. This is probably due to the change in position of the heart with inspiration and expiration. A Q wave is seen occasionally in one or more leads but it is always extremely small and extremely short in duration. If the S wave just reached the lower margin of the isoelectric line, it has been said to have an "amplitude" of 0. In one case this deflection did not quite reach this lower margin, perhaps missing it by $\frac{1}{2}$mm. The T wave was never negative in these normal animals. Left axis deviation was not seen.

The ST segments can best be discussed by reference to figs. 4 and 9. In lead 1, this segment is usually very short. The takeoff may be perfectly isoelectric or it may be depressed 1 mm as shown in fig. 9b. The ST and T wave in this lead is commonly in the shape of an upside down V. (fig. 4 and 9). This pattern in lead 1 is quite consistent. In lead 3, the line often comes up very sharply from the S deflection to the peak of the T (fig. 4 & 9) so that the segment is not defined and nothing can be said of the point of takeoff. Lead 3 maintains this pattern of the fine ST line passing rapidly up to the peak of the T fairly consistently. In lead 2, a similar pattern to that seen in lead 3 is usually seen (fig. 4) or else a slight variation on this pattern, such as a slight rounding of the peak of the T, may be apparent (fig. 9b). Sometimes, however, a configuration similar to that seen in lead 1 is observed (fig. 9a). Occasionally there is slight variation in the ST configuration in the lead so that each is not identical to the other (fig. 9b).

5. Influence of Sex and Weight The animals used in establishing this normal range were females having an average weight of 132 grams. In order to find if these ranges would vary with sex and size, table 2 was compiled. The 10 animals whose tracings are summarized in this table are Wistar males, the average weight 238 grams. Upon examination it will be seen that there is a remarkable similarity between this table and table 1. The only significant difference, in these tracings, was that left

TABLE 2
RANGE OF NORMAL
10 - Male Albino Rats - Wistar

	Average	Range
Weight (grams)	238	200 - 270
Rate (per min)	440 (400 - 460)	375 - 500
PR interval (sec)	0.045	0.04 - 0.05
QRS duration (sec)	0.015	0.01 - 0.02

Amplitude in mm.
Standardization 1 millivolt = 1 cm.

	Deflection	Usual Range	Full Range
Lead 1	P	+ $\frac{1}{2}$	0 - $\frac{1}{2}$
	R	+ 4 - 6	3 - 9
	S	$\frac{1}{2}$ - 1	0 - 2
	T	+ $\frac{1}{2}$	0 - 1
Lead ii	P	+ $1\frac{1}{2}$ - 2	$1\frac{1}{2}$ - 2
	R	+ 7 - 10	6 - 11
	S	+ 0 - 4	0 - 6
	T	+ $1\frac{1}{2}$ - 2	$1\frac{1}{2}$ - 2
Lead iii	P	+ $1\frac{1}{2}$	1 - 2
	R	+ 2 - 6	2 - 10
	S	0 - 3	0 - 8
	T	+ $1\frac{1}{2}$ - 2	$1\frac{1}{2}$ - $2\frac{1}{2}$

axis deviation was seen in 4 of these heavier males. This might have been expected, for not only were these animals plump and their hearts probably more horizontal, but also the tail of these animals could not be transfixed so that they tended to sag out to the left with the tilting of the board. At any rate it was felt that the only significant difference, possibly due to sex and more probably to weight, was a left axis deviation.

6. Influence of Animal Strain. These ranges have been compiled in albino rats of the Wistar strain. The ranges encountered in the tracings of 6 male albino rats of the Sherman strain were compared in order to find if there was any essential variations in the tracings due to a strain difference. The average weight in this small Sherman group was 127 grams. On analysis, it was found that the ranges in the tracings of these young Shermen males fitted well into the ranges shown in Table 1 except that, in 2 animals, the R deflections in lead 2, and in 1, the R deflections in lead 3 were slightly above the upper limit as set forth in table 1. No left axis deviation was seen in these animals. It was felt that these minor differences in the size of the R wave might just reflect an increase in the animal population but, in any event, there was no major variation which was likely to cause confusion if this strain should be used in later experimental work.

7. Subsequent Variation. The question now arises as to possibility of variation occurring in subsequent tracings on the same animal. Constancy of pattern in the same animal is particularly important for, if a major variation should appear, it must be attributable to the experimental procedure and not to variation incidental to repeating the tracing. To check the constancy of pattern, twenty of the female Wistars from the group in table 1, on the day following the original tracing, had a repeat tracing taken. The two tracings taken on each animal were compared. Table 3 is set up to show the number of animals showing the different degrees of variation in amplitude in the different deflections. Time intervals and rate ranges were identical.

TABLE 3

Variation - Subsequent Day.

20 Female Albino Rats - Wistar - from group forming table 1. Repeat tracing compared to original.

		Numbers showing different variations in amplitude in mm. compared to original tracings.				
	Deflection	0mm	$\frac{1}{4}$-$\frac{1}{2}$	1mm	$1\frac{1}{2}$	2mm
Lead 1	P	15	5			
	R	10	7	2	1	
	S	14	6			
	T	17	3			
Lead 2	P	17	3			
	R	8		8	2	2
	S	10	7	2	1	
	T	11	5	4		
Lead 3	P	16	4			
	R	12	1	4	1	2
	S	17	2	1	1	
	T	11	5	4		

It will be seen that there is some difference in amplitudes in subsequent tracings but it will also be seen that these differences are small. Thus, in the amplitude of R1 there was a variation of 1mm or more in only 3 animals. In R2, a higher deflection, there is a variation of 1½mm or more in only 4 animals. None of the T waves showed a variation in amplitude of more than 1mm. The T wave never became inverted, or slurred below the line. The configuration of the ST segment and T Wave is usually fairly similar from one day to the next also. It should be pointed out that, in each animal, the two tracings taken showed some variation in the amplitude of some elements, so that no two tracings were identical in each detail. But, on the whole, there is a remarkable constancy in the tracings taken on the subsequent day and no major variation was seen (fig. 9a and b).

Since some of the projected experimental work might well occupy a months time, it was important to show that young animals over this period of time would not show a spontaneous alteration in the ECG pattern. Accordingly, tracings were taken on 8 of the 21 animals whose tracings are summarized in Table 1, one month following the original tracing. The average weight of these females to start with was 118 grams and the full range 105 - 120 grams. After a month had passed, the average was 148 grams with a range of 130 - 165 grams. From Table 4, it is seen that the rates PR and QRS intervals are similar to those seen in Table 1. However, some variation was found in the amplitude of the different waves. In Table 4, the number of animals showing the different variations in mm. in comparison to the original tracings is seen. The changes are of the same order as were noticed after 1 day (Table 3) a greater percentage, however, of this smaller group showing variation. The T waves never became inverted. Once again then, there is a remarkable constancy in the general pattern. No major variation likely to cause confusion in interpretation occurred. (fig. 9 a,b, and c).

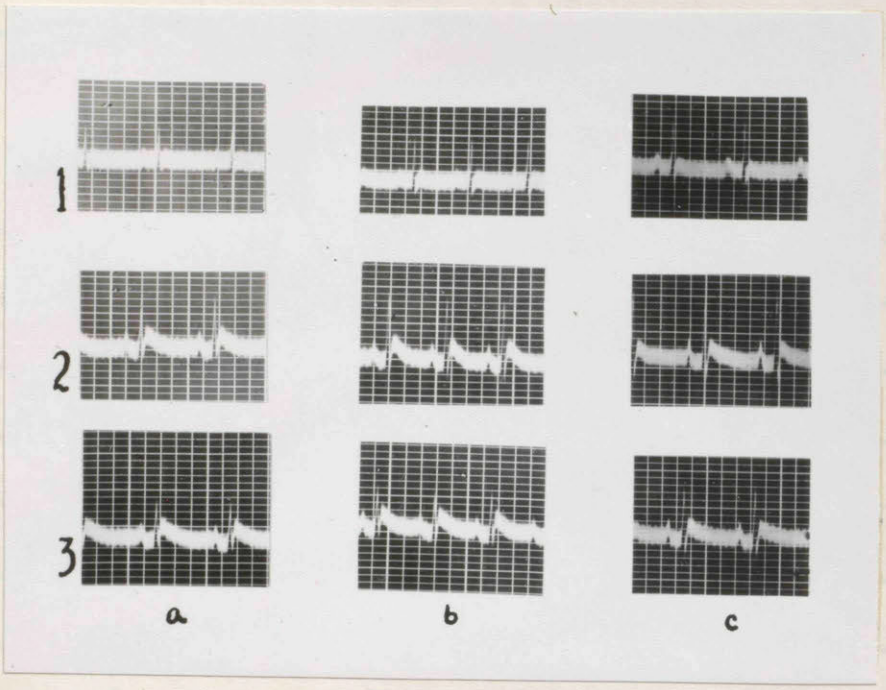

Figure 9.

Repeat tracings same animal under evipal. (a) original tracing (b) Following day (c) 1 month later. Note general similarity between tracings and the slight variations in amplitudes in repeat tracings.

TABLE 4

Variation - 1 month

8 female albino rats - wistar - from group forming table 1. Tracings repeated in 1 month and compared to original.

	Average	Range
Average Weight	148	130 - 165
Rate	380 per min	315 - 461
PR	.045	.04 - .05
QRS	.015	.01 - .015

Numbers showing variations in amplitude in mm. compared to original tracings.

Deflection		0mm	¼-½mm	1mm	1½mm	2mm
Lead 1	P	3	5			
	R	1	3	2		2
	S	4	4			
	T	5	3			
Lead 2	P	3	4	1		
	R	2		3	1	2
	S	3	3	1	1	
	T	3	3	2		
Lead 3	P	5	3			
	R	5		1		2
	S	3	1		2	2
	T	4	2	2		

8. Chest Leads. Some chest leads were also taken on these animals. The technique was as follows. A small manual exploring electrode about 4mm in diameter was used. The lead wire from this small electrode was soldered onto the left arm plate. (fig. 2). The left fore limb was removed from this plate and the left arm lead wire from the amplifier was switched with the left leg lead wire. The fur on the left anterior chest was clipped and the exploring electrode applied with electrode jelly to the ziphi-sternal junction and then to a point on a horizontal plane with this but about 1 cm. out from it.

The greatest difficulty was that, in small animals expecially, the skin is so mobile that it was very difficult to maintain a position. This is very important for the chest and heart are so small that minor variations in the position of the electrode, or for that matter, in the position of the heart, can change the pattern of a complex so markedly that comparison of tracings from animal to animal, or in the same animal in subsequent tracings, is often impossible. (fig. 10b and c). This made it very difficult to establish the normal ranges. However, the chest leads were of interest chiefly in regard to reinforcing the information obtained from lead 1 in the diagnosis of acute heart injury. It was felt that coincident changes in lead 1 and 4 might be helpful in the ECG recognition of damage, particularly if lead 1 was of low amplitude. Thus, what we needed to establish most was whether sharp inversion of the T wave occurred normally in the chest lead. This was not seen. However, some distortion of the ST & T wave below the isoelectric line was seen (fig. 10c). In addition, sometimes in the rush of doing a large number of animals, the left arm and left leg lead wires were not switched and in this case the tracing obtained showed an inverted P, a downward initial deflection and a peaked inverted T wave. (fig. 10d) This error in technique is usually easily spotted. Often the tracings taken from the ziphi-sternal junction are similar to right-sided tracings in the human and those taken more laterally resembled left-sided ones. (fig. 10).

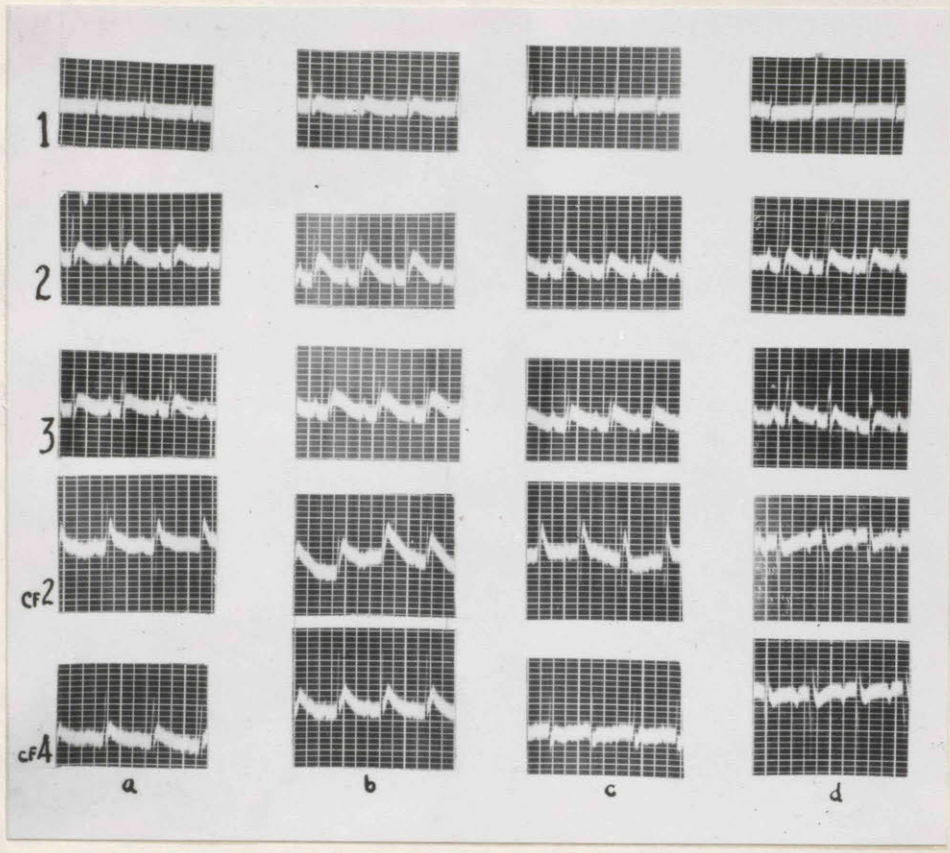

Figure 10.

Chest leads. (a) Note CF2 the right-sided configuration and CF4 the left-sided one. (b) & (c) Tracings taken 1 day apart on same animal with electrode supposedly in same position. Note variation in pattern of CF2 and CF4 from one day to the next. (c) Note St & T in CF4. ST depressed T segment below isoelectric or T small inverted. (d) Tracing taken when lead wires were not switched. Note downward P, R & T.

C. Chemical Methods. It remains now to describe the chemical methods used in this work. The methods, modified by John R. Polley (151) who was previously working in the laboratory, were used exclusively for sodium and potassium determinations.

A blood sample of 1 cc or more was obtained by heart puncture by Dr. S.M. Friedman, the sample being withdrawn into a heparinized needle and syringe. The sample was immediately centrifuged at high speed, following which a 0.4 cc sample of the plasma was pipetted off and added to 3.5 cc of water. Then, 1 cc of 15% trichloro acetic acid was added to precipitate the protein. This was again centrifuged. The supranatant was poured off into a clean dry centrifuge tube and this could be stored. Standards were handled in the same way except that centrifuging was not necessary.

Sodium was determined by precipitation with uranyl zinc acetate in an alcoholic media. To 1 cc of the protein free filtrate, 10 cc of a mixture containing equal volumes of uranyl zinc acetate and 95% ethyl alcohol was added and allowed to stand one hour in the refrigerator (152). After centrifuging and draining, the precipitate was washed on two different occasions with an acetone wash reagent. Finally, the precipitate, being soluble in water, was transferred to a flask by washing out the tube with 30 cc of boiled distilled water. Phenophthalein indicator was added and the acetate group titrated with 0.02N NaOH using a microburette graduated to 0.01 cc. An end-point warning was given by a gradual deepening of the yellow color and the actual end point taken at the first appearance of pink. The amount of NaOH titrated in cc was placed on a calibration curve and the amount of NaCl in mgm% read off the abscissa. The calibration curve was established for each analysis by analysing known sodium chloride solutions ranging in concentration from 450 - 1000 mgm% and plotting this concentration on the abscissa against the amount of NaOH titrated in cc on the ordinate. In order to obtain the sodium concentration in m e/l divide the sodium chloride

value in mgm% by 5.85.

This method is said to be accurate within ± 2% if done in triplicate. When doing a large number of determinations, it was often extremely impractical to do a determination on more than 1 sample. The main difficulty encountered in the use of this method was a precipitate of uranyl zinc acetate appearing in the mixture with 95% ethyl alcohol. This hazard threw the analysis off on two separate occasions.

Potassium was determined by first precipitating the chloride from 1cc of protein-free filtrate with 0.2 cc of 2% silver nitrate. This solution was centrifuged after standing and 1cc of the supranatant drawn off. To this, 0.5cc of cold absolute alcohol was added followed by 1.5cc of freshly prepared silver cobaltinitrite. This mixture was left in the refrigerator over night at a temperature of not less than 3 degrees centigrade. The precipitate formed should be fine and granular or clumped. If needle-like crystals were visible, the sample was discarded. These tend to be present if the refrigeration temperature falls too low. After centrifuging and draining, the precipitate was washed on two different occasions with an alcohol-ether-water wash reagent. Then 5cc of 0.2N NaOH was added to the precipitate and this mixture placed in a boiling water bath of 5 minutes. A blank tube with 5 cc of NaOH was started at this point. After boiling, the material was poured with washing up to 50 cc into a 200cc volumetric flask, and 1cc of 50% HCL added followed by 2cc of 0.5% sulphanilamide reagent and 1.5cc and 0.1% Marshals reagent. After this it was made up to 200cc with water, transferred to a colorimetric tube and read in a photoelectric colorimeter with a 525 mμ filter against the blank. A calibration curve was established for each analysis by analysing known solutions of potassium chloride containing 10 to 30 mgm% potassium and plotting this known concentration of potassium on the abscissa against the colorimetric reading obtained on the ordinate. The potassium content of the serum sample is obtained by placing the

colorimetric reading on the curve and reading off the potassium content in mgm% from the abscissa. To change this value to m e/l divide by 4.

Analysis by this method in triplicate is said to be accurate within ± 3%. Once again, this number of analysis per animal was often impractical. Frequently, duplicate analyses were done, however. One group of analyses was thrown off by not preparing the sliver cobaltinitrite correctly. Two other groups of analyses were thrown off by the overnight temperature in the refrigerator falling too low with the formation of needle-like crystals. As a result, all tubes had to be transferred to a refrigerator several blocks away where the temperature was in a more satisfactory range.

PART I.

To establish the means of elevating the serum sodium and potassium concentrations within the range of ordinary therapeutic possibility and the effect of such elevations on the normal myocardium as measured by the ECG.

Experiment 1. - The acute administration of sodium and potassium.

In the administration of potassium, a dosage must be found which would consistently give definite evidence of an effect on the heart electrocardiographically without causing any of the severe toxic disorders of conduction.

Sodium salts were used in this experiment for two reasons. First, they would be used in an attempt to control possible effects of change in acid-base and water balance coincident with the administration of potassium. Potassium passes into the intracellular fluid and is excreted rapidly, in contrast to the extracellular distribution of sodium and its slower excretion, so that this control is at best a transient one. Second, sodium salts would be administered in large amounts but under the edema level in an effort to produce a specific effect upon the heart.

The intraperitonial route of administration of sodium and potassium was used in the experiment. Zwemer and Truskowski (30) have shown that this

route gives a smooth absorption curve. It is said to give blood levels similar to those obtained with a slow intravenous injection (143). In the rat, this is a convenient mode of administration.

To be certain that any change in the electrocardiogram was not due to the anion content of the administered salt, a chloride and sulphate of each ion was used. All solutions were made up to be isomotic with 900 mgm% sodium chloride except the hypertonic solution of sodium chloride which was to be used in the attempt to show a specific effect. The following concentrations were used:

Salt	Concentration	Calculation
KCL	11.56 gm/L	$9 \times \frac{223.5}{174}$
K2SO4	18 gm/L	$9 \times \frac{374}{174}$
NaCl	9 gm/L	
Na2SO4	14.7 gm/L	$9 \times \frac{284}{174}$
NaCl	36 gm/L	4 x 9

Using these salts in these strengths, an effect on the ECG due to potassium alone could be detected, the anion content being varied and the water and acid base changes being temporarily controlled by sodium. A specific effect due to one or the other anion could be detected. It was hoped that a change due to sodium alone might be detected, for if the change was independant of the anion, and if a change appeared rapidly so that acid base and water change might still be controlled with the potassium, and if this change was accentuated by the hypertonic sodium solution, sodium would be strongly implicated.

Using female albino rats of the Wistar strain, the experiment was set up as follows.

	Animal	Average Wt.	Weight Range	Salt Administered	Dosage
Group 1	6	152 gm	130-180	KCl	3 cc
Group 2	6	133 gm	122-145	K_2SO_4	3 cc
Group 3	6	150 gm.	125-185	NaCl	5 cc
Group 4	6	139 gm	130-150	Na_2SO_4	5 cc
Group 5	6	149 gm	125-182	NaCl (hypert)	5 cc

It should be pointed out immediately that just 12 animals housed 4 to a cage were used in this experiment. After a rest of two days each group of 6 was re-used to form another group, but it was arranged so that the effect of each isotonic cation was tested on 12 different animals. All control tracings on these re-grouped animals were normal, however. Each group received tap water and Purina Fox Chow ad libitum.

The amount of these solutions which was administered was based upon some preliminary work. In 5 female Wistars, averaging 149 grams, after injecting various amounts of isotonic potassium chloride, the quantity giving a distinct effect on the T wave in all leads was found to be 3 cc. This, then, was the quantity of potassium chloride solution to be tested for its uniformity of effect and for the extent of the electrocardiographic change. Potassium sulphate could not be given in an equal volume which would contain, at the same time, an equal quantity of potassium. It was decided to give the potassium sulphate in an equal volume even though a little less potassium would be administered in order that volume change could be controlled.

In further preliminary work on Wistar females, weighing an average of 131 grams, it was apparent that small amounts of sodium chloride had little effect on the ECG. Therefore, the object was to inject as much of the isotonic solution as possible to try and show some specific effect of sodium. Since the rat has a a blood volume of about 6.7 cc per 100 grams (143) these animals would have a

blood volume of about 9 to 10 cc. It was felt that the maximum amount which could be injected intraperitonially would be about 5cc and this was the quantity chosen for both hypertonic and isotonic solutions. 5cc of the hypertonic solution would deliver about 180 mgm of sodium chloride to these animals intraperitonially which is a large dose (143). The only trouble with using 5cc of sodium solutions, while using 3cc of the potassium solution, is the difference in volume and the difference in the amount of cation delivered. However, if any of the changes in the potassium ECG are due to acid-base or volume change, similar changes of a greater degree would be present in the sodium tracings. Thus, it was decided to use a 5cc volume of the sodium salts.

A control tracing was taken on all animals immediately prior to the injection of these salts. In groups 1 and 2, the ECG was repeated after 5, 10, and 20 minutes had elapsed. Preliminary work had indicated these times. In group 3,4 and 5 the ECG was taken 5,10 and 60 minutes after injection so that early and late changes would be detected. To take tracings at these times, small additional doses of evipal were frequently necessary.

About 2 weeks after these ECGs were completed, when the animals had gained an average of about 30 grams, 5cc of isotonic sodium chloride was injected into 10 animals. After 7 mins. blood was taken by cardiac puncture from 5 of these and after 1 hour from 5 more. Of these 10 animals all were from these experimental groups except 3 animals of the same strain, sex and weight which had to be used to augment the total number of animals. 3cc of potassium chloride was injected into 5 animals from these groups and blood taken after 7 minutes by cardiac puncture. Four additional outside animals of the same strain and sex formed the control group for the electrolyte levels.

Results: The results of the single sodium and potassium analysis are presented in Table 5. This table is consulted at this time just to indicate that

TABLE 5.

Following Intraperitoneal Injection

POTASSIUM DETERMINATION

Substance Injected & Concentration	No. of Animals	Time after Inject. in min.	Potassium Concentration (m e/l) Average	Range
Control	4	7	4.3	3.75-4.9
NaCl 9 gm/L	5	7	4	3.5-4.25
NaCl gm/L	5	60	4.3	3.9-4.75
KCl 11.56 gm/L	5	7	7.75	6.6-9.1

SODIUM DETERMINATIONS.

Substance Injected & Concentration	No. of Animals	Time after Inject. in min.	Sodium Concentration (m e/l) Average	Range
Control	4	7	139.5	135.9-143.3
KCl 11.56 gm/L	4*	7	139.8	135.9-144.5
NaCl 9 gm/L	5	7	144.4	140-151.2

* One very high determination was discarded from the group on the basis of experience.

the potassium and sodium concentrations are elevated by this injection procedure. It will be noticed that the sodium results after 1 hour are not presented. This is because 2 of the 5 determinations were ruined by an interfering reagent precipitate. The number of remaining determinations in this 60 min. group was not considered sufficient to be reliable. In addition, 1 sodium value was discarded in the potassium chloride injected animals because it was so extremely high that it is probably that the precipitate interfered here also. But the table does indicate that injection of 3cc of isotonic potassium chloride elevates the potassium in 7 mins. and it seems to indicate the injection of 5cc of isotonic sodium chloride increases the sodium concentration after 7 minutes.

Now, knowing that the electrolyte levels are altered by this procedure, let us go on to consider the cardiographic changes that result in each of the 5 groups.

Because the cardiographic results in group 1 and 2, the potassium groups, are so similar the results in these 2 groups will be presented together. All animals showed similar changes but the degree varied slightly. Figure 11 illustrates the typical changes after 5,10 and 20 minutes. Although a slight prolongation of the PR and QRS intervals was occasionally seen, these conduction intervals still were within normal limits. The distinctive change in lead 1 is an increase in the amplitude of the T wave. The extent of this increase is between $\frac{1}{2}$ to 1 mm. Since the T wave is often so small in the lead, this increased prominence also meant an increase in the duration of the QT interval but this interval is still no longer than that encountered in other leads of the control tracing. In lead 2, a similar increase in the amplitude of the T wave is often seen but, in addition, the configuration of the ST and T is distinctly coved with the takeoff of the ST elevated. This coving and elevation is seen in lead 3 also but not so regularly. The changes in the T waves were maximal in the 5

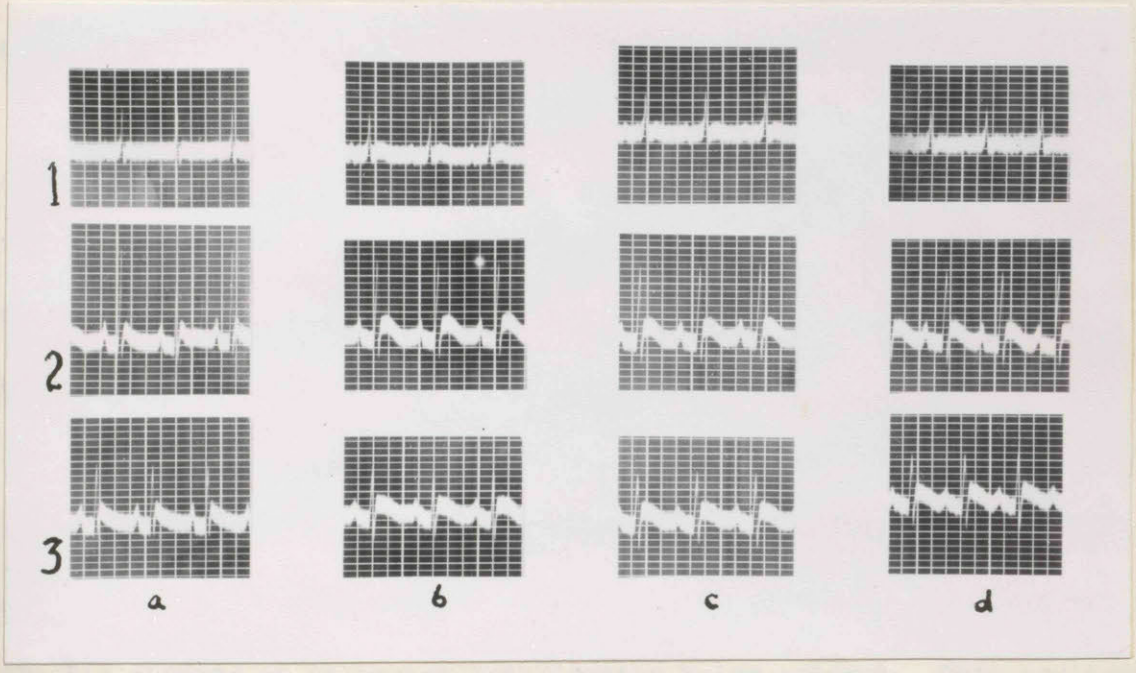

Figure 11.

3 cc of isotonic KCl intraperitoneally. (a) control tracing. PR = 0.04 sec. QRS = 0.015 sec. (b) 5 min. after injection. Note increase height of T waves, coving of ST 2 & 3. (c) 10 min. after. Note PR = 0.05 sec. QRS = 0.02 sec. (d) 20 min. after. Note T changes regressing PR = 0.06 sec.

and 10 minute tracings and were waning by the 20 minute tracing.

The animals in group 3 and 4, the isotonic sodium groups, showed very similar tracings so these will be discussed together. In 2 tracings in group 3 and in 3 tracings in group 4, there was an increase in the height of the T wave in lead 2 of about ½ - 1 mm. This change was apparent at 5 and 10 minutes. At one hour sometimes these changes had regressed and occasionally were exaggerated. Such changes in amplitude of the T in lead 2 were reflected to a slight degree in leads 1 & 3. There was no distinctive alteration in the tracings and no constant change apparent in the tracings of all animals.

The tracings taken on the animals in group 5, the hypertonic sodium group showed similar changes in the height of the T waves. The only difference between this group and the preceding 2 groups is that this change was apparent in the tracings of 5 animals out of 6. Fig. 12 demonstrates a series of tracings showing the increase in the T waves.

Discussion & Summary: Here, then, is a quantity of potassium which in animals of this weight definitely raises the serum level to an extent where a specific distortion of the ST & T waves is present without any of the more drastic changes of potassium intoxication being evident. This particular effect, magnifying T1, and coving and elevating the ST and T of lead 2 and sometimes lead 3, was seen only when the potassium ion was present and was independant of the anion content. Since changes in amplitude of T2 were seen sometimes with the injection of 5cc of isotonic sodium, some of the heightening in lead 2 might be due to volume change or to a change in the base. However, it has been seen that heightening of the T wave is very frequently seen with increasing potassium concentration (84). Neither the peaking of the T wave nor the narrowing of the base of the T wave, previously reported are seen here. The coving and elevation seen here may be a species difference or else it may be that the potassium

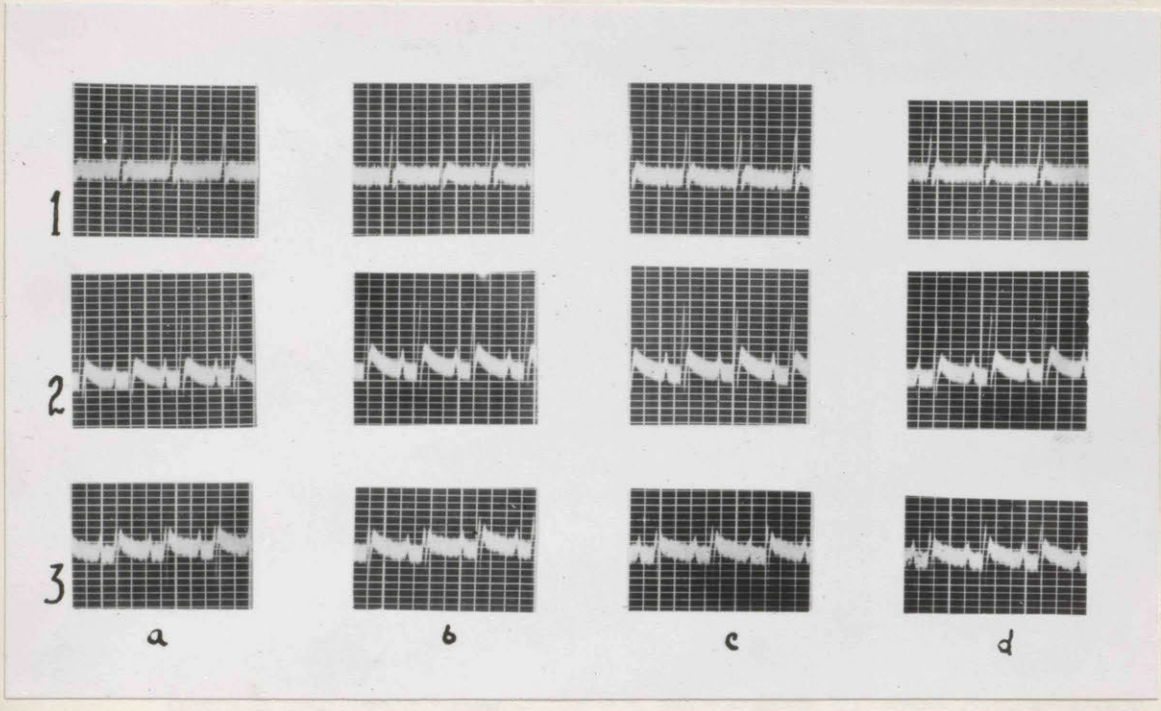

Figure 12.

5 cc of hypertonic NaCl intraperitoneally (a) control tracing (b) 5 min. after injection. Note slight increase in height of T1, increase of $\frac{1}{2}$ - 1 mm in amplitude of T2. (c) (d) 10 and 60 min. after injection. Similar to (b). Note no change in conduction intervals. Compare to figure 11.

concentration has not been increased sufficiently to cause a peaking or narrowing of the T wave. It is interesting that Chamberlain (86) has noted coving in lead 2 occurring occasionally with potassium administration in cats.

This change in the height of the T wave following the administration of isotonic sodium salts is harder to evaluate. For one thing there was no control for the 5cc volume used. For another, the change was not seen constantly in the tracings on all animals. Again, although the hypertonic sodium produced this change in more animals, it did not increase the extent of the change. And finally, this change was often apparent and sometime exaggerated in 1 hour. This might be in keeping with the slower excretion of sodium but it might also be in keeping with a more prolonged change in water balance or in acid base balance with sodium administration. It is thus impossible definitely to attribute this heightening of the T wave in lead 2 to any one factor. It seems probable, however, that there is no specific toxic action of sodium on the heart because an increase in this toxic effect with the large increase in sodium dosage with the hypertonic solution would have been expected. In any event, in this dosage, within the range of therapeutic possibility, sodium does not markedly or constantly alter the ECG.

One of the weaknesses of this experiment is that an external control group having tracings repeated at 5,10,20 and 60 minutes was not done. Previously it was noted that on subsequent days slight changes in the T wave may be present (table 3), but similar work was not done repeating the tracing at close intervals on the same day using small additional doses of evipal. Although it seems improbable, it is possible that this increased height of the T waves might be linked up with the prolongation of the anesthesia.

Experiment II: The prolonged administration of sodium and potassium.

This experiment was designed to determine whether the serum concentration of these ions could be raised with salts administered slowly, over a period of time. If this could be done, the question was whether there was any specific effect of this prolonged alteration of these electrolyte levels on the myocardium

as measured by the ECG.

The salts were administered in the drinking water. Albino rats of the Wistar strain will drink and do well on a solution containing about 1.72% sodium chloride (153). For this work, it was decided to give as many sodium ions in the salts as are present in a solution twice the isotonic concentration (18 grams/L). The salts chosen contained metabolizable anions to minimize any effect of anion administration. Thus, sodium acetate (146) was given in a concentration of 25 grams/L and sodium citrate (146) was given in a concentration of 30 grams/L. Because sodium is essentially an extracellular ion and potassium an intracellular one, the non specific effects of sodium administration can not be controlled over a period of time by the administration of potassium and vice versa. However, the number of potassium ions administered was equated to the number of sodium ions administered, in the hope that the potassium salts would then be accepted as readily as the sodium. Thus, potassium acetate was made up to 30 grams/L and potassium citrate to 31 grams/L.

The choice of the period of time over which these ions should be administered was difficult. From some previous work, (154) it had been shown that with the administration of 1% saline the fluid intake rose until between the 10th and 15th day and then commences to fall. With stronger solutions of saline the intake rises and is maintained at a high level for some time (153) But using a metabolizable salt of this concentration, it was not certain what would happen to the intake. In view of the fact that some of the projected experiments would be long term and others short term, the choice was doubly difficult. But since it was thought that much of the work would be of 2 weeks duration or less and because of the possibility that the intake of these salts might fall as the intake of isotonic saline falls, a period of 12-13 days was decided on.

In this experiment an internal control ECG was not taken. Previously it has been shown that after a period of time some variation in the T wave

might occur (table 4). Thus, slight changes in the amplitude of the T wave could not be considered significant. Tracings from an external control group would serve as a satisfactory control for more serious changes.

The experiment was then set up using female albino rats of the Wistar strain. The animals were housed 6 to a cage and received Purina Fox Chow in addition to the solutions listed below.

	Animals	Average Initial Weight	Average Final Weight	Salt	Conc.
Group 1	6	92	119	Tap water	
Group 2	6	95	119	Sod. Acet.	25 gms/L
Group 3	6	88	102	Sod. Cit.	30 gms/L
Group 4	6	88	109	Pot. Acet.	30 gms/L
Group 5	6	94	113	Pot. Cit.	31 gms/L

The intake of fluid was charted daily in each group. After 12 days, one half of each group had an ECG. After 13 days, the other half of each group was done. On the 15th day, also, blood was taken for serum sodium and potassium analysis.

Results: It will be noticed, in the above table, that all groups gained weight over the period of the experiment but the citrate groups gained slightly less than others groups with a comparable initial weight.

The intake of fluid is tabulated in Table 6. It will be noticed that the animals drank fluid containing the acetate and citrate salts in marked excess of tap water. Notice, also, that the sodium salts increased the fluid consumption to a greater extent than potassium salts did, and that the intake of sodium citrate was greater than that of sodium acetate. This is interesting in view of the fact that the sodium citrate group gained slightly less weight.

TABLE 6

FLUID INTAKE

Administered Salt.	No. of Animals	Average Weight (grams)	Total Fluid Consumption per cage (cc)	Average Daily Consumption per cage (cc)	Average Daily Consumption per animal (cc)
Tap Water	6	92	1350	104	17
Sodium AC.	6	95	2350	180	30
Sodium Cit.	6	88	2690	207	39
Potassium Ac.	6	88	2015	155	26
Potassium Cit.	6	94	1935	149	25

TABLE 7
Following Oral Administration

SODIUM DETERMINATION

Administered Salt.	No. of Animals	No. of days Salt adm.	Average m e/l	Range m e/l
Tap Water	6	13 days	145	143.6-149
Sodium Acetate	6	13 days	143	135-150
Sodium Citrate	5	13 days	124.8	119-131
Potassium Ac & Potassium Cit. * groups combined	6	13 days	128	115-135

* Necessary because several determinations in each group were discarded with reagent precipitate.

POTASSIUM DETERMINATION.

Administered Salt.	No. of Animals	No. of days Salt. adm.	Average m e/l	Range m e/l
Tap Water	6	13	4.6	4 - 5.4
Sodium Acetate	6	13	4.65	4 - 5.5
Sodium Citrate	5	13	5.15	4.9 - 5.4
Potassium Acetate	6	13	6.19	5.5 - 6.7
Potassium Citrate	6	13	6.25	4.9 - 7

The first attempt at sodium and potassium analysis was unsuccessful because, in the case of sodium, an interfering precipitate in the reagent was formed and, in the case of potassium, many needle like crystals were formed following refrigeration at too low a temperature. Both analyses were repeated on single samples. The results are listed in Table 7. It will be seen that, in spite of this large sodium intake, the serum concentration is not elevated at the time of sampling. This may be because compensation is effective with an increased renal excretion, retention of water and a shift of intracellular fluid to the extracellular phase. It is interesting to note that sodium citrate, in spite of the highest fluid consumption and the smallest weight gain, actually appears to lower the sodium concentration, as do the potassium salts.

From Table 7 also, it is apparent that the potassium salts by mouth, over this period of time and in this dosage, did raise the serum concentration of potassium somewhat in most animals.

Careful examination of the ECG failed to reveal any distinctive alteration. All the experimental tracings were either similar to the control tracings or else were well within the range of normal which was earlier established .

Summary. This experiment has shown that the serum potassium concentration can be raised over a period of 13 days by oral administration of a metabolizable salt but that this increase, acting for an undefined part of the 13-day period, caused no alteration in the electrocardiographic pattern. It also has shown that the serum sodium concentration on the 13th day was not increased by this procedure and that the high sodium intake for this length of time had no effect on the ECG.

PART II. - The effect of variations in electrolytes on the myocardium of adrenalectomized animals.

The experiments in this section were designed first to determine whether as the serum and cellular potassium concentration rose and the serum and cellular sodium fell with adrenalectomy, a typical electrocardiographic pattern developed. If it did, it seems desirable to find what influence the acute administration of sodium salts or potassium salts, as in Experiment I, would have on this pattern. If, for instance, sodium improved the pattern, it might indicate that the adnormalities were due to the low serum or cellular sodium. Thus, by this procedure, it might be possible to attribute the changes in the ECG to changes in concentration of one ion or the other. To start with, then, we had first to establish the electrolyte and cardiographic pattern following adrenalectomy.

The experiment was set up using 30 male albino rats of the Wistar strain, housed 5 to a cage, drinking tap water and eating Purina Fox Chow ad libitum.

	No. Animal	Average Weight	Procedure
Group 1	10	118 grams	control
Group 2	22	103 grams	adrenalectomy

Every 2 days for 6 days, 3 control animals and 6 adrenalectomized animals were to have an ECG and blood samples taken by heart puncture for sodium and potassium analysis. The animals were to be selected at random and discarded after the puncture.

This experiment was started with the animals being adrenalectomized. On the 2nd day, ECG and blood samples were taken as planned. However, when the cardiographic film was developed, it was found that the tracings were hopelessly distorted by alternating current interference. At this time, the laboratory was being rewired for fluorescent lighting. It was thought that this change was responsible for the sudden appearance of interference. Despite most strenous efforts, this interference could not be eliminated and nothing but grossly distorted tracings could be obtained. This experiemnt had therefore to be abandoned.

As a matter of fact, some 2 weeks were to elapse before this unexpected complication was overcome. By this time, attention had to be turned to other work, hoping at a later date to return to this experiment. However, this proved to be impossible.

PART III. - The effect of varying the potassium concentration on an enlarged heart.

Experiment 4. - Because DCA administration in high doses to young rats causes a hypertension and enlargement of the heart (26)(27), because focal myocardial necroses following the administration of DCA has been reported (41)(22), and because of the marked alterations in intra- and extra-cellular electrolytes which occur with this drug (20(21), it was decided to find if these changes were reflected in the ECG so that later, the effect of administered salts on this pattern could be judged. Accordingly, I joined in an experiemnt being done by Doctors S.M. and C.L. Friedman. This experiment had been set up by them as follows, using male albino rats of the Wistar strain.

	No. of Animal	Average Weight (grams)	Treatment
Group 1	10	78	Untreated Control
Group 2	10	68	DCA-Saline
Group 3	10	57	Pregnenalone
Group 4	10	76	DCA-Saline Pregnenalone

The animals were housed 4 to a cage and given Purina Fox Chow ad libitum. Group 1 & 3 drank tap water and group 2 & 4 1% saline.

On the day 1 of the experiment, two one-third portions of a 75 mgm. DCA pellet were implanted under the skin of the back in the animals in group 2 and 4. On the 14th day, an additional one-third portion of a 75 mgm pellet was implanted.

On day 2, pregnenalone (2 mgm) in oil was injected subcutaneously into the animals in group 3 and 4.

On the 15th day, blood pressure determinations were done by the

Doctors Friedman under an ether anesthetic. Immediately after the procedure, an ECG was taken while the animals were still under the ether anesthetic. The following day, renal function tests were done by the Doctors Friedmans and blood samples were taken for potassium determinations.

Results. Except for the cardiographic results, the findings of importance to this work are presented in Table 8. It should be noted that this B.P. procedure used by the Doctors Friedman is very well standardized. They consider that, in the animals of group 2 and 4, the blood pressure is definitely elevated. Notice that the heart weights are also definitely increased in these groups receiving DCA-saline.

The potassium analysis in this experiment were done in duplicate. From Table 8, it will be seen that the average concentration, in the 2 DCA saline groups, is depressed in comparison to the control though not markedly. The range of potassium concentration in these animals is also, for the most part, lower than the control range. The pregnenalone group shows a slightly lower average concentration than the control but the range falls almost into the control range.

The electrocardiograms were taken under ether because initially ether was the anesthetic used for taking the ECG, its disadvantages at that time not being fully realized. Internal control tracings were not taken because the cardiographic equipment was not available in time. However, it was felt that any gross distortion such as the pattern of ventricular strain or the flat T waves of low potassium would be apparent.

Of the 10 animals in group 1, the control group, there were 6 ECGs taken. In each of the other groups, there were 8 tracings taken. The fact that the animals were under either at the time of the tracing makes interpretation very difficult. For instance, the only major change present in group 4 was a prolongation of the PR interval (over .06 secs.) appearing in 3 tracings (fig. 13a) But it has been previously noted that an ether anesthetic may sometimes cause

TABLE 8

	Group 1 Untreated Controls	Group 2 DCA-Saline	Group 3 Pregnenalone	Group 4 DCA-Saline & Pregnenalone.
No. Animals-	10	10	10	10
Average Initial Weight (gm)	78	68	57	71
Average Final Weight (gms)	120	112	109	106
B.P. in mm. Hg. (Friedman)	86.8	117.5	93.5	113.2
Heart Weight mgm/100 cm2	173.6	198.6	179.6	205.8
Average Serum K conc. m e/l	6 determ. 5.7	5 determ. 4	7 determ. 4.83	7 determ. 3.9
Serum K conc. range m e/l	4.4 - 6.7	2.8 - 4.9	4.1 - 5.8	2.5 - 4.9

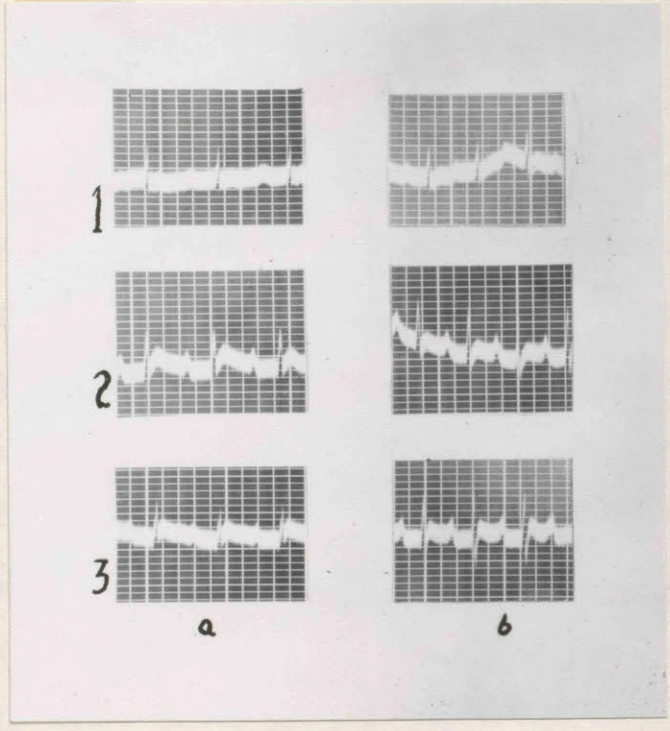

Figure 13.

(a) ether anesthetic; DCA - saline - pregnenalone. Note prolonged PR interval 0.07 - 0.08 sec. (b) PR = 0.06 sec. T 1 & 2 flattened.

dropped beats though this was not seen in the control group here. In addition, ether may cause a precipitant drop in serum potassium concentration (157). Whether this disturbance in A-V conduction is due to the low serum potassium (135) caused by ether or DCA, or whether it is a direct effect of the ether alone is not the question. The point is that DCA effect can not be definitely said to be the cause of this prolongation of the PR interval.

Low T waves were seen in 2 tracings from group 4. (fig. 13b) This was not seen in another group even though the B.P., heart weight, and electrolyte changes were affected about equally in group 2 & 4. In addition, an internal control was lacking as well as a range of normal variation with ether. Thus, no confidence can be placed in these low T waves either.

There was no other essential change seen in any of these tracings.

Summary. All that can be said of this experiment is that this dose of DCA and saline for this length of time, did not produce any regularly occurring alteration in the ECG pattern although an electrolyte change was produced. No confidence can be placed in the changes that did occur in some tracings, because of the ether anesthetic.

Experiment 5. Since in the previous experiment there were some changes of uncertain significance seen in a few tracings following DCA administration, it was thought that the effect of DCA should be re-assessed under evipal anesthesia where the range of normal had been well worked out. In addition, in experiment 4, it was apparent that the majority of the experimental animals had a normal tracing. It therefore seemed important to extend the period of time over which the DCA was active in the hope that a larger percentage of animals would show a change in the ECG. Although this time factor would not be so important if the ECG abnormalities were due to a functional disorder connected with the electrolyte change, it would be very important if the alterations were due to a structural

change connected with the hypertension or a prolonged electrolyte alteration.

Certain workers, including Selye (28), have shown that potassium administered with DCA will prevent the fall in serum potassium concentration but does not prevent the occurrence of hypertension and other lesions. Provided that electrocardiographic abnormalities were caused by prolonged DCA administration, it was wondered if the administration of potassium would prevent the appearance of the abnormal electrocardiographic pattern.

Just at this time, Doctors S.M. and C.L. Friedman had an experiment well underway which was suitable for determining the effect of prolonged administration of DCA in large doses on the ECG and the effect of potassium on this electrocardiographic pattern if one were to develop. This experiment was set up as follows with male albino rats of the Wistar strain.

	No. of Animals	Average Weight Initial (grams)	Procedure.
Group 1	10	65	untreated control - tap water.
Group 2	10	66	drinking 1% KCl
Group 3	12	68	DCA & tap water
Group 4	12	70	DCA & 1% KCl

The animals were housed 4 to a cage. They received Purina Fox Chow ad libitum in addition to the fluids detailed above.

On day 1 of this experiment, a one-third portion of a 75 mgm pellet of DCA was implanted under the skin of the back of the animals in Group 3 and 4. Over a period of 28 subsequent days, 4 additional one-third portions of a 75 mgm pellet of DCA were implanted one at a time at intervals varying from 4 to 14 days into each of the animals.

The potassium was administered as 1% KCl.

Internal control ECGs were not taken on these animals for the experiment was underway before it was realized that it would fit in with this work. However, it seemed likely that any gross abnormality developing in the ECG of the treated animal would be apparent in comparison to the external control and in comparison to the known range of normal.

At 35 days, the blood pressures were taken by Dr. Friedman and were found to be distinctly elevated in both DCA groups (Table 9). At this time also ECGs were taken on 4 animals in group 1 and on 4 in group 2, while tracings were taken on 3 animals in group 3 and on 3 in group 4. Because all these tracings were within normal limits, it was decided to carry on with the experiment for an additional 2 weeks. Thus, on the 37th and 42nd day, another one-third portion of a 75 mgm pellet of DCA was implanted into the animals of group 3 and 4.

On the 50th day, ECGs were taken on 10 animals in each group except in group 4 where 9 tracings were taken. Blood pressure determinations, using ether, had been done by the Doctors Friedman on the previous day. These workers, during the course of renal function studies, on the 50th day, obtained blood samples for potassium analyses. The animals were killed and heart weights obtained.

Results & Discussion. The results important to this work are presented in Table 9. The weight gains, as judged by the average, are almost equal, the DCA group gaining slightly less than the others. It will be seen that the blood pressures are distinctly elevated in both groups receiving DCA. In these groups also, the heart weight is markedly increased. The potassium determinations were done on 8 samples. The first analysis run in duplicate, showed a separation in values of such a degree that it was felt that the analysis was unreliable. Thus, a second determination was done on a single sample and these results are shown in Table 10. In most cases, values from this second single determination agreed closely with one or the other values obtained on the first duplicate analysis. These agreeing values were felt to be reliable. If those values from

TABLE 9.

	Group 1 Control	Group 2 KCl	Group 3 DCA	Group 4 DCA & KCl
No. of Animals	10	10	10	9
Average Initial Weight (grams)	65	66	68	70
Average Final Weight (gms)	237	233	209	224
B.P. mm.Hg. (SMF) 35 day	103.8	106.2	135	123.4
50 day	108.8	109.6	131.8	144
Heart mgm/100 cm2 weight	199	197	245	249
Average Serum K conc. (m e/l)	8 determ. 4.1	8 determ. 4	8 determ. 3.3	8 determ. 3.4
Range of Serum K. conc. (m e/l)	3.4 - 5	3.25 - 5.4	2.5 - 4.7	2.4 - 5

the single determination, which did not closely agree with one of the previous duplicate values, were discarded, there was no significant alteration in the average figure or in the range as presented in Table 10.

In some cases, DCA administration caused a lowering of potassium below the range of normal as shown here but not in all cases as the average and range shows. The oral administration of 1% potassium chloride in group 2 did not increase the serum potassium concentration as measured on the 50th day in contrast to the alteration caused by administering a more concentrated metabolizable salt seen in Experiment II. The administration of this amount of potassium in group 4 was not sufficient to prevent the slight fall in potassium concentration caused by DCA in this dosage over this length of time.

The ECGs in the control group and in the potassium chloride group were within normal limits, as were the blood pressures and heart weights. In each of the DCA-treated groups all tracings were normal with one exception in each group. These two abnormal tracings are very dissimilar.

The abnormal tracing in group 3 is shown in fig. 14b along with a tracing taken on the 35th day (fig. 14a). The latter tracing appears to be within normal limits. The blood pressure on the 35th day in this animal was 148 mm Hg. and on the 50th day 148 mm Hg. On the 50th day, also, the serum potassium concentration was 2.5 m e/l. Between the 35th and 50th day the tracing changed. In lead 1 and 2 and, to a lesser extend, in lead 3, although the rhythm is regular and the PR interval constant, the complex of the one beat is somewhat different from the next but similar to the 3rd, while the second beat is similar to the 4th. In lead 1, the difference is apparent in a deepening of the S wave, an increased height of the T wave, and a slight change in the shape of the ST and T configuration. In the second lead, the S wave is deeper on every alternate beat and the T slightly higher. In the 3rd lead, every other R wave is splintered.

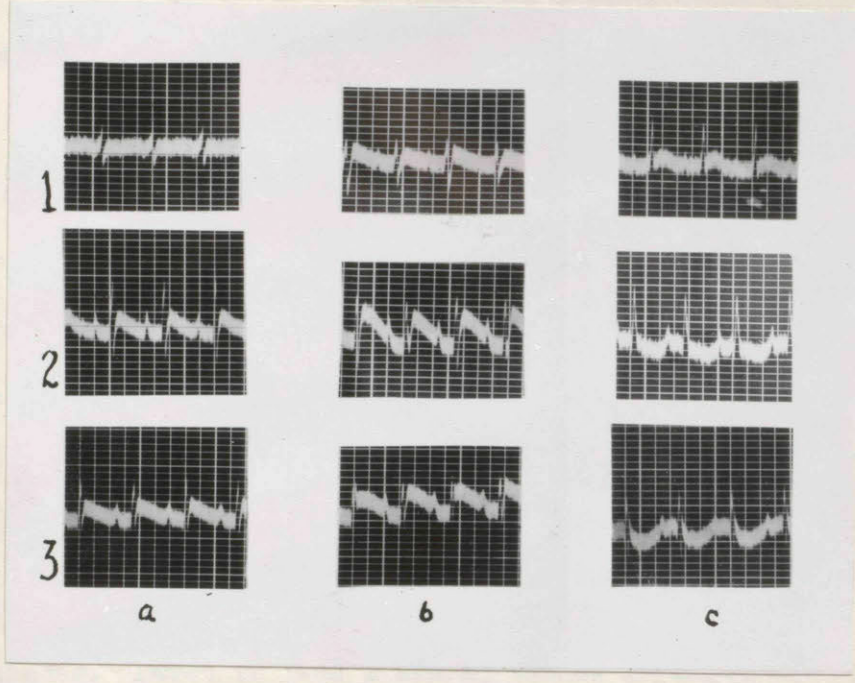

Figure 14.

(a) Tracing taken 35th day of DCA administration. Probably within normal limits. (b) Taken on 50th day. In lead 1 note increase depth of S wave every other beat and slight increase in height of T. Similarly lead 2. In lead 3 notice slight splintering of R wave every alternate beat. In all leads note increased height of T waves compared to previous tracing. (c) Another animal after 50 days. Note depression of ST2 & 3 and inversion of T2 & 3.

In comparison to the previous tracing, all T waves are markedly increased in amplitude. In conjunction with the other changes, this marked increase in amplitude may be of some importance.

The cause of this variation is pattern is not certain. In view of the increased amplitude of the T wave, it seems hardly likely that the changes are due to a functional alteration connected with the lowering of the serum potassium concentration. The animal was not obviously anoxic as the tracing was taken, so that this was probably not the cause of the increased amplitude of the T wave. It does seem possible, however, that the electrocardiographic changes are connected with a structural defect associated with chronic hypertrophy, for this pattern may represent a variation in the conduction pathway in the ventricles and ventricular conduction disorders are often seen in hypertensive heart disease (155).

The abnormal tracing in the DCA-KCl group is shown in fig. 14c. There is no previous tracing on this animal but such changes have not been seen under other circumstances. Here, in lead 2 and 3, the ST is depressed and the T wave small inverted. The potassium concentration in this case was 3 m e/l and it seems possible that this is responsible for the change, although other factors might as easily have been important. Other animals had potassium levels as low or lower than this and showed no similar change but there is a variation in the potassium concentration at which different subjects show ECG changes.

There is one factor that further complicates the interpretation of these tracings. It will be recalled that blood pressure determinations under ether were done in these animals on the day prior to the taking of the ECGs. It is conceivable that this ether anesthetic might have been responsible, at least in part, for some of these changes, although it is improbable in that similar changes were not seen in the control group.

Thus, this experiment shows that DCA in large doses over a period of 50 days does not always lower the serum potassium, and does not cause any regularly occurring ECG abnormalities, only 2 out of 19 treated animals showing any marked changes. The prolongation of the PR interval, seen under ether in Experiment 4 in the DCA-saline group, was not seen here with larger doses of DCA alone over a longer period of time using evipal. The diminished height of the T wave, in 2 tracings in Experiment 4, may still be due to a variation occurring with the ether anesthetic, though it seems a little more likely now that the finding was connected with the low potassium. It seemed obvious that, if an abnormal ECG pattern was to be produced fairly regularly by DCA due to the effect of either the sustained electrolyte changes or hypertension on the heart, the drug would have to be active longer than 50 days or else reinforced with saline over a long period. Also it was suggested that, if changes in serum potassium with doses of DCA of this order were to be prevented, larger amounts of potassium would have to be administered in the drinking water.

It was planned now to procede to renal hypertension and to DCA produced hypertension which had been operative for a longer period of time. If an abnormal pattern could be produced in a large enough number of animals to be significant, the effect of the administration of various salts administered according to experiment 2 or to experiment 1 would be tested. Unfortunately, this plan could not be carried out.

PART IV:- The effect of varying the electrolyte concentration on the acutely damaged heart.

On page 29 the theoretical possibilities concerning the potassium concentration in the zone surrounding the necrotic area have been discussed. Briefly, knowing that in the alarm phase of the general adaptation syndrome there is an increase in serum potassium, and knowing that disintregating muscle cells lose potassium, it was wondered how important those alterations were to the

function of the acutely injured myocardium particularly the myocardium surrounding the injury. Two possibilities were suggested. First, the fibres in the zone around the injury are further handicapped by the loss of potassium and potassium administration in non-toxic doses might be beneficial. On the other hand, the second proposition suggests that there is an accumulation of potassium in large amounts in the exudate surrounding the injury, which further impedes the function of the surrounding fibres, and an agent lowering the potassium might be beneficial.

To start with, then, how was the myocardium to be injured? Without an elaborate set-up for maintaining respirations, it seemed obvious that an operative procedure involving thoracotomy would have to be done very quickly. Under the circumstances, tieing off the anterior descending branch of the left coronary in these small animals seemed almost impossible. The idea was to cauterize this artery as close to its origin as possible. In a number of animals the following operative procedure was worked out.

The animal was anesthetized with evipal 12 mgm per 100 grams. Ether was not used because of its possible distortion of the ECG and because of danger near the open flame. With the evipal, caution had to be used in administering the anesthetic for, if respiratory depression developed, the increased respiratory effort compensating for the thoracotomy did not appear, and this lack of respiratory response seemed to be fatal in some of the preliminary work.

The animal was placed on its back and turned a little to the right. The left fore-limb was taken up over the left shoulder and clipped to the towelling there. The fur was cut over the left anterior chest. All instruments were boiled. An incision was made about 1 cm to the left of the mid-sternal line from the lower margin of the thorax to just below the left shoulder. The muscle was separated until the ribs and intercostal muscles were well exposed. The tip of a pair of pointed scissors was inserted between the ribs on a level with the

lower inside margin of the cardiac notch. Then the thoracic wall was cut upward on a line with the left extreme of the notch. The chest was then spread by means of artery forceps placed on each cut margin. It is important not to spread these margins too widely, for the fine pleural and pericardial membranes are attached at the mid sternal line and, if these membranes are stretched too much, the pleura from the right side may bulge dangerously to the open side with respiration. In a preliminary operation, these stretched membranes ruptured.

Following this incision, the heart is easily visualized in most animals In some large animals with a deep thorax, the heart may be very deeply placed in the chest and be relatively inaccessible or else it may move up toward the incision and move back deep into the thorax with respiration. This latter type, particularly, is difficult to handle rapidly.

As soon as the heart is exposed, a round probe with a flat tip 1 mm in diameter is heated rapidly to red heat in an open flame. This hot tip is then applied to the heart. At first, the upper part of the intraventricular grove was aimed at, in an attempt to cauterize the anterior descending branch of the left coronary. This proved to be hazardous, however, for the right ventricle is so thin walled, even a touch of the hot probe punctured it and the animal died of haemorrhage. After a time, therefore, the region to the left of the interventricular grove and just down from the auricle was injured in the hope that large branches of the vessel would be caught, (Experiment 6), but, if this was not regularly accomplished, the degree of injury was variable so that a more localized area of damage of more equal magnitude was preferred, and thus the thick tip was injured (Experiment 8 & 9). In searing the tip, there was very little danger of piercing the heart or of injuring a large arterial branch. It should just be mentioned that in introducing the hot probe into the chest, care must be taken not to injure the pleural membrane from the other side.

As soon as the heart is injured, the thorax is rapidly closed with silk sutures, three usually being sufficient. Caution must be exercised in this for, if the needle is large, as it was in Experiment 6, too deep a bite may be taken which may catch the heart or pericardium in the suture. A small no. 16 curved surgical cutting needle seemed to be the best.

The skin edges are temporarily held together with artery forceps and, as quickly as possible, a needle on a 5cc syringe is inserted through the chest wall and the air sucked out. Frequently, frothy pink foam is seen in the syringe as the lung re-expands and hits the needle. From the time that the thorax is opened, until this procedure is done, about one and a half minutes elapse. The skin is then closed with clips. Very little bleeding is apparent in this operation. If any is seen, it can be quickly stopped by applying an artery clamp briefly.

In the following experiments in which this operative technique was employed, there were only 2 immediate operative deaths in 65 operations (3%). Of course, the big question is the artificiality of such an experimental procedure. In the human, acute myocardial injury is usually caused by acute anoxia while here it is caused by heat. In human infarction, moreover, the worst damage is deep seated while here it is superficial. The human is subjected often to severe pain while these animals are anesthetized. On the other hand, the animals undergo an operation which must be severely shocking in itself. However, in spite of these differences, the procedure was adopted because it seemed probable that in acute myocardial injury the resulting changes in and around the area of injury are probably very similar regardless of the immediate cause of injury whether it is anoxia or heat. This was, of course, an assumption but in the hope that it was a reasonable one, these experiments were undertaken.

Experiment 6. - This first experiment was a preliminary one to determine first, if the thoracotomy per se, had any effect on the ECG. The next question was whether this type of myocardial injury produced any diagnostic change in the ECG and, if so, when was this change fully apparent. Another problem was whether there was any change in the serum potassium level at the time when the ECG changes were pronounced. And finally, was there any alteration detectable in the adrenal caused by the procedure?

The animals used were male albino rats of the Wistar strain. They were made up into groups as follows:

Group	No. of Animals	Av. Weight (grams)	Weight Range (grams)	Procedure
Group 1	10	228	140 - 350	Untreated control
Group 2	8	272	190 - 360	Thoracotomy - sham operated.
Group 3	8	295	270 - 330	Myocardial Injury

The animals were housed 4 to a cage and received Purina Fox Chow and tap water ad libitum.

A control 4 lead ECG was taken on all animals the day previous to operation. The sham operated group had the chest open for approximately as long as the group in which the myocardium was injured. All burns were placed just lateral to the interventricular groove about half way between the tip and the auricle. All experimental animals had a tracing taken on the 1st, 2nd, and 3rd post-operative (P.O.) day, while 6 controls were checked on P.O.2 and 2 on P.O.3. On the 3rd day, because distinctive ECG changes were seen by this time, blood was taken for potassium analysis. The animals were killed, then, and autopsied. The gross appearance of the heart was noted especially. The adrenals were weighed. Then the hearts and adrenals were sectioned and stained with H & E.

As said before, internal control tracings were done on all animals. Unfortunately, 6 of these tracings in the thoracotomy group were ruined during

development of the film owing to a technical error.

A 4th lead was taken on these animals at the level of the ziphi-sternal junction about 1 cm off the mid-line. Several factors combined to make these tracings extremely variable. In addition to those factors mentioned previously (page 43), in these cases, the heart may be shifted due to the thoracotomy, the electrode may be shifted by the position of the incision, and the chest wall may be swollen. Thus, changes in this lead must be distinct, and even at that, they are considered to be only confirmatory of some change occurring in lead 1.

Results: Before going on to consider the cardiographic and pathological changes in these groups in detail some figures will be presented illustrating the ECG findings of acute myocardial injury in the rat.

In fig. 15 a & b, two tracings on an animal in group 3 are shown The control tracing is within normal limits. On P.O.2, a Q wave is prominent in the standard leads. R2 hardly reaches above the isoelectric line. T 1 and 2 and, to a lesser extent, T4 are sharply inverted. This is the most markedly abnormal pattern of injury which has been seen. In fig. 15 c,d,e, the more usual pattern which develops in the myocardial injury group is seen. Here, on P.O.1, ST1 is elevated while T1 is small inverted. By P.O.3, the T waves in lead 1 and 4 have become deeply inverted and T2 has become flat. The pattern seen in these tracings is the more usual pattern of this acute anterior myocardial damage. In the summary which appears in table 10, the large letter T represents such marked and typical findings as those shown in fig. 15 and indicates, at least, that T1, and often T4 as well, has become distinctly and constantly inverted.

The series of changes in fig. 16 are not nearly so striking. Here the changes are of a lesser degree and are less constant. On P.O.1, ST1 is variable but is sometimes slightly elevated. On P.O.2, T1 is variable being small inverted or flat. On P.O.3, the ST and T segments are slurred and depressed and

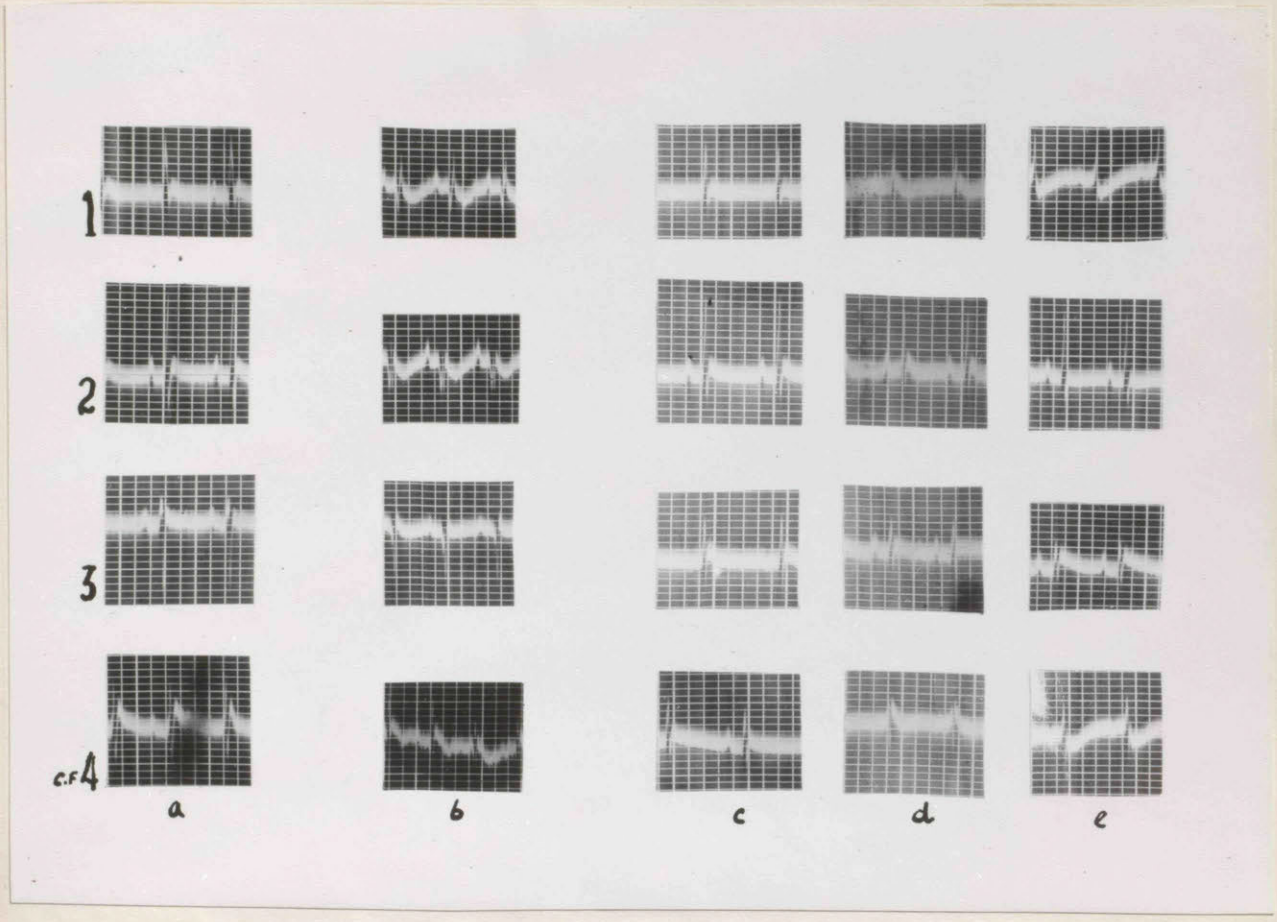

Figure 15.

Pattern of acute myocardial injury. (a) control (b) PO2 Note prominent Q1 & 2 & 3 and inverted T1, 2 & 4. (c) (d) (e) another animal. (c) control (d) PO 1 ST 1 elevated. T1 small inverted. (e) PO2 T1 & 4 deeply inverted T2 flat.

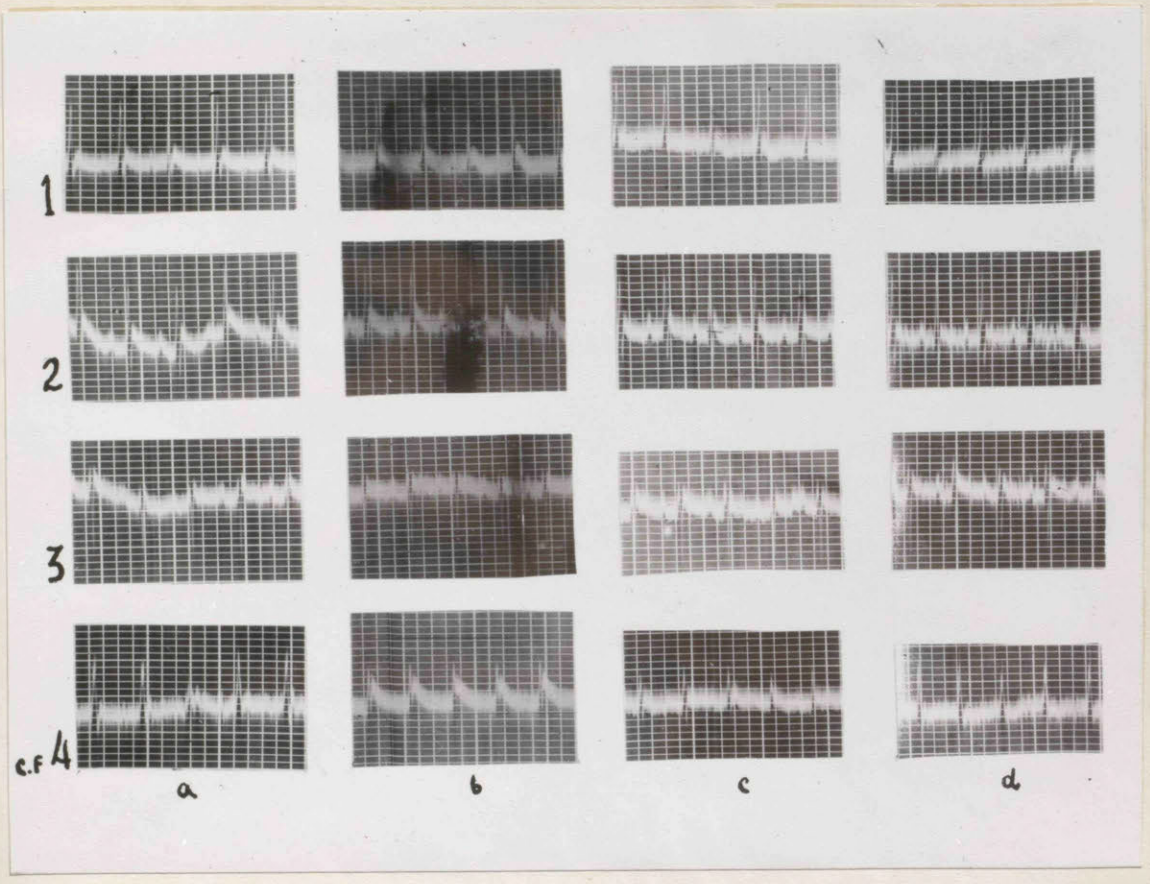

Figure 16.

(a) control (b) PO 1 ST1 slightly elevated. T1 may be very small inverted in last complex. (c) PO 2 ST1 + T1 variable. ST1 occasionally elevated T1 small inverted or else flat. (d) PO 3 ST + T segments in lead 1 slightly depressed below isoelectric line. T4 occasionally small inverted.

T4 is small inverted. Tracings in which there are only minor changes in lead 1, and in which there are some supporting changes in lead 4, sometimes more marked thar here, fall into one category. This category, where the changes are not so marked or constant, are considered to be suggestive of myocardial damage and the tracings are designated by the letter S in table 10.

A series of tracings which does not show any definite (T) or suggestive (S) abnormalities in the ECG but only such variations as may occur in uninjured repeat tracings are considered within normal limits and are referred to as N in Table 10. (fig. 20).

Table 10 has been set up in an attempt to correlate the pathological and cardiographic findings. The external control group has not been tabulated here in that all tracings were within normal limits and in that there was no abnormal findings in the heart grossly or microscopically. We have already seen how the ECG findings are to be classified by the letters T, S, and N. A number appearing after these letters will indicate the P.O. day on which the most marked change in the T wave is apparent.

At autopsy, in the gross, the induced injury on the myocardium usually appeared as a distinct white spot about 1½mm in diameter surrounded by an area of purplish or reddish discolouration. This type of injury, which was usual, is referred to in table 10 as + 2. If this burn penetrated the myocardium so that there was a distinct defect in the surface, it is classified as + 3. If this burn was smaller, as if only a light touch had been made, it is graded as + 1. If, as occasionally happened, a second light burn was placed on the myocardium because of uncertainty regarding the first attempt, a + 1 was added to the grade. If an accident occurred, such as catching the myocardium in a stitch by taking too deep a bite with the needle, the severe inflammatory injury is considered + 4.

Another factor, of which note must be made, is the degree of pericarditis. This was difficult to gage sometimes because the haemopericardium, resulting from

the heart puncture procedure, occasionally obscured it. A + 1 is used to designate the slight almost unavoidable adhesion of the parietal pericardium to the site of incision. A + 2 indicates that this adhesion shows thickening and inflammatory change. A + 3 indicates that this adhesion also involves the visceral pericardium so that there is a band of tissue from the incision to the heart, often at the site of injury. Included in this grading, also, are those hearts which are free in the pericardial sac but in which there is a grossly visible and distinct fibrinous visceral pericarditis. A + 4 indicates that the heart is firmly adherent to the incisional site but not caught in a stitch.

The microscopic findings are difficult to tabulate. For one thing, only 2 sections were cut off each block in the same place, so that the microscopic findings can not be graded, serial sections being missing. Usually, under the microscope in this type of injury, an arc of very deep staining muscle bundles with no nuclei is seen extending in from the epicardial surface. Around this is an area of inflammatory cell infiltration, empty muscle sheaths and fibroblastic proliferation. In table 10, "injury confirmed" will indicate this picture. Other findings are presented in an abbreviated synopsis.

On consulting table 10, it will be seen 2 of the animals in the thorocotomy group show the typical ECG changes of myocardial damage. At autopsy, it was found that in using a large curved needle, a suture had been passed through the tip of the left ventricle in the animals. The whole tip was swollen pale and necrotic-looking and in the microscopic section, practically the full thickness of the ventricles was destroyed, the muscle bundles being replaced by inflammatory cells and fibroblasts. In addition, there were two other animals showing a series of tracings in which there were minor changes suggestive of myocardial injury (S). At autopsy, in one, there was evidence of a fibrinous visceral pericarditis and the microscopic showed an inflammatory infiltration into the myocardium beneath this pericarditis. In the other, there were no visible involvement of the visceral

TABLE 10
see text.

	Animal No.	ECG	PATHOLOGY Gross Degree Injury	Gross Degree Pericard.	Microscopic
Thoracotomy	634-1	N		+ 1	
	-2	N		+ 1	
	-3	N		+ 1	
	-4	N		+ 1	
	635-3	S		+ 3	Patch peric. with inflam infiltr.
	-4	T3	+ 4	+ 4	Severe inflam in ventr. wall
	636-2	S		+ 2	Patches peric. with inflam. infiltr.
	-4	T2	+ 4	+ 4	Severe inflam. in ventr. wall.
Myocardial Injury	637-1	T3	+ 2	+ 4	Injury confirmed.
	-2	T3	+ 2	+ 3	" "
	-3	S3	+ 2	+ 1	" "
	-4	T2	+ 2	+ 2	" " some deep inflam. infiltr.
	638-1	S3	+ 2	+ 1	" "
	-2	S2	+ 2	+ 2	" "
	-3	T3	+ 2	+ 3	" "
	-4	N	+ 2	0	" "

pericardium but in microscopic section there were 2 spots of pericarditis with inflammatory infiltration beneath. All these thoracotomy animals were operated on in succession and the suggestion is that there was some change in technique so that the myocardium was injured by the needle. At any rate, it was felt that there was sufficient evidence of myocardial injury found at autopsy to account for these ECG changes.

In those 4 thoracotomy animals in which there was no evidence of myocardial damage, the tracings were all within normal limits. It is unfortunate that the internal control tracing is not available but certainly it can be said that in these 4 animals there is no suggestion of a variation from the normal range.

In the myocardial injury group, it will be seen that 4 animals show definite change (T), 3 show suggestive changes (S), and 1 shows no diagnostic change (N). Most of these animals showed about the same degree of myocardial injury, except for 2 animals 637-1 and 637-4. In the first of these, the tip of the heart was involved in a dense adhesion to the site of the incision, and under the adhesion, the myocardium appeared white and necrotic. In 637-4, the heart & visceral pericordium were free from adhesion, but the tip of the heart beyond the injury appeared white and necrotic and, on microscopic, deep inflammation was seen. Possibly, in this case the major blood vessel to the tip was involved.

Thoractomy, without injury to the heart, does not seem to distort the ECG. But because the heart was injured in 4 instances in the thoracotomy group, the results of adrenal weight and potassium concentration in these injured animals are included in the myocardial injury group. This decreases the number of thoracotomy animals to the extent that the values in this group are not sufficient to be statistically significant.

In Table 11, the average adrenal weights and the results of the potassium determinations are given. The adrenal weights in the myocardial injury

group are practically the same as in the control group. In addition, microscopic examination with an H & E stain revealed no significant difference in appearance in the 2 groups. After 3 days, there was, then, no change in the adrenals as revealed by these methods of examination.

The blood for potassium determination was taken 3 days after injury when most of the ECGs were showing most distinctly evidence of myocardial damage. All samples were analysed in duplicate and these duplicates were so close they were averaged. It seems obvious, from Table 11, that the serum potassium concentration in the myocardial injury group is not significantly different in average or in range from the controls.

Discussion: It might be wondered why all the animals in the myocardial injury group did not show marked electrocardiographic changes indicative of injury. There is no ready answer to this question. The degree of injury is about the same, except for the 2 animals mentioned above. The positions of these injuries were all about the same, and a slight difference in exact position did not seem to be responsible for one animal showing a typical change and another not. The intensity of surrounding reaction in the myocardium may be the responsible variable but this can not be judged from out data. The exact time of the tracing may also be a factor, that is, the tracing may not have been taken at a time when injury would have been most apparent electrocardiographically. There is a suggestion from the data in Table 10, that the degree of pericarditis developing may be the variable factor. Thus, when there was no evidence of pericarditis, no electrocardiographic evidence of damage developed (638-4). In the myocardial injury group when pericarditis was minimal (+ 1) the changes were only suggestive (S) in spite of definite myocardial injury (637-3, 638-1). But, on the other hand, the changes were only suggestive with the same degree of injury and a greater degree (+ 2) of pericarditis (637-2). Also, in animal 637-4, a + 2 pericarditis associated with a more serious injury, showed marked changes. If

the pericarditis is severe and involves the myocardium, it, no doubt, increases the myocardial injury and contributes to the distortion of the ECG (635-3). But the degree of pericarditis does not seem to be the only variable explaining the difference in the cardiographic patterns developing.

In the future an attempt would be made to decrease the degree of of pericarditis by exercising extreme caution in suturing. Also, the position of injury, would be changed to the tip to avoid involving a large artery. It was hoped that these maneuvers would reduce the variability in the extent of injury.

Summary: Thus, in this experiment, several important facts have been demonstrated. First, with myocardial injury, there was no change in the serum potassium concentration at a time when changes in the ECG were marked. Similarly, no change could be demonstrated in the adrenal weights or appearance under H & E stain at this time. In addition, the patterns of acute myocardial injury in the ECG were seen and these patterns were classified. This pattern was usually most marked by P.O.3 as far as the T wave inversion was concerned. In 1 case, myocardial injury did not alter the ECG. There was an indication for shifting the position of the injury. And finally, it seemed fairly certain that pericarditis involving the myocardium may produce abnormal patterns and contribute to the pattern of myocardial injury.

Experiment 7. The purpose of this experiment was to test the effect of raising and lowering the potassium concentration on the electrocardiographic pattern of the acutely damaged heart. There had been no demonstrable change in the serum potassium concentration at a time when there was a marked ECG change following injury. Nonetheless, it was felt that there might still be marked local change in potassium concentration and distribution around the area of damage. Some indirect evidence for this local change in potassium, further inhibiting myocardial function, might be provided, if raising or lowering the potassium improved,

or, on the other hand, exaggerated, the ECG pattern of acute injury.

The Experiment was set up as follows using male albino rats of the Sherman Strain.

	No. of Animal	Average Weight (grams)	Weight Range (gms)	Procedure
Group 1	7	99	88 - 106	Control
Group 2	7	122	108 - 138	Myocardial Injury untreated
Group 3	8	124	112 - 145	Myocardial Injury potassium
Group 4	8	126	108 - 140	Myocardial Injury DCA

The animals were housed 3 or 4 to a cage. They all received Purina Fox Chow ad libitum. All animals, except those in group 3, drank tap water. An internal control 4 lead ECG was taken on all animals on the day prior to operation.

These animals were considerable smaller than previously used so that more caution was exercised in injuring the heart - that is the hot probe was touched more lightly to the surface of the heart. The burn in this experiment was placed in the region of the tip of the left ventricle. For suturing the chest, a small curved needle was used and extreme care was taken in this step so that neither the heart nor the pericardium would be caught in the suture.

Because the animals in the potassium treated group were roughly comparable in weight to the animals used in experiment 1 & 2, they received potassium in the same dosage intraperitoneally each time and in the same concentration in the drinking fluid. Thus, the animals in group 3 received an injection of 3cc of potassium chloride intraperitonially three times a day at roughly 6 hour intervals in the concentration used in experiment 1 (11.56 grams KCl/L). In addition, the group was given potassium citrate water to drink in the strength used in Experiment 2 (30 grams K cit/L) and this they consumned at the rate of

40 - 45cc per cage per day for 3 days. The total fluid intake, therefore, for these animals per cage, was approximately 90 - 95cc per day compared to an average water intake in the control group per cage per day of 100cc.

DCA was used to lower the serum potassium concentration. Three one-third portions of a 75 mgm pellet of DCA were implanted into each animal in group 4 at the time of operation under the same anesthetic. Each pellet was implanted in a different position under the skin of the back.

An ECG was taken each day for 3 days post-operatively on all experimental animals. The tracings were taken on the animals in the same order in which they were operated so that there was practically the same time interval between the operation and the tracing in each animal. In the external control group, 7 animals had a tracing taken on P.O.2 and 2 on P.O.3.

On the 3rd P.O. day, after the ECG had been taken, blood samples were taken for potassium analysis by heart puncture. These samples were drawn about 6 hours after the last potassium injection. Shortly thereafter, the animals were killed and an autopsy done. Myocardial sections were cut and stained with H. & E.

Results: Two animals in the external control group died, one with the evipal and one with a severe "twisting disease". One animal in the DCA-treated group died on P.O.2 and one animal in the KCl-treated group on P.O.3. At autopsy, the lungs of both these injured animals were found to be dull red and one section oozed pus. In both animals there was also a purelent pericarditis and pleuritis. Here, then, was another factor, infection, which would have to be controlled in future.

Table 12 was composed to demonstrate the pathological findings in relation to the ECG. The same grading and symbolism is used here as was used in the previous experiment. Marked ECG abnormalities, such as were seen in fig. 15, are represented by the letter T. Less distinctive changes, as seen in fig. 16,

are suggestive of myocardial injury and are designated by the letter S. And a series of tracings showing no more change than is compatible with normal variations are symbolized by the letter N. The day upon which the T wave change is most marked in designated by a post-script number.

The pathological changes are presented in the same way also. The usual finding is a white spot about 1½mm in diameter with a surrounding purplish or reddish discoloration. These are graded ± 2. Less injury than this are referred to as ± 1. Those burns which are deep with an apparent defect in the surface continuity are graded as ± 3. Occasionally in operating on these animals (1 case), not being certain that the hot probe touched the heart, a second definite burn was placed on it. If such is the case, 1 point is added to the grade.

Pericarditis is designated as ± 1 for an adhesion of the parietal pericardium to the incisional site. ± 2 indicates that the parietal pericardium is more firmly adherent and, in addition, shows evidence of inflammatory thickening. If the visceral pericardium and heart are involved in this inflammation so that there is a band connecting the heart to the incisional site or else a distinct fibrinous pericarditis is visible grossly, it is graded ± 3. If the myocardium is firmly adherent to the incision, it is graded ± 4.

Sections were examined under the microscope but, so often the cut had apparently missed the area of injury, that these findings are not included in the table. In those slides where the injury was seen, the microscopic picture was identical to that seen in the previous experiment.

Table 12 does not include group 1, the external control group. In this group, there was no marked variation in the series of tracings and neither was there any significant finding at autopsy.

Table 12 presents some very interesting findings. In group 2, the untreated injury group composed of 7 animals, it can be seen that 3 animals developed marked ECG changes in lead 1 & 4 classified as T. In these animals

the injury was + 2 to + 3 and the pericarditis + 3 to + 4. One animal showed less marked but suggestive (S) changes with a + 2 injury and a + 2 pericarditis. Three other animals with a + 2 injury and a + 1 to + 2 pericarditis showed no diagnostic change. Thus, 4 animals out of 7 showed some cardiographic evidence of injury.

In group 3, the potassium treated injury group, composed of 8 animals one of which died on the 3rd P.O. day of purelent pericarditis pleuritis and pneumonia, a much greater number showed marked ECG evidence of damage. Six animals show the marked typical changes and are classified as T. These animals showed an injury ranging from + 2 to + 3 and a pericarditis ranging from + 1 to + 3. One additional animal shows suggestive changes with an injury of + 2 and a pericarditis of + 1. There is only 1 animal who does not show a change suggestive of damage and at autopsy this animal showed a + 2 injury and a + 3 pericarditis. It should be noted that 2 animals (685-2 and 686-1) showed major ECG changes with a degree of injury and pericarditis that in the other groups did not cause such marked abnormalities. Thus, in this group of 8, all but 1 showed some cardiographic evidence of damage.

In the group treated with DCA, out of 8 animals, one of which died on the 2nd P.O. day of a purelent infection, there are only 2 animals showing distinct changes. Both of these show an injury of + 3. One shows only + 1 pericarditis while the other has a pericarditis of + 2. In the remaining 6 tracings, there is no diagnostic change despite an injury ranging from + 2 to + 3 and a pericarditis ranging from 1 to + 3. Thus, in the group, there are only 2 out of 8 showing ECG evidence of injury.

One thing shown in the table on close examination is that there is almost the same degree of injury and of pericarditis in each group. Just to illustrate this, the average injury in group 2 is 2.14, in group 3, it is 2.25, and

TABLE 12
see text.

Group	Animal No.	ECG & day	Degree Injury	Degree Pericarditis
ii	683-1	N	+ 2	+ 2
Injury	-2	T2	+ 3	+ 3
Untreated	-3	T2	+ 2	+ 3
	684-1	S2	+ 2	+ 2
	-2	N	+ 2	+ 1
	-3	T2	+ 2	+ 4
	-4	N	+ 2	+ 1
iii	685-1	T2	+ 2	+ 2 purelent - died P03
Injury	-2	T2	+ 2	+ 1
&	-3	T2	+ 3	+ 3
KCl	-4	T2	+ 3	+ 2
	686-1	T2	+ 2	+ 2
	-2	T2	+ 2	+ 3
	-3	S	+ 2	+ 1
	-4	N	+ 2	+ 3
iv	687-1	N	+ 2	+ 3
Injury	-2	T2	+ 3	+ 2
&	-3	N	+ 2	+ 2 purelent - died P02
DCA	-4	N	+ 2	+ 1
	688-1	N	+ 2	+ 2
	-2	N	+ 2	+ 3
	-3	T3	+ 3	+ 1
	-4	N	+ 2	+ 1

and, in group 4, it is 2.25. The average pericarditis in group 2 is 2.28, in group 3, it is 2, and in group 4, it is 1.88. Although averaging is an artificial device here, in that it doesn't show the various combinations of injury and pericarditis, it serves to indicate that the groups are closely comparable pathologically.

In table 13 the serum potassium concentrations in each group are presented. The potassium determinations were done on duplicate samples. The duplicate values obtained were so close as to render the determinations reliable. In the control group, there are only 5 animals, because 2 died, but, because these control values in average and in range are very close to the large control group shown in Table 11, these concentrations probably represent a reliable sample. It will be noticed that the potassium level has not been elevated by the injection of potassium three times daily and its administration in the drinking water. But it should be emphasized that the samples were drawn 6 hours from the last injection. In addition, the animals had been anesthetized prior to the sampling, when the ECGs were taken, and had, therefore, probably not been drinking a great deal that day. At any rate, all that can be said of these animals is that the potassium intake was high. It seems fairly certain however, that three times daily, following the intraperitoneal injection of potassium, there would be a transient rise in serum potassium concentration as this group is comparable in weight to the group in experiment 1.

It will be seen from table 13 that there is a significant decrease in the average serum potassium concentration and a lower range of concentration in the DCA treated group. Thus, DCA in this dosage, in 3 days, exerts a potassium effect.

Discussion & Summary. One thing that this experiment seems to indicate is that a high potassium intake and transiently high serum potassium effects the heart adversely as measured by the ECG in comparison to the control group. In the

TABLE 13

POTASSIUM DETERMINATIONS

Group	Group 1 Control	Group 2 Injury Untreated	Group 3 Injury & KCl	Group 4 Injury & DCA
No. of Samples	5	7	7	7
Average K. conc. m e/l	4.95	5.03	4.74	3.37
Range K conc. m e/l	3.87-6	3.37-6.25	3.87-5.6	2.5-4.4

control group, 4 animals out of 7 showed ECG evidence of damage, 3 of them showing marked evidence. In the potassium-treated group, not only did more animals develop cardiographic changes (7/8), but also more showed marked ECG abnormalities (6/8), even when the injury and pericarditis were not expecially severe. This finding is particularly interesting in view of the fact that Sharpey Shafer (138) found that the administration of potassium in acute myocardial infarction in the human exaggerated the pattern of infarction, and increased the inversion of the T waves.

On the other hand, lowering the serum potassium with DCA in this dosage seems to be of some benefit to the heart as measured by the ECG. In the control group, 4 animals out of 7 presented some ECG evidence of myocardial injury and 3 of these showed marked changes. In contrast, in the DCA treated group, only 2/8 developed ECG abnormalities or, if the animal dying on P.O.2 is discarded, 2/7 This difference is not marked but it is suggestive.

The numbers used here are probably not sufficient to be certain, but the suggestion seems to be that raising the potassium intake to a high level is a further handicap to the acutely injured heart as measured by the ECG while lowering the potassium is of some benefit electrocardiographically. This effect of raising and lowering the potassium on the ECG in acute injury is rather a paradoxical one. In the normal and chronically injured heart, potassium administration increases the amplitude of the T wave, while here it seems to exaggerate the inversion of the T wave. On the other hand, in most cases, lowering the potassium tends to a flattening and inversion of the T wave, while here lowering the potassium tends to maintain the T wave. Thus, this evidence may possibly indicate, indirectly, that in myocardial injury there is an accumulation of potassium locally which is further interfering with the action currents of the heart. If this were the case, these experimental findings would be understandable.

It seems probable that the result seen following potassium treatment is reliable in that it essentially agrees with Sharpey-Shafers findi-

human. On the other hand, the effect of DCA is no more than suggestive. It might be wondered if the effect of DCA is due to its potassium lowering effect or to some other effect such as increasing the sodium. Or else, it might be said that the effect of DCA is due to its favouring the development of counter shock phenomena. But Selye (105) points out that DCA seems to be valuable in this respect only in certain special conditions, such as potassium intoxication and severe dehydration. The value of DCA in potassium intoxication might fit in with a beneficial effect suggested here, if this theory of localized potassium concentration were accepted. The fact that the findings in this experiment are complimentary may indicate that the DCA effect is due to lowering the potassium.

Experiment 8. The purpose of this experiment is to confirm the beneficial effect of DCA on the electrocardiographic pattern of acute injury and to investigate it further. It was decided that the experiment should be done on a large group of animals to try to cover more fully variability in the extent of injury and pericarditis. In addition, it was decided that penicillin should be given to try to prevent bacterial infection which was a variable factor killing 2 animals in the previous experiment. Further, it seemed that a more severe injury was needed, so that not only could survival rate be gaged, but also so that a greater percentage of animals would develop the pattern of injury. The experiment would be allowed to run longer because of this survival rate study and also because, by prolonging the duration, the speed with which the abnormal ECG returned to normal could be seen.

The experiment was set up using male albino rats of the Wistar strain as follows:

	No. of Animals	Average Wt. (gms)	Weight Range (grams)	Procedure
Group 1	6	101	88-108	Uninjured Control - penicillin
Group 2	16	96	76-124	Injury & Penicillin
Group 3	16	94	76-110	Injury & Penicillin & DCA

The animals were housed 3 - 4 to a cage and given Purina Fox Chow and tap water ad libitum.

In the week previous to operation, all animals had a 4 lead electrocardiogram taken as an internal control. Three hours before the operation commenced, all animals received 6000 units of procaine penicillin in 2% aluminium monostearate. The usual commercial strength of this preparation is 300,000 units of penicillin per cc. To dilute this, sesame oil was used, on the advice of the manufacturer, 1 cc of this commercial penicillin was made up to 10 cc, so that 1 cc contained 30,000 units. Then, 2/10ths of a cc was injected under the skin of the back so that the delivered dosage was 6000 units. The external control group also received this dosage so as to control any possible effect of this drug on the ECG. This preparation is said to maintain penicillin blood levels for about 96 hours (156). An additional dose was administered on the 1st P.O. day.

At operation, the same hot probe was used, but a greater effort was made to burn the heart severely in the region of the tip. This was accomplished by holding the probe on the cardiac surface longer and by exerting pressure on it. Once again, the greatest care was taken in closing the thorax to avoid catching the heart or pericardium in the suture.

In group 3, immediately following the operation, while the animals were still anesthetized, 3 one-third portions of a 75 mgm pellet of DCA were implanted under the skin of the back, each being inserted in a different position.

Following the operation, the 4 lead ECGs on the experimental animals were repeated on P.O.1,2,3,4, and 6. The cardiograms were taken in the same order as the operations were performed so that there was approximately the same length of time between operation and tracing in each animal. The control group, receiving penicillin, had repeat standard ECGs done as follows; on P.O.1 there was 2 animals done, on P.O.2 there were 4, and on P.O.4 the full group was done.

On the 7th day, all animals were weighed, killed and autopsied. A

section of the heart was taken for microscopic study.

Results: Two animals in group 2, the untreated-injury - penicillin group, died during the course of experiment. One animal was found dead on P.O.2 At autopsy, there was no evidence of infection and the only finding of note was the cardiac injury. The second animal died on the P.O.4 but this result is not so clear cut as the other death. This animal, weighing 116 grams, was given 11 mgm of evipal. For at least 20 minutes after this, it was under surveillance and appeared in good condition. Very shortly thereafter, the animal was found dead. Usually, if evipal alone is responsible for death, it kills the animal in 10 to 15 minutes after the injection. Also, it should be noted that this dose is not a high one and had been well tolerated 4 times previously. The suggestion is that, because of the myocardial injury, the animal's resistance was very low and the added insult of evipal was just too much.

In table 14, the initial weights are compared to final weights. It will be seen immediately that the injured animals have gained weight almost as quickly as the control group. In addition, it will be seen that both injured groups have gained weight at almost the same rate.

The ECGs of the control group all remained within normal limits. These animals presented no significant finding at autopsy.

The electrocardiographic and pathological findings will be summarized in several tables and figures to follow. The microscopic sections were unfortunately of little value in this study because serial sections were not taken and very few of the single sections transected the actual area of injury. In those in which the area of injury was sectioned, there were a few deep-straining muscle bundles apparent in an area of empty sheaths and fibrobastic proliferation. At the epicardial surface an accumulation of inflammatory cells was visible. This whole area of damage extended irregularly into the myocardium.

TABLE 14

WEIGHT TABLE

Group	No. of Animal	Initial Weight		No. of Animal	Final Weight	
		Average (grams)	Range (grams)		Average (grams)	Range
Control	6	101	88-118	6	153	130-180
Injured - Untreated	16	96	76-124	14	143	130-164
Injured DCA	16	94	76-110	16	142	122-158

In table 15, the untreated-injury - penicillin group, and in table 16, the DCA-injury - penicillin group, the electrocardiographic patterns which developed are opposed to the gross pathological findings. These findings at autopsy are not so easy to classify, principally because after 7 days the injury was not so well defined, and because after 7 days adhesions involving the heart were often very firm and, in separation, the surface was sometimes torn. In some cases also, the injured area seemed to be covered with a thick gelatinous material making the injury appear less defined.

The burn usually appears as a yellow point about 1mm in diameter. Around this are zones of reaction, the whole being about 2 - 3mm in diameter. Sometimes around the point a hyperemic zone is visible and then next a pale area. Sometimes tiny red points are visible in the surrounding area. This type of finding is graded + 2. If a definite indentation at the point of injury is apparent, it is graded as + 3. A question marks beside the figure means it was very difficult to estimate the burn exactly because of tearing in separating an adhesion.

The pericarditis is graded as before. + 1 indicates a fine adhesion of the parietal pericardium. + 2 indicates inflammatory involvement of the parietal pericardium. + 3 indicates that there is an adhesion or pericarditis involving the visceral pericardium and heart, and + 4 indicates a firm adhesion of the heart to the incisional site.

The ECG pattern developing is represented as before by a T, which means a marked alteration of pattern particularly an inversion of T1 and 4, by an S, which means minor changes suggestive of myocardial damage and by an N which means no significant alteration. The post script number indicates the P.O. day on which the changes, particularly in the T wave, are most marked.

These tables 15 and 16 should be examined together. It should be noted that, as groups, the degree of injury and degree of pericarditis are very similar.

TABLE 15.

Group 2 - Injury - Penicillin Group

Animal No.	ECG's & PO Day	Degree of Injury	Degree of Pericarditis.
691-1	T2	+ 2	+ 2
-2	S2	+ 3	+ 2 Died PO3 (see text)
-3	T2	+ 3	+ 4
-4	T2	+ 2	+ 3
692-1	T2	+ 3	+ 3
-2	T2	+ 2	+ 4
-3	T3	+ 3	+ 4
-4	T3	+ 3	+ 4
693-1	T2	+ 2	+ 3
-2	T3	+ 3	+ 3
-3	S2	+ 2	+ 1
-4	T2	+ 2	+ 1
694-1	T	+ 2	+ 1 Died on PO2
-2	T2	+ 2	+ 3
-3	T3	+ 2?	+ 4
-4	T2	+ 2	+ 1

TABLE 16

Group 2 - Injury - Penicillin - DCA Group.

Animal No.	ECG & day	Degree of Injury	Degree of Pericarditis.
696-1	T2	+ 2	+ 4
-2	T2	+ 2	+ 2
-4	T2	+ 2	+ 3
697-1	T2	+ 2	+ 4
-2	T2	+ 2	+ 3
-3	T2	+ 3	+ 2
-4	N	+ 3	+ 2
698-1	S2	+ 2	+ 4
-3	T2	+ 3	+ 1
-4	N	+ 2	+ 1
699-1	T2	+ 2	+ 1
-2	T2	+ 3	+ 1
-3	T2	+ 2	+ 4
-4	S3	+ 3	+ 3
700-1	S2	+ 2	+ 2
-2	S2	+ 2?	+ 4

If these grades are averaged again, the degree of injury in the untreated group (table 15) is 2.37, while in the treated group (table 16) it is 2.31. The average degree of pericarditis in the former group is 2.69, while in the latter it is 2.56. In table 15, there are 10 animals with a + 2 injury and 6 with a + 3, while in table 16, there are 11 animals with a + 2 injury and 5 with a + 3. In each group also, there are 5 animals with a + 4 pericarditis and 4 with a + 1. In the untreated group, there are 5 animals with a + 3 pericarditis and 2 with a + 2, while in the treated group, there are 3 with a + 3 and 4 with a + 2. Thus at autopsy, in the gross, the groups seem to be comparable.

The electrocardiographic findings in these comparable groups are interesting. In the untreated group (table 15), 14 out of 16 developed a definitely abnormal pattern (87.5%), 9 by the second day, 4 by the 3rd, while 1 died on P.O.2. Two additional animals showed less marked but suggestive changes. (12.5%). Thus, 16 animals out of 16 gave ECG evidence of myocardial injury.

In the DCA treated group, 10 animals out of 16 developed marked ECG abnormalities all of which developed by P.O.2 (62.5%). Four additional animals developed less marked suggestive changes, 3 by P.O.2, and 1 by P.O.3 (25%). There were 2 animals who showed no ECG evidence of damage (12.5%) though definite evidence of damage was present at autopsy.

Table 17 has been compiled in an attempt to show the cardiographic progress of each animal over the 6 day period. All tracings on all animals obviously can not be presented. In lieu of this, table 17 has been composed. The P.O. days are represented in the vertical columns. The T or S is listed in the vertical column corresponding to the day on which the most marked change was apparent and preceding the T or S are the figures representing the degree of injury and the degree of pericarditis, in that order, taken from table 15 and 16.

The letter R represents full recovery of a normal pattern. Because T1 is so often small, in the order of 0 - 1 mm, and because variation in the

height of the T wave does occur normally, the tracing is said to have returned to normal if the affected T1 segment has become perfectly isoelectric, for a distortion of this T segment below the line is not seen in normal animals. Lead 4 can not be included in the estimation of recovery because it is so poorly standardized. Fig. 17 illustrates a typical tracing (T) which has returned to normal (R). The letters PR indicate "partial recovery" - that is the abnormal pattern is regressing but the T segment is still distorted and below the isoelectric line. Figure 18 illustrates a series of tracings which are judged as having partially recovered (PR). Note that the ST & T segments in lead 1 are still below the isoelectric line. The R and PR are entered into the column representing the P.O. day on which the improved pattern was apparent. An A appearing in the 6th day column indicates that the tracing still remains grossly abnormal. (fig. 19). An N indicates that the slight variations throughout the series of tracings are within normal limits (fig. 20).

The untreated and treated animals are placed in adjacent columns and grouped on the basis of the degree ECG abnormalities to fascilitate comparison. Of those animals with a T pattern, it will be seen that the DCA treated animals recover a normal pattern more quickly than the untreated group. Thus, in the DCA group having a T pattern, at 3 days one animal shows recovery and at 4 days a total of 4 animals have recovered a normal pattern. If recoveries in the S group are included, it is seen that 2 more animals have recovered by 4 days making a total of 6 (42.5%). In the untreated group, in contrast, there is no full recovery by the 4th day in the T classification but 1 animal in the S group has recovered for a total recovery of 1 (1/16 = 5.5% or 1/14 not counting dead animals = 7.1%).

By the 6th P.O. day, in the DCA group, 7 out of 10 animals having the T pattern have fully recovered and 4 out of 4 having the S pattern have, also, making a total of 11 animals fully recovered as gaged by the ECG (78% = 11/14).

TABLE 17.
Progress Table - see text.

Untreated POST OPERATIVE DAY					DCA - Treated. POST OPERATIVE DAY				
Animal No.	2nd	3rd	4th	6th	Animal No.	2nd	3rd	4th	6th
691-1	2:2:T	PR	PR	R	696-1	2:4:T		PR	R
-3	3:4:T			PR	-2	2:2:T			PR
-4	2:3:T		PR	PR	-4	2:3:T		R	
692-1	3:3:T		PR	PR	697-1	2:4:T			PR
-2	2:4:T	PR	PR	PR	-2	2:3:T		R	
-3		3:4:T	PR	PR	-3	3:2:T		R	
-4		3:4:T		PR	698-3	3:1:T	R		
693-1	2:3:T		PR	PR	699-1	2:1:T			R
-2		3:3:T		A	-2	3:1:T		PR	PR
-4	2:1:T		PR	R	-3	2:4:T	PR	PR	R
694-1	2:1:T	died							
-2	2:3:T	PR	PR	R					
-3		2:4:T		A					
-4	2:1:T		PR	R					
691-2		3:2:S	died		698-1	2:4:S	PR	R	
693-3	2:1:S	R			699-4		3:3:S	PR	R
					700-1	2:2:S	PR	PR	R
					-2	2:4:S		R	
					697-4	3:2:N			N
					698-4	2:1:N			N

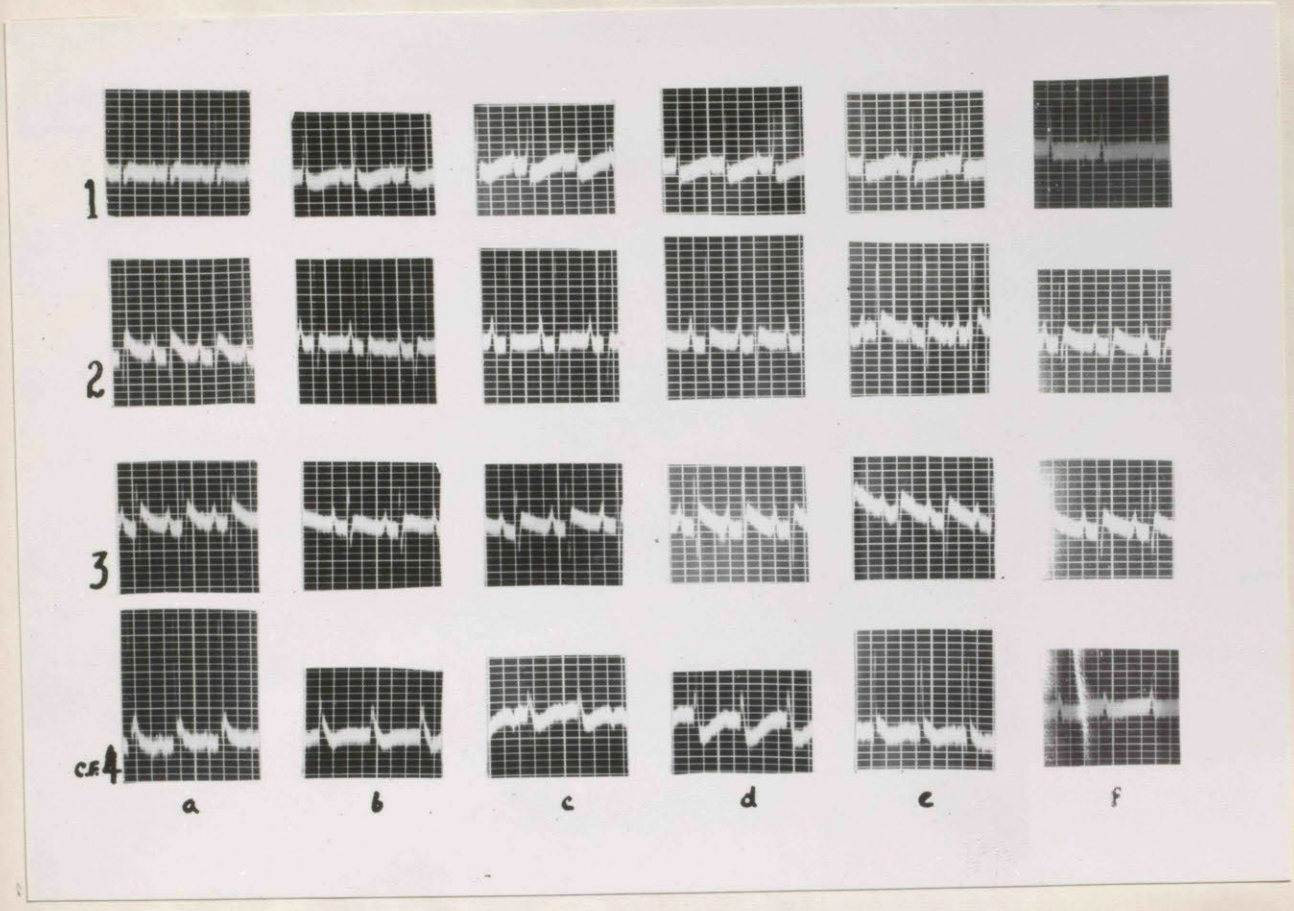

Figure 17.

(a) control (b) PO 1 Note inverted T1 & 4. (c) PO 2 T1 & 4 more inverted (d) PO 3 T4 more inverted. (e) PO 4 - Partial recovery. T1 & 4 less inverted (f) PO 6 - Recovery. T segment in lead 1 isoelectric.

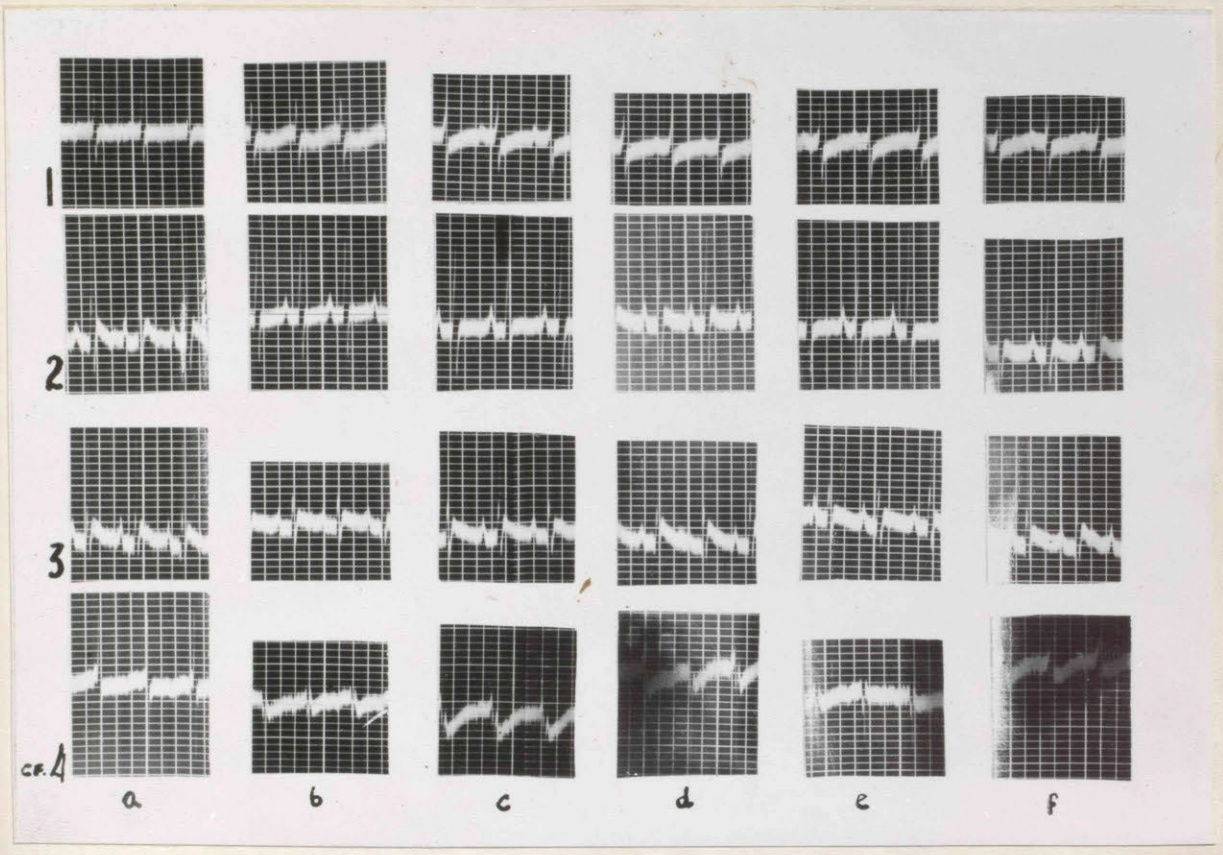

Figure 18.

(a) control (b) PO 1 - T1 + 4 small inverted. (c) PO2 - T1 + 4 more inverted (d) PO3 Similar to previous tracing. (e) PO 4 - T4 less inverted. (f) PO 6 Partial recovery. ST and T segments in lead 1 still depressed below the isoelectric line.

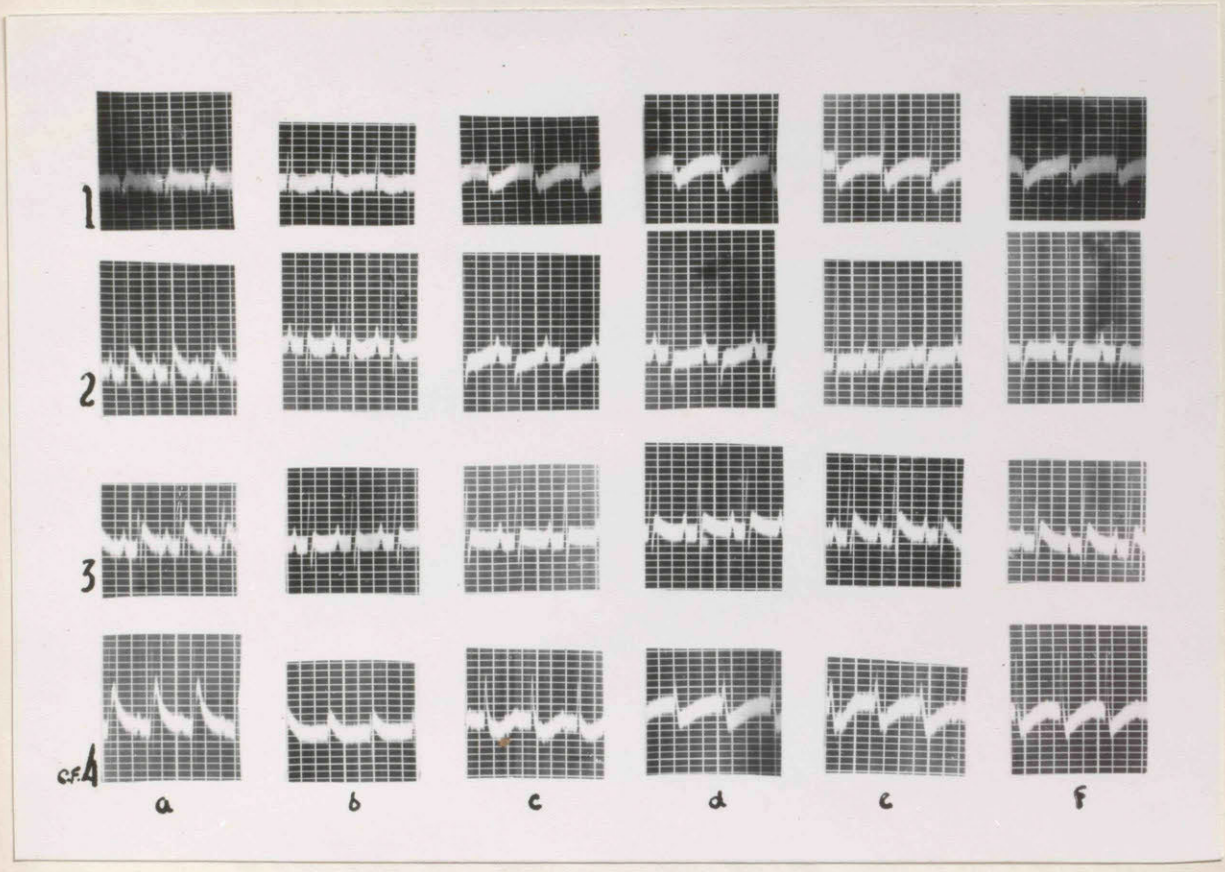

Figure 19.

(a) Control (b) PO 1 - T1 small inverted. (c) PO 2 - T1 & 4 deeply inverted. T2 & 3 flatter. (d) PO 3 (e) PO 4 (f) PO6 - tracing still remains grossly abnormal.

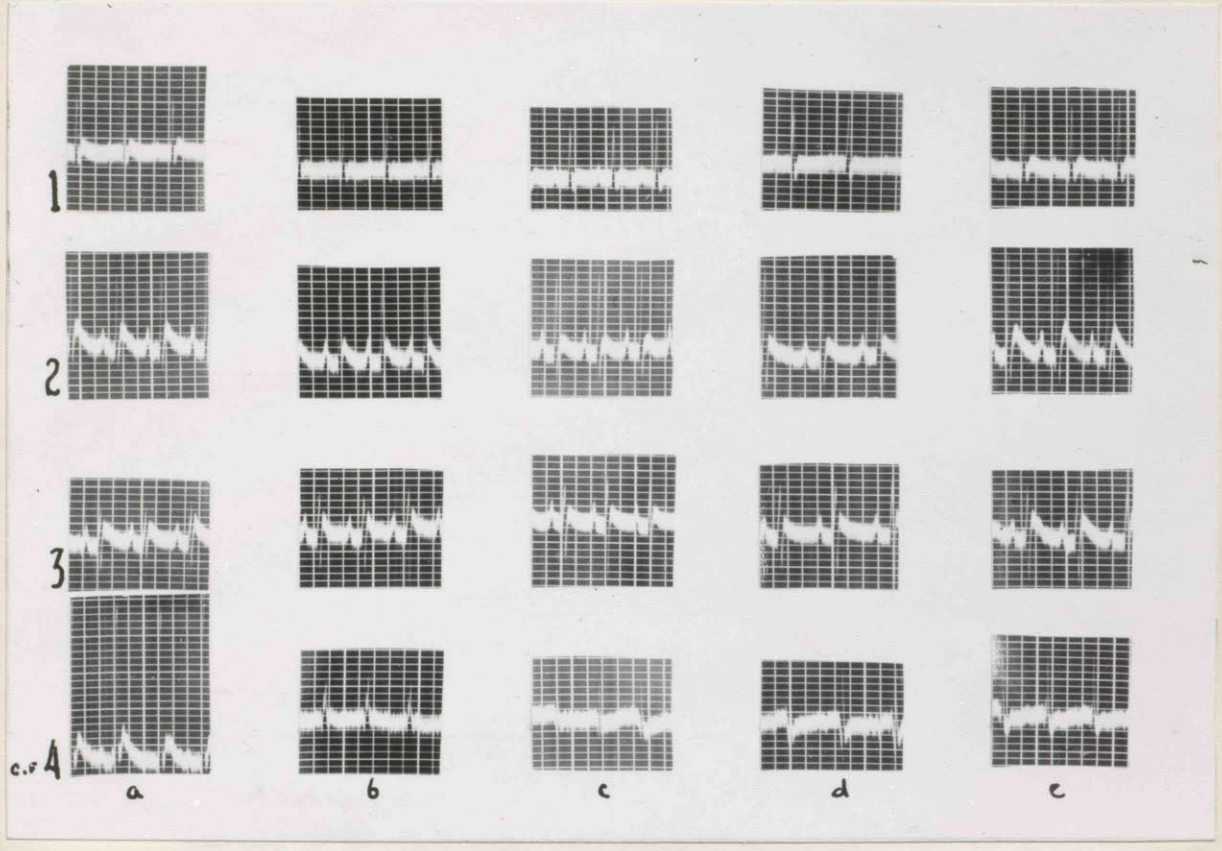

Figure 20.

(a) control (b) PO 1 (c) PO 2 (d) PO 3 (e) PO 4. Series of tracings shows slight variations but all within normal limits.

This leaves only 3 animals in the DCA treated group that have only partially recovered. No tracings remain grossly abnormal. It will be recalled that 2 animals never showed an abnormal pattern.

In the untreated group, by the 6th P.O. day, only 4 out of 14 animals having a T pattern had fully recovered so that the total showing recovery, including the 1 in the S group, by 6 days is 5 (5/16 = 31% or 5/14 if the dead animals are not counted = 35%). 7 animals showed partial recovery and 2 tracings remained grossly abnormal. In addition, it will be remembered that 2 animals died.

It is interesting to compare the progress of animals in each group having the same degree of injury, pericarditis and ECG change. Thus, compare animal 692-2 in the untreated group to animal 696-1 and 699-3 in the treated. Each animal shows a T pattern by P.O.2, but by the 6th day, both the DCA animals have fully recovered while the untreated animal has not. A similar comparison can be made between untreated animals 691-4, 693-1 and 694-2 and the treated animals 696-4 and 697-2. The treated animals both show full recovery by the 4th day while only 1 of the 3 untreated animals shows recovery by the 6th day, the other two showing only partial recovery.

Summary & Conclusions. To summarize the results of this experiment on quite a large number of animals, it should be emphasized that the DCA-treated animals developed the marked pattern of acute injury less frequently than did untreated animals (62% to 87%). In addition, more animals in the DCA group developed minor suggestive changes than in the untreated group (25% to 12.5%). Then again, 2 animals in the treated group did not even develop an abnormal pattern in contrast to the 2 animals which died in the untreated group. It should be noted especially that, in those DCA animals having an abnormal pattern, the normal ECG pattern is regained more readily than it is in untreated animals (at 6 days 78% to 35%). Two animals in the untreated group have a markedly abnormal

pattern by the 6th day whereas no such prolonged change is apparent in the treated group.

PART IV - CONCLUSIONS. It is essential at this point that caution be exercised in the interpretation of these results for several reasons. It is well to re-emphasize the artificiality of this type of cardiac injury. This experimental work was done in the hope that the change in the heart around an area of injury, regardless of whether the cause of the injury was anoxia or heat, were similar. This assumption is a very broad one and one for which no justification has been presented. Thus, the results do not justify conclusions beyond those applicable to acute myocardial injury caused by an epicardial burn following thoracotomy. Next, attention must be directed to the fact that, although such results are apparent in the albino rat, they are not necessarily applicable to other species. Thus, statements concerning this work must be confined to the albino rat. The last caution is probably the most necessary one to sound. This concerns electrocardiography. First, are those hearts which showed marked cardiographic changes more handicapped than those developing less marked changes or those not developing changes? Although there is some justification for the broad statement that the more abnormal the ECG the more handicapped the heart, it can by no means be said to be always applicable. In addition, it should be pointed out that, although the electrocardiographic apparatus records electrical events objectively, the interpretation of the tracing is an intensely subjective matter. This becomes especially important in the interpretation of border-line tracings. In these experiments, after certain criteria were laid down, the tracings were classified. But another observer might classify the border-line tracings differently. This device of classification on certain rigid criteria ma be deceptive. For instance, in experiment 8, tracings in which the T segment remained distorted below the isoelectric line were classified as PR and those in which the T segment became perfectly isoelectric were classified as R

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Using these criteria, the results obtained in experiment 8 are tabulated. Now, although there was often a distinct difference between tracings in the 2 classifications, in the border-line cases, where the T segment was just slightly below the isoelectric line, there was only a minor difference. Suppose those T segments which are almost isoelectric, but not quite, are classified as R, a marked change in the statistics results. There would be 5 more Rs in the untreated group and 2 more in the DCA treated. Now, not counting the dead animals, 10/14 in the untreated group would have recovered (70%) whereas in the treated group 13 out of the 14 animals showing an abnormal tracing would have recovered (93%). The comparison of these percentages does not count the 2 animal dying in the untreated group and the 2 animals not developing a change in the treated group, nor does it show the greater rapidity with which the DCA-treated group recovered, but the comparison, although it is still indicates a favourable effect of DCA, is not so distinctly in its favour. Thus, this emphasizes how this type of classification may be deceptive and in addition, how dependant these results are upon interpretation.

Bearing these limitations in mind, certain conclusions are suggested by these experiments. The electrocardiogram of the albino rat whose heart has been injured by an epicardial burn following thoracotomy seems to show a pattern of injury more frequently if potassium is administered and less frequently if DCA is given than injured control animals do. In addition, DCA-treated animals seem to recover a normal electrocardiographic pattern more rapidly. It was postulated that following myocardial injury, potassium accumulates in excess around the zone of injury further impairing function and it is possible that the detrimental effect of potassium administration and favourable effect of DCA administration, with the resultant lowering of potassium, indirectly, lend some credence to this postulate.

These experiments are certainly encouraging to further work. They should probably be repeated using not only cardiographic evidence but such other evidence as survival rate and performance tests. They should be repeated on another species in which coronary occlusion is a practical procedure, and yet in whom the many factors such as age, breed, et cetera, are controlled, and in whom the ECG is well standardized. If similar results were obtained, it might then be worth extending the thoughts expressed here to the treatment of acute coronary occlusion in the human.

Summary of Experimental Work.

1) The choice of animals was considered.

2) The electrocardiographic apparatus was described, a suitable anesthetic found, and a standard posture adopted. The normal ranges in the albino rat were established with the influence of sex, weight, and strain being considered. The variations in the normal pattern which occurred on a subsequent day and after a month in the same animal received attention. Chest leads were briefly discussed.

3) The means of administering potassium and sodium, both acutely and chronically, within the range of therapeutic possibility were established, the effect on the serum concentrations and on the normal heart as measured by the ECG being noted.

4) Experiments to find the effect of variations in the concentrations of these ions on a cardiographic pattern developing in the adrenalectomized animal, had to be discontinued in its initial phases.

5) An experiment to find the abnormal cardiographic pattern developing after the administration of DCA and saline for 12 days, with a view to finding later the effect of administered salts on this pattern, revealed abnormalities in only a few tracings despite the fact that the potassium concentration was diminished and the heart weight and blood pressure increased in the groups receiving DCA-saline. Because ether anesthetic was used, little confidence could be placed in the electrocardiographic findings.

6) In another experiment, DCA was administered in large doses, as well as potassium chloride alone and in conjunction with DCA, all over a 50 day period. The serum potassium concentration was somewhat diminished in each group receiving DCA whereas the heart weights and blood pressures in these groups were increased. However, only 2 abnormal tracings were

seen. Further work was projected but not undertaken.

7) A method of inducing a myocardial injury is described which involves thoracotomy and an epicardial burn.

8) A preliminary experiment was performed to establish the cardiographic pattern of this acute injury. Potassium concentrations were determined and the adrenals weighed, at the time when the ECG patterns were marked, but no change was found. The effect of thoracotomy per se was considered. The cardiographic and gross anatomical findings were classified.

9) An experiment to show the effect of potassium administration and of DCA administration, for its potassium lowering effect, on this pattern of acute myocardial injury was undertaken. Potassium administration increased the number of animals showing marked electrocardiographic abnormalities in comparison to the injured control group and DCA diminished the number showing cardiographic changes. All three groups were roughly comparable pathologically in the gross. The theoretical implications of this result were considered.

10) A further experiment on the favourable effect of DCA was undertaken in a large number of animals, not only to confirm the effect suggested in the previous experiment, but also to judge the effect of DCA on the recovery of the electrocardiographic pattern. DCA appeared to exert a favourable effect in this type of myocardial injury in that fewer animals developed the pattern of severe injury and those that did, recovered more rapidly in comparison to the untreated injury group. The groups were fairly comparable as to the degree of injury in the gross

11) Certain cautions in the interpretation of these experiments were sounded

and yet a hypothesis that would explain these results was suggested. Plans for further work were projected.

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