THE ROLE AND POSSIBLE SIGNIFICANCE OF POTASSIUM, CALCIUM AND MAGNESIUM IN THE CARDIAC ACTIONS OF DIGITALIS GLYCOSIDES.

by

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CONTENTS

INTRODUCTION

Despite extensive laboratory and clinical investigations, the exact role of electrolyte changes in the cardiac action of digitalis is still obscure. While it is well established that potassium and calcium are essential ions for the maintenance of normal heart function, the relationship and possible significance of these ions in the pharmacological and toxicological actions of digitalis have been rather controversial. The primary objective of this investigation was to elucidate, if possible, these potassium-calcium-digitalis interrelationships. In connection with these studies some observations were also made concerning the possible significance of the magnesium, the lactate and the sodium ions as well as dextrose, in regard to digitalis action.

The main cardiac action of the digitalis glycosides is to improve myocardial contractility, but there is now no doubt that changes in heart activity can lead to associated or secondary changes in coronary blood flow. Indeed, recent studies from this laboratory, indicate "that, apart from the mechanical influences associated with changes in the heart contractions, the rate of coronary flow can be strikingly influenced by changes in potassium/calcium ratios in the perfusing fluids", in isolated rabbit heart experiments (Melville and Mazurkiewicz, 1956).

In assessing the role of electrolytes in the cardiac actions of digitalis, it is necessary therefore to consider the concomitant changes in coronary flow, and so far as we are aware, there are no previous systematic studies in the literature along these lines. Moreover, some recent observations of Wiggers (1954) would suggest that when the force of ventricular contractions is increased (such as can be readily induced by the digitalis glycosides) the coronary flow rate may actually increase.

It is conceivable therefore that in the weakened and "failing heart", the observed favorable therapeutic action of digitalis might be due, both to the improved heart contractions, as well as to the resultant augmentation of coronary flow. This has also been a debatable point, and it was hoped that studies along the lines indicated above might throw some light upon this question.

There are numerous investigations in the literature concerning the effects of digitalis on cardiac potassium equilibrium. In many of these however, the changes occurring in the heart have only been inferred from the observed changes in plasma potassium, when electrical or other changes in the heart function ensued. Owing to the widespread presence of potassium in body tissues, it is difficult to evaluate such findings, and in order to overcome these difficulties, the isolated perfused heart was selected for this study. Moreover, in this preparation, it is also possible to follow sequential changes in potassium balance and to correlate these with the characteristic mechanical and electrical alterations in the heart, as induced by digitalis under various conditions of perfusion.

Finally, both potassium and magnesium have been reported (Historical Review) to counteract the arrhythmias resulting from excessive digitalis therapy; and, calcium excess in the body has long been reputed to enhance digitalis toxicity. However, there is still no satisfactory antidote for digitalis, and the problem of the relationship of ionic changes to the processes of recovery in the over-digitalized heart also warrants investigation. In the course of this work therefore, some preliminary observations were also made in an attempt to asses the role of various electrolytes--potassium, calcium, magnesium and lactate— in the recovery processes or resuscitation of the digitalis-poisoned heart.

HISTORICAL REVIEW

The general physiological role of potassium has been reviewed by Danowski and Elkinton (1951), and the literature concerning the effects of potassium and calcium on heart function has been summarized more recently by Mazurkiewicz (1955) and Korol (1956). The present review will therefore be confined to studies dealing primarily with the interrelationships of ionic changes and the cardiac actions of digitalis.

A. Studies on Potassium-Digitalis Interrelationships.

The complex pharmacological actions of digitalis in relation to potassium alterations have been reviewed by Cushny (1925), Lendle (1935) and more recently by Lown and Levine (1954).

Effects on Isolated Perfused Hearts. Clark (1912) first observed that alterations of potassium concentrations in the perfusing fluid did not markedly influence the effects of low concentrations (0.8 to 4 γ/ml.) of digitoxin on the isolated perfused frog heart when the heart is perfused with recirculated perfusate. In addition, neither decreased sodium chloride (to 0.1 per cent) nor addition of low concentrations (0.04 to 0.02 per cent) of lactic acid appeared to affect the response to digitoxin. It was therefore concluded that the action of digitoxin is mainly dependent on the presence of the calcium ions in the perfusing fluid.

Hagen (1939) studied the influence of digilanid-C in isolated perfused rabbits' hearts in which the perfusing Ringer-Locke solution was also continuously recirculated, according to the technique described by Locke and Rosenheim (1907). After ten minutes of "stabilization", and again, after one hour of perfusion with added digilanid, samples were taken from the left ventricle for potassium and water analyses. With "low" doses of the drug, there was a slight, but significant increase of potassium (+6.87 per cent) concentration in the ventricle, but with "high" doses there was a definite loss (-14.72 per cent). These alterations were not accompanied

by any change in the water content of the heart.

Friedman and Bine (1947) utilizing the perfused embryonic duck heart, observed that omission of potassium from the perfusing fluid enhanced the effects of lanatoside-C leading to arrhythmias and early arrest. On the other hand, excess potassium depressed the heart beat and inhibited the actions of the glycoside. These authors also reported that only toxic concentrations led to loss of potassium, and suggested that excess potassium might inhibit digitalis action by: "(1) itself depressing the irritability of the heart, and (2) by serving as a source of potassium to a heart apparently losing potassium after exposure to toxic amounts of digitalis".

When the effects of a low concentration (0.4 γ/ml.) of ouabain were studied on isolated rabbits' hearts by Baker (1947), it was observed that either increased calcium or decreased potassium hastened cardiac depression, while either decreased calcium or increased potassium reduced the effects of the drug. The author postulated that the effects of changes in potassium were greater than those of corresponding changes in calcium. The validity of this conclusion is however doubtful, since the heart was kept for rather prolonged "stabilization periods"; and the low ouabain concentrations employed by this worker would certainly lead to marked variability in the end-point employed (50 per cent decrease in contraction response).

While studying the possible role of acetylcholine in the mechanism of digitalis action, Holland, Greig and Dunn (1954), using guinea pig hearts, observed that low concentrations of lanatoside-C retarded potassium loss from the heart, while high concentrations caused a significant loss (-14.6 per cent from auricle and 9 per cent from ventricle). By lowering the concentrations of the potassium in the perfusing medium to 2.7 mM, the loss of potassium following the glycoside was augmented (27 per cent from the auricle and 20 per cent from the ventricle). Under these conditions

acetylcholine, or a sodium-deficient medium (51 mM) or eserine, could partially or completely reverse this potassium loss. The authors concluded that the T-wave depression produced by digitalis may be due to the inhibition of cholinesterase activity in the presence of the glycosides, but that the R-ST segment changes were due to the digitalis effect on some other metabolic process. The basis for these conclusions is however not clear. Moreover, Kahn and Acheson (1955) have tested the effects of various cholinesterase inhibitors with cardiac glycosides upon the active cation transport across the erythrocyte membrane, and concluded that "cholinesterase activity was not involved".

Effects on Heart-Lung Preparation. Contrary to the findings of Hagen (1939) on the isolated perfused rabbit heart, Wood and Moe (1938, 1940 and 1942) observed that both "therapeutic" and "toxic" doses of lanatosides (A, B or C) led to decreased myocardial potassium without any significant effect on water content. These authors could also not correlate the onset of irregularities with the degree of potassium loss from the heart. They suggest that the "therapeutic" and "toxic" effects of digitalis are independent of potassium myocardial changes. There are no other studies utilizing the heart lung preparation, and these findings require confirmation.

Effects on the Heart in Situ. Calhoun and Harrison (1931), following the subcutaneous injections of whole leaf digitalis preparations in dogs, observed that "low" doses induced a slight and insignificant decrease of potassium in the ventricles. These authors also concluded that skeletal muscle potassium is not influenced by "toxic" doses of digitalis (two experiments).

From rather scant observations on dogs under pentobarbital anesthesia, Camp (1939) inferred that digitalis might produce an early sensitization of the heart to injected potassium. Since no analyses of either the blood

or the heart for potassium were made, the data presented by this worker hardly justifies the inference.

Adequately-controlled experiments by Boyer and Poindexter (1940) indicate however that divided or single "therapeutic" doses of 'digifolin', that is, doses that produce characteristic electrocardiographic changes without any evidence of intoxication, induced a significant increase in the potassium content of the cat's heart muscle. The muscle potassium increased from 61.5 mEq./kg. in the controls to 69.4 mEq./kg. in the digitalized cats.

Following toxic administrations of the tincture of digitalis to pentobarbitalized dogs, Sherrod (1947) observed that the dog's auricles exhibit a decrease in both potassium and calcium contents and some gain of sodium, but this latter does not appear to compensate for the loss of potassium and calcium. It was also observed that there was a gain in sodium in the ventricular muscle in the absence of any other electrolyte change. None of the tissues from the digitalized hearts showed any significant change in the water content. These findings would rather suggest that movement of electrolytes might be an important factor in digitalis action.

Utilizing the Kollf-type of artificial kidney, Lown, Weller, Wyatt, Hoigne and Merrill (1952) showed that acetylstrophanthidin exhibited an increased toxic effect, when the animals were depleted of potassium. Conversely, increasing the plasma potassium raised the toxic dose of the glycosides. However, the toxic end points of digitalization as employed by these workers, were ventricular premature beats and tachycardia, but as is well known, these effects might well be due to the potassium alterations per se, rather than to any direct glycosidal action.

Zeeman, Hirsh and Bellet (1954) observed no significant difference between the average lethal dose of lanatoside-C in untreated control dogs and animals treated with desoxycorticosterone acetate. In the latter group there was presumably a total body depletion of potassium, although closer

scrutiny of the authors' data do not reveal any significant difference in serum potassium in the two groups of animals.

Unterman, De Graff and Kupperman (1955) showed that in adrenalectomized rats with characteristic hyperpotassemia, there was an increased sensitivity to ouabain, but desoxycorticorterone acetate-treated rats showed no such change in sensitivity. It is rather surprising that in the latter type of experiment, ouabain did not induce a greater degree of potassium depletion, since Aikawa and Rhoades (1955) have shown that digitoxin reduces plasma potassium, and leads to a general decrease in potassium concentrations in body tissues.

Using K^{42} as tracer, Conn (1956) investigated the potassium movement in the hearts of dogs. From calculations of the kinetics involved in the potassium distribution in the left ventricle, it was concluded that digitoxin reduced interstitial-intracellular potassium transfer rates and also diminished cellular and interstitial potassium. These data do not explain whether the cellular potassium loss is due to an inhibition of influx or an increased outflow from the cell, or a combination of both. Further application of this technique to the digitalis problem might be well worth while.

The coronary arterio-venous differences of sodium and potassium in anesthetized dogs have been employed to measure myocardial ion exchange, by Regan, Talmers and Hellems (1956). These findings indicate that acetyl-strophanthidin leads to an abrupt increase in potassium and a decrease of sodium in the coronary sinus blood. Both cations resume approximate control values within 30 minutes, and there was no substantial transfer of water. This led to the hypothesis that the effect of digitalis on the myocardium might be due to both sodium and potassium movements. However, since all the calculations of these authors are based on the assumption that digitalis does not effect coronary flow, the validity of their conclusions must be questioned.

Clinical Evidence. Sampson and Anderson (1932), Thomson (1939) and Stewart and Smith (1941) have all reported favorable effects following oral administration of potassium salts to man, in various types of cardiac arrhythmias. In extension of these findings, Sampson, Alberton and Kondo (1943) also reported good effects with potassium therapy in 32 patients with ectopic beats due to administrations of lanatosides (A, B and C). All patients were given large enough doses of the glycosides to produce constant nausea, and marked slowing of the ventricular rate—one third developed ectopic beats. These latter could all be abolished with 5 to 10 gm. of potassium salts given orally. However, curiously enough, this action developed without any significant change in the blood serum potassium. Levine, Merrill and Somerville (1951) more recently reviewed the effects of potassium excess in man, and it would appear that rather similar types of cardiac irregularities can be induced by either hypopotassemia or hyperpotassemia.

Enselberg, Simmons and Mintz (1950), and Lown, Saltzberg, Enselberg and Weston (1951) have described various aspects of potassium and digitalis action on man. The former authors administered potassium salts (2 - 10 gm.) on 40 different occasions to 31 patients with various arrhythmias, mostly associated with digitalis. In general, the arrhythmias were corrected, but the conduction disturbances were aggravated. Similarly, the latter group of authors reported on ten patients with congestive heart failure, who were in negative potassium balance following either (a) administration of mercurial diuretics combined with ammonium chloride, or (b) desoxycorticosterone, or (c) intravenous infusions of glucose and insulin. The amounts of the digitalis necessary to induce toxic characteristic electrocardiographic changes were determined, before and after potassium depletion. Toxic effects appeared following smaller doses of digitalis in patients who were presumably in negative potassium balance, despite the fact that serum potassium levels

were not significantly affected. The authors concluded that the increased myocardial sensitivity to digitalis was probably a result of alterations in intracellular potassium distribution.

In 1952 Clarke and Mosher reported some tissue analyses of autopsy material obtained from seven normal hearts, eleven hearts from patients in congestive failure, and five hearts from patients who had been previously digitalized. The four chambers and the septal tissue were analysed separately for sodium, potassium, chloride and water. The results indicated that the total water content of normal, diseased or digitalized hearts were the same. However, in congestive heart failure, there was a sodium gain and a reciprocal potassium loss. Digitalis therapy restored these values as well as the sodium-potassium ratio to near normal levels.

Effects on Miscellaneous Isolated Tissues. Using isolated strips of turtle ventricle electrically stimulated, Wedd (1939) studied the effects of various concentrations of digoxin. It was concluded that low or "therapeutic" doses exert their effect without influencing potassium movement but loss of myocardial potassium occurred only with high or "toxic" concentrations of the drug.

Cattell and Goodell (1937), and Cattell (1938) postulated that observed sartorious muscle responses to ouabain were due to the release of potassium from the muscle, but Guttman and Cattell (1940) later showed clearly that there was no relationship between the actions of the glycoside and intercellular potassium concentrations.

From studies on the cats' papillary muscle strips, Garb and Venturi (1954) concluded that changes in potassium concentration did not influence the positive inotropic action of ouabain. However, the onset of ouabain arrhythmias could be delayed by the use of high potassium concentrations.

Red blood cells have been utilized by several workers (Schatzmann, 1953;

Schatzmann and Witt, 1954; Kahn and Acheson, 1955) in attempts to elucidate the effects of the cardiac glycosides and their aglycones on the active transport of sodium and potassium. There is general agreement that the glycosides are capable of completely inhibiting the active phase of cation transport. However, similar inhibition was not observed with other compounds capable of exerting ionotropic effects on the heart, e.g. adrenaline and veratrum alkaloids. Cation transport studies of Johnson (1956) on frog sartorious muscle showed that ouabain and strophanthidin inhibited the net outward transport of sodium and the net inward transport of potassium. The exact significance of these findings in relation to the in vivo effects of digitalis, is still uncertain.

In summary, it appears that studies involving different experimental arrangements, can lead to conflicting results regarding the effects of digitalis on ionic equilibrium in the heart. However, most authors agree that "toxic" doses of the digitalis glycosides lower the potassium concentration of cardiac muscle. Several observations cited indicate that "low" or "therapeutic" doses of digitalis do not effect or may actually increase the level of myocardial potassium, but this point requires further investigation. It has been reported that administration of potassium either lessens, has no effect, or augments the cardiac toxicity of digitalis. However, in connection with this problem, the fact that potassium per se is a potent myocardial depressant is often ignored. Indeed, the role of the electrolyte itself under these conditions is difficult to asses, and in some instances, the potassium may be exerting its antagonism entirely through its direct myocardial depressant effect, rather than through its specific chemical role.

B. Studies on Calcium-Digitalis Interrelationships.

Synergistic Action. Many experimental studies in the literature suggest that calcium and digitalis may be additive or synergistic in their cardiac actions (Werschinin, 1910; Clark, 1913; Burridge, 1915; Geiger and Jarisch, 1922; Schoen, 1923). The literature on this question as reviewed by Billigheimer in 1929, led to a similar conclusion.

Loewi (1918) observed that the less calcium contained in the perfusing fluid of the frog heart, the higher the dose of strophanthin necessary to lead to arrest of the heart. It was therefore concluded that strophanthin renders the myocardium more sensitive to the presence of calcium.

Studies on the hypercalcemia induced in intact dogs by rapid intravenous infusion of calcium chloride or by the administration of parathyroid hormone, showed that when these are followed by infusion of ouabain, there is a marked synergism between calcium and ouabain on the heart (Gold and Edwards, 1927). Finally, Baker and Baker (1955) have also studied the electrical responses of the ouabainized isolated frog heart to calcium and concluded that "ouabain favors the development of increased ventricular automatism in the presence of large concentrations of calcium".

"One-Way" Synergism. Fisher (1928b) however, noted that calcium in the concentrations used in Ringer, or in higher concentrations, had no effect whatever if given before or during digitalis administration in the Straub-perfused frog heart. On the other hand, a marked calcium effect became apparent when digitalis was employed before increasing the calcium concentration. It was therefore concluded that digitalis sensitizes the heart to calcium, but that this is only a "one-way synergism", because conversely, calcium does not sensitize the heart to digitalis. Thus, the author concluded that the two drugs exert their actions on different parts of the heart.

Bower and Mengle (1936) also observed in dogs that after previous

administration of 'digalin', 30 to 40 per cent only of the usual lethal dose of calcium was required to induce cardiac arrest. However, if 30 per cent of the lethal dose of calcium was given first, subsequent 'digalin' administration led to no unusual toxicity.

Golden and Brams (1938) and La Barre and Van Heerswynghels (1939) have demonstrated that when calcium is injected at varying time intervals following digitalis, toxic effects developed most readily, when an interval of 30 minutes elapsed between the digitalis and calcium injections. These observations might lend some confirmation to the "one-way" synergism theory.

No Synergistic Responses. Konschegg (1913) has reported that during perfusion of the frog heart with calcium-free Ringer and ouabain solution, the time required for cardiac arrest was not prolonged, as compared with that observed with ouabain in normal Ringer solution. It was also noted that perfusion with calcium-free Ringer can lead to cardiac arrest, but this latter can be reversed with strophanthin. This was interpreted to mean that the action of strophanthin is independent of calcium. However, Handovsky (1923) could not observe reversal of the calcium-free arrest by strophanthin, but did observe some temporary recovery in the potassium-free arrested heart. This author also reported that the strophanthin effect could be much improved by the addition of glucose (one per cent) to the normal Ringer.

Since changes arising from the use of calcium-free Ringer solution could be counteracted by maintaining proper osmotic pressure by addition of cane sugar to the solution, Pietrkowski (1918) concluded that the glycosides act directly on the heart muscles and the action did not depend on calcium.

Nyiri and DuBois (1930) have also reported some differences in the actions of calcium and digitalis on the frog heart. Thus, the calcium effect was immediate, whereas digitalis required a definite latent period. Also, calcium toxicity could be readily reversed by perfusion with normal

Ringer, or sodium chloride, or potassium-sodium chloride solution, but the digitalis-arrested heart again recovered much more slowly. In a second group of experiments, the combined effects of the various ions and digitalis were studied. It was noted that digitalization of the heart could occur within the usual time irrespective of calcium concentrations. However, one cannot observe the effect, if the heart muscle has lost its ability to contract, but, if at any given time, one re-establishes the inotropic properties in the course of the digitalization, the corresponding effect for the time of perfusion can be observed. It was concluded by these authors that full digitalis action can occur in the absence of calcium, and that calcium and digitalis are not essentially related in their pharmacological actions in the isolated frog heart.

Calcium-digitalis interrelationships were later re-investigated by Nahum and Hoff (1937) on unanesthetized rabbits. Their results also led to the conclusion that there was no evidence of any additive effects of the two agents. However, these findings have been severely criticised by Gold and Kwit (1937) on the grounds (a) that the rabbit is an unsuitable animal for digitalis studies, showing a rapid excretion and a high tolerance, (b) that the concentrations employed were too small, and finally, (c) that Nahum and Hoff had ignored a "strong synergistic effect", which Gold and Kwit considered to be shown by the results.

Using morphinized dogs, Smith Winkler and Hoff (1939a) could again not demonstrate any synergistic or additive effects of calcium and digitalis. The electrocardiographic changes were correlated with the calcium levels in the serum, and the results indicated that the lethal effects of calcium and digitalis were neither synergistic nor even completely additive.

Finally, Blumenfeld and Loewi (1945) have reinvestigated the calcium-digitalis action in the isolated frog heart. Their results rather indicate that the "systolic" or "diastolic" standstill of the heart, as produced by

various concentrations of digitalis, cannot be reproduced with calcium ions. In addition, the cardiac arrest following digitalis cannot be prevented by decreasing the calcium concentration of the perfusing solution.

Clinical Studies. Edens and Huber (1916) have examined the blood calcium in patients showing digitalis hypersensitivity. They found rather high values in many of these cases, and therefore suggested that this was a contributing factor to the increased tendency to bigeminal rhythms following digitalis therapy in some cases. Bower and Mengle (1936) have recorded two deaths following intramuscular administration of Digalin and intravenous injections of calcium gluconate in man. However, no conclusive evidence is available from clinical reports on this subject.

In summary, therefore, either complete synergism, "one-way" synergism or total independence of actions, have been advocated for calcium-digitalis interrelationships by various groups of workers. It is therefore evident that this problem requires further study.

C. Studies on Effects of Magnesium on Heart Action and on Magnesium-Digitalis Interrelationships.

Effects of Magnesium on the Heart. A general review of the cardiovascular and other actions of the magnesium ion has been published by Engbach (1952). As early as 1907, Matthews and Jackson reported that when magnesium sulphate was administered to the exposed hearts of frogs, turtles and birds (roosters and geese), there was a very marked depression leading to standstill. This depression could be temporarily counteracted by artificial mechanical stimulation, or exposure to solutions of barium chloride or calcium chloride, although adrenaline could not reverse this depression.

The electrocardiographic changes following intravenous injections of magnesium sulphate into unanesthetized dogs have been described by Miller

and Van Dellen (1938) and Smith, Winkler and Hoff (1939b). The data show that magnesium caused an initial tachycardia, which gradually gave way to a bradycardia. The electrocardiograms indicated that intracardiac conduction is depressed, giving rise to increased P-R intervals, occasional S-A and A-V block and widening of the QRS complex. There was however, a poor correlation between magnesium serum levels and the observed electrocardiographic changes. In addition, calcium failed to alter the magnesium effects on the heart.

Van Dellen and Miller (1939), noted that following atropine (0.2 mg./kg.) or vagotomy, the overall electrocardiographic findings induced by magnesium were not effected. Thus, the effects of magnesium were not due to any central vagal action, but to a direct effect on the heart.

The coronary action of magnesium was investigated by Elek and Katz (1942). Using a 25 per cent solution of magnesium sulphate they observed augmentation of coronary flow ranging from 27 to 89 per cent following injections of 0.15 to 2 ml., i.e. 37.5 to 500 mg. into isolated perfused fibrillating dogs' hearts.

An extensive clinical investigation by Bernstein and Simkins (1939) indicated that injections of a ten per cent solution of magnesium sulphate in either patients without cardiovascular diseases (34 cases) or, patients with various types of cardiac diseases (66 cases), produced no uniformity in the responses. These authors concluded that such intravenous injections of magnesium exert no deleterious effects on the human heart.

Boyd and Scherf (1943) investigated the effects of intravenous injections of magnesium sulphate in ten cases of paroxysmal tachycardia, and in one case of auricular flutter. It was reported that injections of 10 or 20 ml. of a ten per cent solution lessened three out of eight attacks, whereas a 20 ml. dose of a 20 per cent solution, stopped completely all attacks. Transitory appearance of disturbed conduction and ventricular extrasystoles were also

reported by these workers.

Magnesium-Digitalis Interrelationships. In 1935, Zwillinger suggested intravenous magnesium sulphate for the treatment of premature beats and paroxysmal tachycardia. He reported in one patient, that paroxysms of ventricular tachycardia and fibrillation due to excessive injections of strophanthin were abolished by intracardiac injections of 10 ml. of a 15 per cent solution of magnesium sulphate, but the patient succumbed seven hours later. The author also reported that rabbits and frogs, injected with the usual "lethal doses" of digitalis could be saved by treatment with repeated small doses of magnesium. No details of these experiments were however given by the author.

Rothberger and Zwillinger (1936) also noted that magnesium could counteract the barium chloride or strophanthin tachycardia induced in morphine-chloralosed dogs.

Using the heart-lung preparation, Stanbury and Farah (1950) investigated the interaction of magnesium and digoxin action. It was observed that magnesium slowed the rate of the S-A node by a direct action independent of nervous influences, but that a significant increase of the level of the magnesium ion in the blood in 14 heart-lung preparations, had little effect on the "toxic" or "lethal" dose of digoxin. In two experiments magnesium chloride was added quickly to the preparation already showing toxic digoxin effects, and in these cases, only a temporary restoration of normal rhythm ensued.

Greiner and Garb (1950) have reported that magnesium (2 - 14 mM. concentration) produced a slight and gradual increase in the threshold of electrical excitability of the cat papillary muscle preparation. Similarly, Garb (1951) observed that neither "magnesium-free Locke solution" nor "ten times the physiological concentrations of magnesium" in Locke solution, exerted any

effect upon either the electrogram or myogram in this preparation.

Miller and Van Dellen (1941) concluded that in two digitalized dogs, magnesium sulphate exerted an additive effect in altering the T-wave producing further A-V delay. They noted that magnesium led to an initial brief tachycardia, which they attributed to a blocking of the vagal effect of digitalis. The results also suggested that magnesium does not overcome the effect of digitalis poisoning, but rather increased the degree of block and the occurrence of the ectopic impulses. This view is rather opposed to those of all earlier published studies in the literature, and in view of the small number of animals studied, requires further investigation.

Enselberg, Simmons and Mintz (1950) have confirmed that rapid intravenous injections (20 ml.) of 20 per cent magnesium sulphate abolished or significantly reduced various types of digitalis arrhythmias, but concluded that the antidotal value of magnesium in these arrhythmias, was limited by its brief action and the occasional undesirable effects (flushing, feeling of suffocation, paresthesias), which it produces.

Szekely (1946), Szekely and Wynne (1951), reported further clinical and experimental studies in which they confirmed that the magnesium action was exerted directly on the heart, and that the intravenous administration of magnesium sulphate might be a useful procedure in overcoming extrasystoles due to overdigitalization, but the effects may be only temporary.

In summary, there is no doubt that high concentrations of magnesium can be shown to possess a marked myocardial depressant action. The antagonism between digitalis and magnesium is however, limited and the observed short duration of this antagonism may well be due to this direct myocardial depressant effect of magnesium, rather than to any specific ionic magnesium-digitalis interrelationship.

D. Observations on Changes in Coronary Flow as Related to Ionic Effects and to Digitalis-Ionic Interrelationships.

On the basis of the electrocardiographic changes observed following the application of potassium chloride solution (20 per cent) to the dog's heart *in situ*, Wiggers (1929) suggested that these alterations resembled those induced by coronary occlusion. Gruber and Roberts (1926) observed that when isolated cats' and rabbits' hearts were perfused with potassium-free Ringer solution, the coronary flow rate was decreased. They also noted that calcium-free Ringer increased coronary flow rate.

A more detailed study of this problem was made by Katz, Rodbard, Friend and Rottersman (1938) who, using the isolated fibrillating dog heart, noted that when the potassium concentration in the perfusing defibrinated blood was increased slightly (4 per cent above normal), there was a slight increase in coronary flow. However, increasing the concentrations by 50 and 150 per cent above normal potassium levels, produced a decrease in the coronary flow. With higher potassium concentrations, these workers could develop complete coronary closure. Similarly, increasing calcium concentrations up to 24 fold, and sodium up to approximately three fold, both caused coronary dilatation. In view of the high concentrations employed, the latter two ions may have produced their effects only through their hypertonic actions, and it is difficult to draw conclusions from these data.

An attempt to evaluate the effects of potassium-calcium ratios on coronary calibre was made by Cohen(1936), who recorded the effects of various ions on isolated coronary rings of cattle. The rings were placed in normal saline solution and various concentrations of calcium and/or potassium were added to the bath. The results indicated that added calcium when not accompanied by potassium, increased the "tone" of the coronary arteries, and on decreasing the calcium concentration, there was a reversal

of this effect. Added potassium alone, in low concentrations, had a variable effect but high concentrations always depressed the coronary "tone". However, with calcium present in the solution, low potassium only depressed the "tone" of the coronaries. Similarly, studies on magnesium indicated that this ion depressed coronary "tone".

Finally, Melville and Mazurkiewicz (1955, 1956), recording coronary flow on the isolated rabbit heart, indicated that excessive doses of potassium could induce coronary dilatation or a "paradoxical" coronary constriction, while calcium excess caused dilatation. Curiously enough, decreasing or completely omitting calcium or potassium from the perfusing fluid, both increased coronary flow. These workers have concluded that changes in the potassium-calcium ratios can therefore influence the coronary flow.

Digitalis and Digitalis-Ionic Effects. The effects of digitalis upon the coronary circulation have been extensively investigated. Because of the difficulties involved in assessing the multiple factors affecting coronary changes, a wide variety of methods have been employed. Since no single technique takes into consideration all the factors influencing coronary flow, the results on the whole have been contradictory. Some of the varied techniques and the results obtained both in connection with the actions of digitalis and digitalis-ionic interrelationships in respect to the coronary circulation are summarized hereunder.

Effects on Isolated Coronary Vessels. Eppinger and Hess (1909), using isolated coronary arteries, reported that strophanthus, Digalin and digitoxin produced constriction. However, the opposite effect was observed by Cow (1911), who showed that coronary arterial strips were dilated with digitalis.

Rabe (1912), using isolated strips of coronaries, concluded that the coronary arterioles were more sensitive to digitalis than the vessels of the limbs. Thus, it was found that coronary constriction occurred with strophanthin (1:20,000,000) while a dilution of 1:1,000 produced little or slight effect

on the peripheral vessels. Similarly, Digitalin constricted the coronary arteries in 1:5,000,000 concentrations, but a concentration of 1:10,000 was necessary to effect peripheral constriction. A variety of responses were obtained by Voegtlin and Macht (1913) using coronary arteries of the ox and pig in oxygenated Locke solution. They concluded that: (1) coronary artery constriction was caused by Digitalin, digitoxin (Merck), Digitalin (Kiliani) and tincture of digitalis; (2) coronary dilatation by Digalin, and infusion of digitalis, and (3) no change in coronary artery occurred with strophanthin (Merck) and strophanthin (Boehringer).

These earlier papers indicate already the controversial nature of the responses of the coronaries to digitalis. This simple method also does not give information concerning the changes in smaller coronary vessels, which are the main component of the coronary bed. They also throw no light on changes in the rate of coronary flow in the beating heart.

Effect on Isolated Perfused Hearts. Loeb (1904), using the isolated cat's heart perfused with physiological saline, obtained variable results. He concluded from five experiments, that strophanthin (0.15 to 0.2 mg./100 ml. added to the perfusing fluid) produced no change in flow. Digitoxin (0.5 - 0.6 mg./100 ml.) led to rapid constriction.

With the isolated dog heart, Lindner and Katz (1941) reported that injections of massive doses (25 mg.) of k-strophanthin caused variable constriction and dilatation. In other experiments with both Digifolin (Ciba) and ouabain, variable and unpredictable results were also obtained.

Effects in Heart-Lung Preparation. Bobo (1927-28) has shown that 0.0025 mg. of g-strophanthin per 100 ml. of blood, as well as injections of the tincture or infusion of digitalis (in equivalent doses) reduced heart size, but the arterial pressure, cardiac output and work of the heart remained constant, while the coronary sinus flow increased, following a delayed onset.

He concluded that since blood pressure remained constant, the increased coronary flow was due to a decrease in the coronary resistance and that digitalis caused direct coronary dilatation.

Fisher, Guggenheimer and Müller (1928) also employed a similar technique using strophanthin in doses of 0.05 to 0.25 mg. per 500 ml. blood, and found no change with the lower doses, but with higher "lethal doses" decreased coronary flow ensued.

Ruhl and Wiehler (1934) similarly studied the effects of strophanthin (doses not given) and reported variable effects on coronary sinus outflow, with a tendency towards constriction. Ginsberg, Stoland and Siler (1938) carried out an extensive study on 53 heart-lung preparations, again using the Morawitz cannula to record the coronary sinus outflow. A variety of digitalis preparations were investigated by these authors, who concluded that digitalis acted directly on the coronaries and not on the extrinsic nervous mechanism controlling coronary flow, since no difference in response was observed after atropine. In general, their findings indicated that under all conditions employed, the most consistant effect was a decrease in coronary flow rate during an initial period of about ten minutes, followed by an increase which persisted throughout the remainder of the experiment.

Effects on the Heart in Situ. Bond (1910) reported no change in the rate of outflow from a sectioned coronary vessel in anesthetized dogs, after intravenous injections of strophanthin. On the other hand, Meyer (1912) reported an increase in coronary flow in curarized cats, in which a cannula was inserted into a superficial coronary vein and the outflow measured. From studies on urethanized cats, Sakai and Saneyoshi (1915) concluded that "therapeutic" doses had no effect, but "high" doses led to cardiac arrest within 15 minutes and produced initial constriction followed by marked dilatation just prior to death.

Numerous reports in the literature (Fenn and Gilbert, 1932; Gilbert and Fenn, 1932, and Ginsberg, Stoland and Siler, 1938) indicate variable coronary responses in anesthetized dogs following administration of various digitalis preparations.

Hildebrandt and Osterwald (1938), using the Rein thermostromuhr on anesthetized dogs, observed no change in right coronary flow, after injections of "therapeutic" doses (0.15 to 0.5 mg.) of strophanthin, but higher doses produced a decrease followed by an increase in coronary blood flow.

In attempting to avoid the usual experimental complications, Essex, Herrick, Baldes and Mann (1938), Essex, Herrick and Vischer (1938) and Dearing, Essex, Herrick and Barns (1943) have used unanesthetized trained dogs, with a thermostromuhr previously attached to the circumflex branch of the left coronary artery. Different digitalis preparations studied led to the observation that "therapetuic" doses had no detectable effect on the coronary flow. However, "toxic" doses decreased coronary flow, following a delay of four to six hours after intravenous administration. This decrease persisted for several days. The changes in coronary flow could not be correlated with the changes in heart rate or blood pressure.

In a preliminary report on morphinized anesthetized dogs, Page, Wendel, Sheldon and Foltz (1950) determined coronary flow by the nitrous oxide technique, and concluded that small doses of ouabain had no effect on coronary vessels when comparisons are made before and 30 minutes after the drug administration. Recently Regan, Talmers and Hellems (1956) also found no change in myocardial oxygen A-V difference after acetyl-strophanthidin was given to dogs.

Clinical Observations. There are numerous clinical studies in the literature concerning the possible influence of digitalis on coronary flow. One of the earliest of these was made by Forthergill (1871). In his review he

suggested that the improved cardiac and general circulatory actions following digitalis therapy lead to secondary improvement in the coronary circulation. Moreover, from some observations on two cases of angina pectoris, it was concluded that digitalis might have a beneficial effect on that condition.

Eggleston (1920) and Cushny (1925), in extensive reviews of digitalis action, concluded that in small "therapeutic" quantities, digitalis had no effect on coronary circulation in man. On the other hand, Fenn and Gilbert (1932) reported that some patients on digitalis therapy developed angina pectoris, and when the drug was withdrawn, the attacks ceased. They then performed an extensive series of experiments on dogs, and concluded that digitalis can produce anginal pain by constricting the coronaries, but this does not occur in all cases and was unpredictable.

Kountz and Smith (1938) reported large increases in coronary flow following digitalis in perfused, revived human hearts. Gold, Otto, Kwit and Satchwell (1938) investigated 120 ambulant cases of arteriosclerotic heart disease with cardiac pain. Large enough doses of digitalis leaf preparations were administered to lead to toxic symptoms in 28 per cent of their cases. The digitalis was given for periods from one to 68 weeks (average eleven weeks), and 15 per cent of the patients reported an increase in pain, while 30 per cent reported a decrease. Similar results were, however, obtained with a 'placebo'. These workers concluded that since these patients were unusually susceptible to cardiac ischemia, the results indicated that digitalis, even in large doses, rarely, if ever, produces discernible constriction of the coronaries in man.

In 1939, Gold again reviewed the literature on this question and reconfirmed his opinion that digitalis does not exert any direct constriction of the coronary circulation. On the contrary, Levy, Bruenn and Williams (1940) also administered digitalis leaves to a series of patients with coronary sclerosis and anginal attacks. After five days of treatment, the

patients were subjected to an "anoxemia test", consisting of inhalation of ten per cent oxygen and 90 per cent nitrogen. The authors found that the appearance of pain occurred more rapidly and concluded that digitalis increases the tendency to pain in certain patients, susceptible to anginal attacks.

Bing, Maraist, Dammann, Draper, Heimbecker, Dailey, Gerard and Calazel (1950) catheterized the coronary sinus in twelve patients and measured the coronary flow by means of the nitrous oxide method. Ten minutes after intra-arterial injections of 0.65 mg. k-strophanthin, negligible effects on the coronary flow were recorded. Thus the authors concluded that strophanthin exerts no effect on the coronaries.

In summary, the variable results described above might be due to the various methods employed for the measurement of changes in the coronary circulation after the administration of digitalis. The use of isolated coronary vessels appears to be rather unphysiological. Measuring flow from a severed superficial coronary vessel is not a reliable index to the flow of blood in the coronary system. The isolated heart preparation can give inaccurate results due to insufficiency of the aortic valves. Furthermore, the heart with empty chambers, beating outside the body is considered too abnormal by some workers to give any conclusive findings. The coronary blood flow in the heart-lung preparation, using the Morawitz cannula or the thermostromuhr may be altered by the effects of the preparatory operative procedures, by the anesthetic agent, or by the fact that the total coronary flow is not measured. Indeed, the clinical data is controversial and not convincing in either direction. Thus, though a large variety of techniques are available, there is no conclusive evidence concerning the digitalis effects on the coronary vessels. Furthermore, there are no previous systematic studies in the literature concerning the coronary flow changes associated with potassium, calcium and magnesium actions in respect to the

overall cardiac actions of digitalis.

E. "Resuscitation" Following Digitalis Toxicity.

The extensive earlier literature concerning the recovery or restitution of perfused frog's hearts following digitalis arrest, has been reviewed by Rothlin (1933), and Lendle (1935). The ease with which this action could be achieved varied greatly with the different preparations and experimental conditions employed (Straub, 1910; Clark, 1912; Issekutz, 1915; Rothlin, 1927; Lenz, 1926; Fisher, 1928a; Graf, 1929; Gander, 1932, and Beaune and Balaceanu, 1933). It appears that with sufficient time complete recovery could ensue, since a second period of poisoning followed exactly the same pattern as the first. The effects of the agents were equally reversible when Ringer solution or blood plasma were used, or when the glycoside was given to the intact frog, and the arrested heart subsequently excised and perfused (Kingisepp, 1935).

No relationship was noted between reversibility of action and the cumulative action in the different preparations (Walter, 1918; Gottlieb, 1918). The former worker reported that after strophanthin, recovery occurred in 2.5 to eight hours, whereas, after digitalis leaf preparation, the recovery time was more rapid (two to four hours). The possibility that the presence of the genin in the infusion might enhance reversibility was suggested by Giacomi (1926). Straub (1931) found that the binding of effective amounts of digitalis by heart muscle occurred within the initial 60 seconds of contact between the muscle and the drug. Thus, after 60 seconds of exposure to digitalis, and replacement of the perfusing fluid with normal Ringer, the process of poisoning continued, as if the drug had not been removed. Different glycosides showed different binding capacities, which seemed to be dependent on water solubility and stability of the particular molecule.

Frommel (1928a and b), postulated that the observed differences in

reversibility between the actions of whole leaf extracts and digitoxin, depended on the presence of the potassium in the former.

Another relationship between potassium and recovery was shown by Scarinci (1953). His experiments indicated that after the frog's heart (Straub perfusion) was stopped with ouabain (1:30,000) solution, washing with Ringer solution without potassium, restored the contractions of the heart. If the heart, beating on this potassium-free solution, is then re-perfused with normal Ringer, the organ again went into arrest. Thus, this alternate perfusion with potassium-free Ringer and normal Ringer can alternatively stop and start the frog heart under these conditions.

Attempts to antidote digitalis toxicity in experimental mammalian hearts have been rather disappointing. The literature on this question has been reviewed by Cohen (1952). Kyser, Ginsberg and Gilbert (1946) suggested from experimental observations on 32 digitalized dogs that atropine might aid in modifying digitalis myocardial lesions. However, the clinical reports are conflicting. In seven patients, Resnik (1924) converted digitalis induced transient auricular fibrillation in man to a regular sinus rhythm, with an injection of 2 mg. of atropine. Similarly, Gold and Otto (1926) stopped digitalis bigeminy in five cases with the subcutaneous injection of 4 mg. of atropine sulphate. However, neither vagotomy nor atropinization appear to lessen digitalis toxicity in experimental dogs (Robinson and Wilson, 1918).

Both quinidine and procaine amide have also been studied as possible antidotes in digitalis poisoning with variable results (Berry, 1951; Kayden, Brodie and Steele, 1951; Scaffer, 1951; Stearns and Callahan, 1951). Finally, as already pointed out, both potassium and magnesium have been employed to counteract digitalis arrhythmias and toxicity. (See Sections A and C.)

However, the mechanism of "resuscitation" is still obscure, and the

possible role of potassium or other chemical agents in this process is still rather uncertain.

METHODS

The isolated rabbit's heart perfused with oxygenated Locke solution by a modified Langendorff procedure was used in most experiments. A few confirmatory observations were also made on the isolated perfused cat's heart. Full details of the perfusing procedure have been described by Lu and Melville (1950) and more recently by Melville and Mazurkiewicz (1956). Korol (1956) has also given a detailed account of the procedure and the experimental arrangement. In brief, the method permits simultaneous kymographic recordings of coronary inflow and associated heart contractions (amplitude and rate). In each experiment, after the heart was attached to the apparatus, a preliminary "equilibration period" of 15 minutes of perfusion with normal Locke solution was always allowed to elapse before any observations were recorded. It must be emphasized that with this method of perfusion of the isolated heart, special care must be taken to make sure that the aortic valve is functioning efficiently after the preparation is set up. Consequently, during this preliminary equilibrating period the coronary flow was checked at frequent intervals, and unless a uniform and steady flow rate within"normal limits" was being maintained after the initial ten minutes, the heart was discarded. In order to facilitate changing from one type of perfusing fluid to another, two or three perfusion reservoirs were attached to the apparatus and kept in a large water-bath, maintained at 37° C. by a thermostat. The reservoirs were placed at a height of approximately 60 cm. above the heart.

The electrograms of the isolated heart were also recorded on a Sanborn Visocardiette, using the standard lead II position, and attaching the right arm (RA) lead to a fine silver wire tied around the aortic stump over the thread attaching the aorta to the perfusion cannula. The left leg (LL) lead

was connected to a similar sterling silver wire (0.01 inch diameter) electrode connected to the heart. A similar technique has been previously reported by Korol (1956). Since the primary interest in these experiments was in arrhythmias, the generic terms of QRS and ST-T changes were adhered to rather than detailed descriptions of the action potentials or the monophasic curves.

The cats used in the experiments were lightly anesthetized with ether before removing the heart; and the heparin (1 mg. per kg.) injected intracardially. The remainder of the procedure was identical to that employed in the rabbit experiments.

Samples of the fluid entering the heart and the fluid leaving the heart were taken at the times marked by the signal line as shown in the individual kymograph records. A one in 50 dilution with distilled water was made of the samples and the potassium determinations were carried out by standard flame photometric procedure.

Stock standard solutions were prepared for use on the flame photometer. These standards were prepared so that the final concentrations of sodium ion was identical to that in the perfusing solution. Attempts were made to use two standards with concentrations of potassium below, and two standards with concentrations of potassium above, the unknown solution.

Similarly, standard preparations of sodium were used for sodium determinations. Calcium determinations were done by the method of Clark and Collip (1925). The Beckman D.U.Spectophotometer with flame attachment was used for both the sodium and potassium determinations. All analyses were done in random order in duplicate, or triplicate, if the first two values varied more than three units per cent transmission. The concentrations of the ions in the experimental samples were obtained from curves of the standard solutions by plotting milliequivalents per liter on the abscissa, against observed per cent transmission on the ordinate.

The "net gain" or "net loss" of ion from the heart was calculated as the difference between the amount of potassium entering and that leaving the heart during a ten second period of time. This time was the average time usually required for collecting enough perfusate for analysis. Correction for variation in rates of coronary flow was made by multiplying the potassium concentration (mEq. per liter) of the sample by the associated coronary flow (ml. per ten seconds) as calculated from the records obtained at the time the sample was collected. Consequently, the amount of potassium entering or leaving the heart is expressed as microequivalents per ten seconds (γEq. per 10 sec.).

In presenting the results, the data from several experiments were grouped together and mean changes reported. In the graphs the average times for specific criteria (e.g. cardiac standstill, net potassium changes) are presented. Standard errors, correlations and probability determinations were carried out wherever practical, Snedecor (1955) was used as a standard reference for these determinations.

When modified Locke solutions were employed no attempt was made to maintain isotonicity. It has been reported by Butcher, Wakim, Essex, Pruitt, and Burchell (1952) that addition or subtraction from the standard perfusion fluid of 34 mEq./l. of sodium and chloride had little or no effect on the electrocardiograms recorded from the isolated rabbit, dog or turtle hearts. Since the osmotic changes produced by altering the concentration of potassium during the experiments reported here were maximally of the order of 11.2 mEq./l., failure to balance to isotonicity should not alter significantly cardiac function.

Ouabain U.S.P. (S.B. Penick and Co.) was dissolved in the Locke or modified Locke as indicated in the different experiments. The other chemicals used were: (1) Merck reagent grade magnesium chloride ($MgCl_2.6H_2O$). This

was dried overnight at 100° C. prior to use. The concentration of magnesium given has therefore been calculated for the formula $MgCl_2.4H_2O$. (2) Sodium lactate 1/6 Molar (Abbott*). And, (3) miscellaneous drug administrations, such as adrenaline((l-adrenaline bitartrate**) or noradrenaline (l-noradrenaline bitartrate monohydrate**), potassium chloride or calcium chloride solutions for injections, were freshly prepared, as needed, either in Locke solution or Locke as modified for the perfusion.

In the experiments in which sodium lactate was employed, the normal isotonicity was restored by omitting an equivalent calculated amount of sodium chloride. Similarly, when the sodium concentrations were reduced, sucrose was used to restore isotonicity.

* Courtesy of Abbott Laboratories, Montreal, Que.

** Courtesy of Sterling-Winthrop Research Institute, Rensselar, N. Y.

RESULTS

1. OBSERVATIONS ON THE EFFECTS OF VARYING CONCENTRATIONS OF OUABAIN ADDED TO NORMAL LOCKE'S SOLUTION UPON THE CORONARY FLOW, HEART ACTION AND ASSOCIATED ELECTROLYTE MOVEMENTS.

(a) Comparisons of the Effects of Different Concentrations of Ouabain.

Since the development of the effects of the glycosides on the heart requires time, the concentrations of the perfusing fluid employed and the rate of perfusing, are obviously important factors. Prior to investigating the influence of the various ionic changes, it was therefore important to make a comparison of the effects of different concentrations of ouabain, when perfused under the conditions of these experiments.

In Figure 1, are presented graphically the average times (minutes) required of perfusion to reach cardiac standstill (mechanical arrest), when the hearts of rabbits or cats were perfused with different concentrations of ouabain, added to normal Locke's solution. The standard error in each case is also shown. A total of 31 different hearts were used, comprising five (rabbits) with the highest concentration (30 γ / ml.); two groups of nine animals (seven rabbits and two cats in each group), with both the 15 γ/ml. and 7.5 γ/ml. doses; and three and five rabbits with 3.75 γ/ml. and 1.88 γ/ml. respectively. Although only four cats' hearts were included in this group, the results obtained with them did not appear to be strikingly different from those observed in corresponding rabbit experiments, and therefore the findings in both species are considered together. A greater number of cat experiments would however be necessary before a final conclusion regarding species differences could be drawn. Since this was only an incidental aspect of this work, it was not considered necessary to extend these observations further.

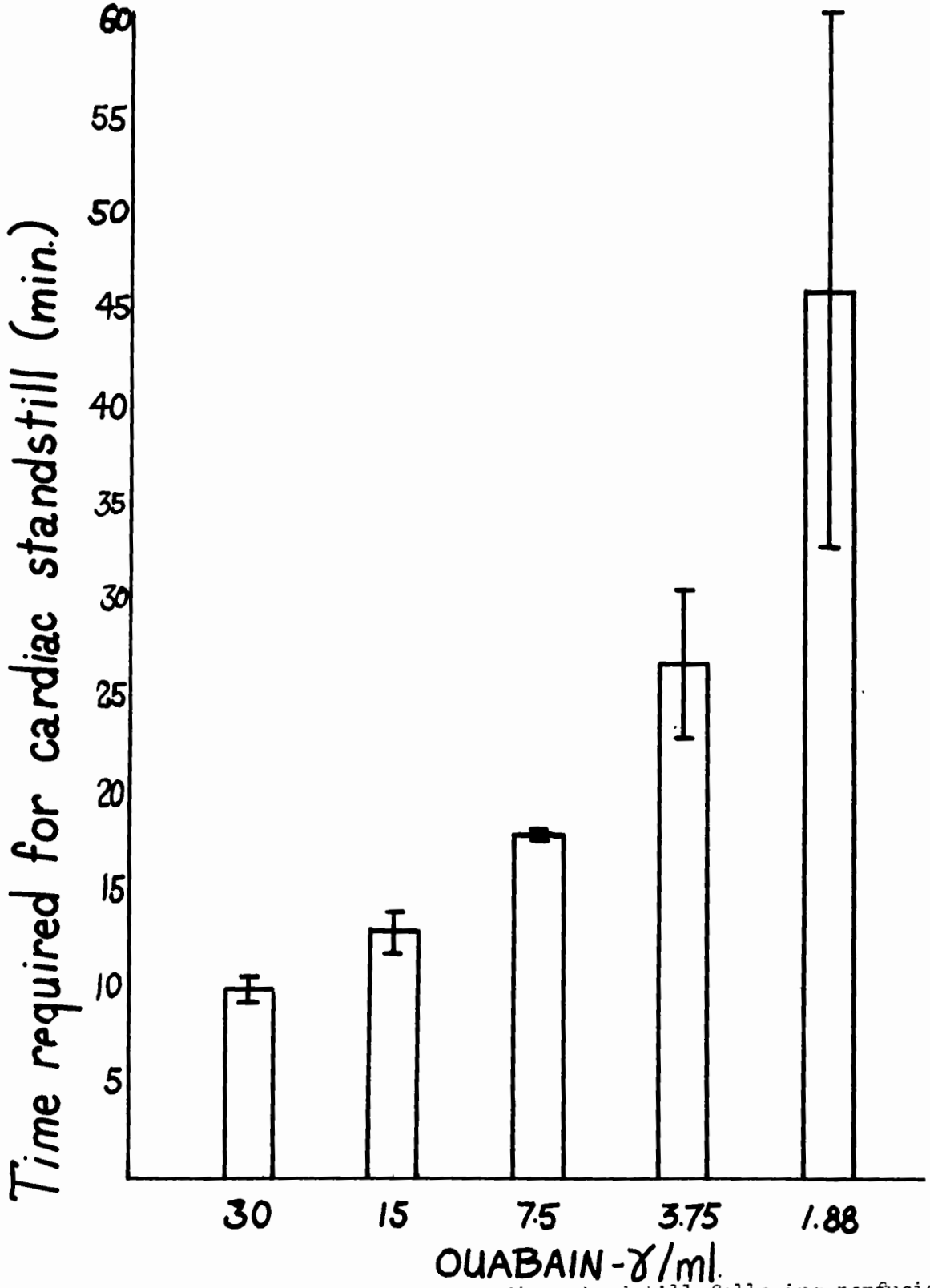


Figure 1. Graph of average times to cardiac standstill following perfusion with decreasing concentrations of ouabain (γ/ml. added to normal Locke). Standard errors also shown.

It is evident from Figure 1, that as the concentration of ouabain in the perfusing fluid is decreased, the average time required for cardiac standstill increases, and in addition the average variation from experiment to experiment is greater, as shown by the standard error in each group. The average periods required for cardiac standstill in the different groups were: 9.0 ±0.75, 12.8 ±0.83, 17.56 ±0.63, 26.7 ±4.08 and 46.0 ±14.1 minutes for ouabain concentrations 30, 15, 7.5, 3.75, and 1.88 γ/ml. respectively, as shown in Figure 1. The variation with the lowest concentration (1.88 γ/ml.) was so marked that one of the five perfused hearts in this group did not reach standstill even after 92 minutes of perfusion.

For the purpose of making comparative studies of changes in the heart when ouabain is perfused under the different experimental conditions employed in this study, it is evident that the concentration leading to the least variation from experiment to experiment was an intermediate one (7.5 γ/ml.). In many of the earlier studies reported in the literature (see Historical Review), workers have selected for comparison what has been defined as "therapeutic" or "toxic" concentrations of digitalis preparations. Such terminology is however rather vague, and the fact that only slight variations in toxicity from experiment to experiment were observed at this dose level, permit a more exact basis for comparative studies than hitherto reported in the literature. It is conceivable that the marked variations in the individual experiments at the lower concentrations, might be due to lack of a constant rate of fixation or accumulation of ouabain in the heart at the normal rate of coronary inflow, and consequently, would not be suitable for such comparative studies.

Figures 2, 3 and 4 show typical examples of the concomitant changes in coronary inflow and heart contractions (both mechanical and electrical)

as recorded during perfusion with high (30 γ/ml.), medium (7.5 γ/ml.) and low (1.88 γ/ml.) concentrations of ouabain, respectively.

As can be seen from Figure 2, during perfusion with high ouabain concentration, there is a rapid increase in the amplitude of contractions, associated with a slight initial decrease in coronary flow rate (B)---the corresponding electrogram shows no significant change in form, but a slight bradycardia. With continued perfusion at C, D, and E, one observes continued augmentation of the amplitude of the contractions, associated with various types of arrhythmias ---shifting pacemaker (C) and ventricular tachycardia (D and E), continuing until mechanical arrest ensued (G). Concomitantly, there is a progressive increase in coronary inflow which also persisted to G---the electrical record at this time showed terminal fine fibrillating movements. This figure also demonstrates that when the perfusing fluid was changed to potassium-free Locke, there was a transitory period of ventricular fibrillation (H) followed by arrest of the heart, from which recovery could not be obtained by changing the perfusing fluid to normal Locke. Similar results were obtained in another similar experiment.

Apart from delay in the appearance of coronary dilatation and the slower development of final cardiac arrest, a rather similar sequence of changes was observed with all lower concentrations, and typical examples of these are shown in Figures 3 and 4. These speak for themselves.

In Figures 3 and 4 may also be seen recovery from ouabain when the perfusing fluid was changed to normal Locke. Similar recovery was obtained in nine different experiments. Furthermore, when the ouabain-arrested heart (Figure 5) is perfused with Locke solution containing fifty per cent of the normal potassium (2.8 mEq./l.), as shown at (K), there was a rapid onset of idioventricular beats, with temporary reversion to cardiac standstill, but on continued perfusion with this solution, there was progressive

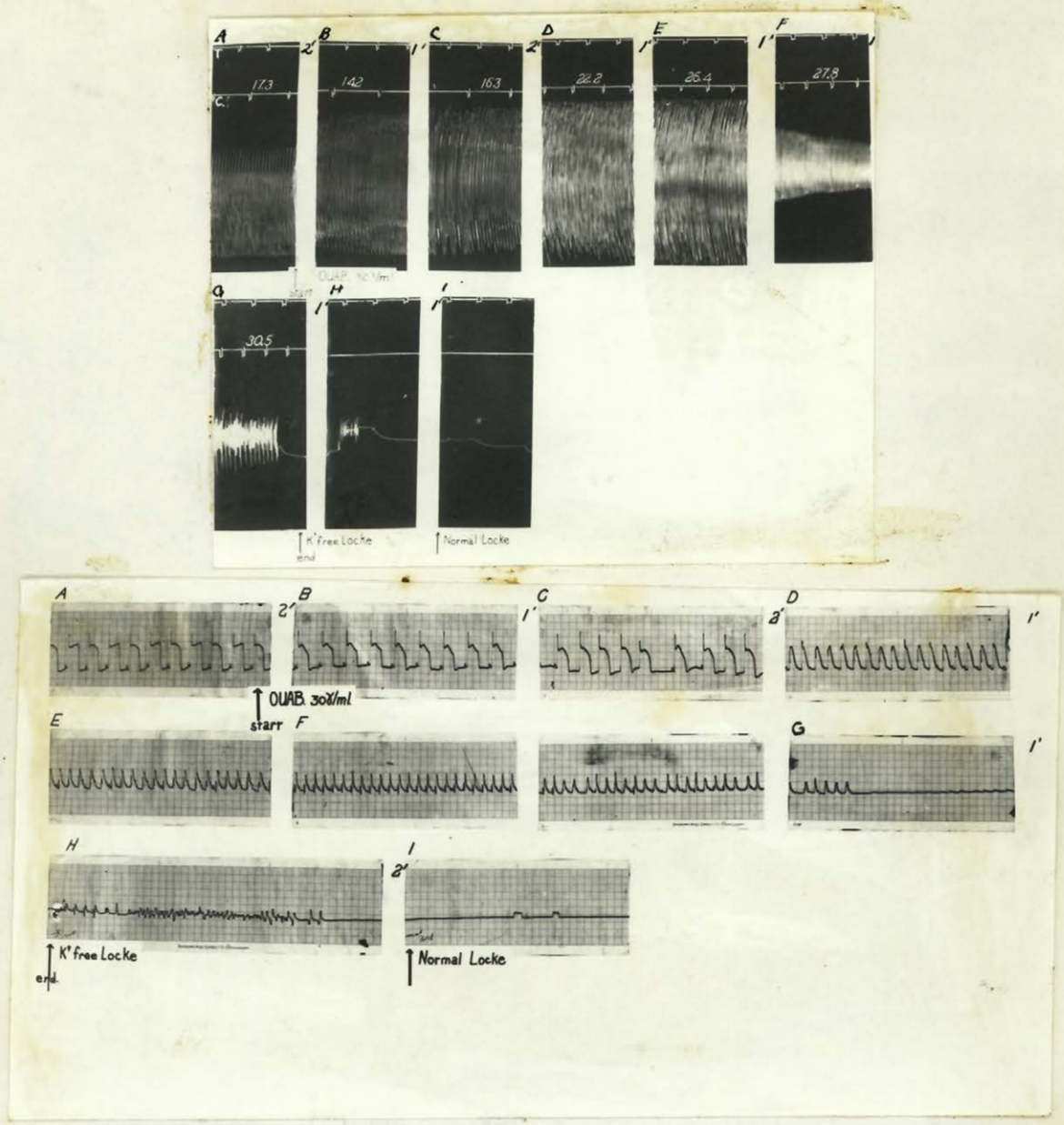


Figure 2. Isolated rabbit heart. Perfusion with ouabain (30 γ/ml.) added to Locke. Sections A to I of kymographic tracings (upper record) and associated electrograms (lower record). T - time—10 seconds; the intervals between the sections are shown in minutes. C.F. - calculated coronary inflow in ml. per min. Each mark on the line corresponds to the passage of the "indicator" in front of each photoelectric cell, alternately; and each interval represents a fixed inflow (3.75 ml.) into the coronary vessels. The lower tracings on the kymographic record shows the heart contractions, with systole upwards and diastole downwards.

Upper and Lower Records. At first arrow (start, Ouab. 30 γ/ml.) the perfusing fluid was changed from normal Locke solution to Locke containing added ouabain. At second arrow (end, K^+ free Locke) the ouabain perfusion was stopped and the heart perfused with potassium-free Locke. At third arrow (Normal Locke) perfusion was changed to normal Locke.

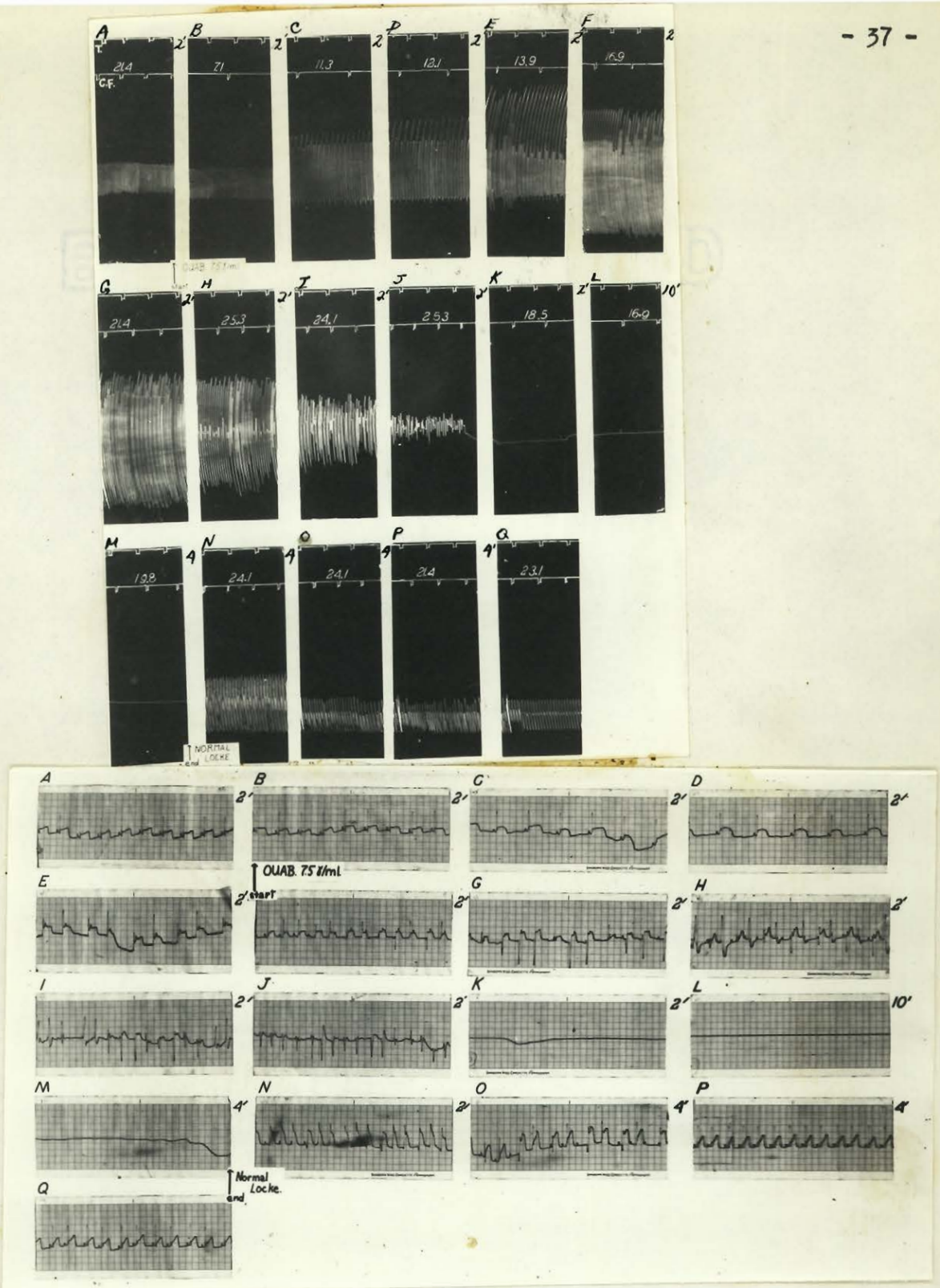
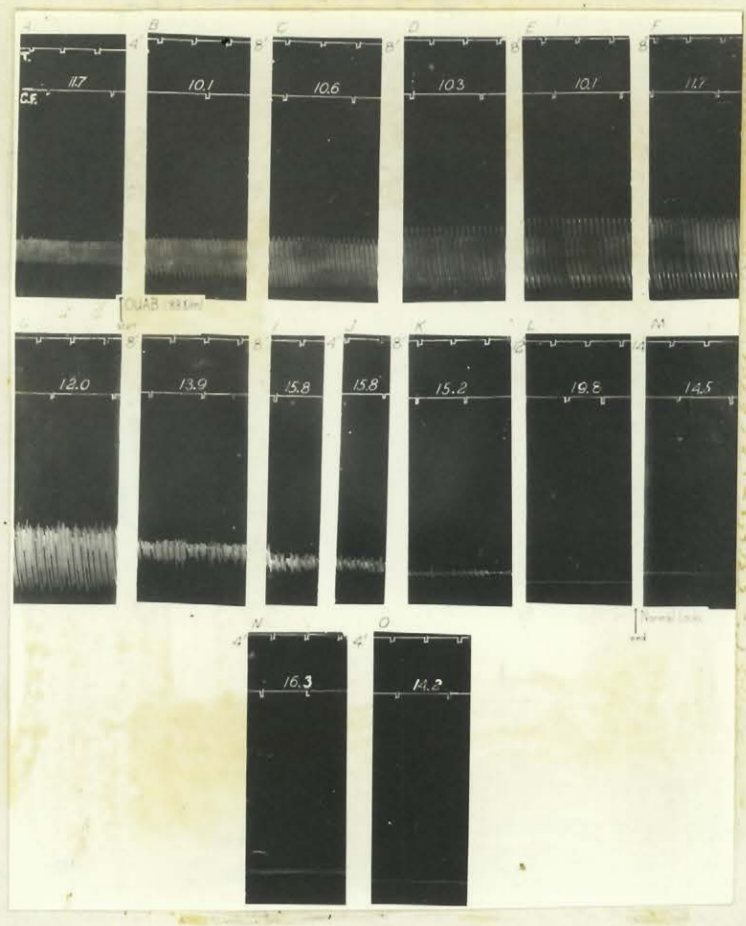


Figure 3. Isolated rabbit heart. Perfusion with ouabain (7.5 γ/ml.) added to normal Locke. See legend of Figure 2.

Upper and Lower Records: At first arrow (Start, Ouab. 7.5 γ/ml.), the perfusing fluid was changed from normal Locke to Locke containing added ouabain. At second arrow (end, normal Locke) the ouabain perfusion was changed to normal Locke.



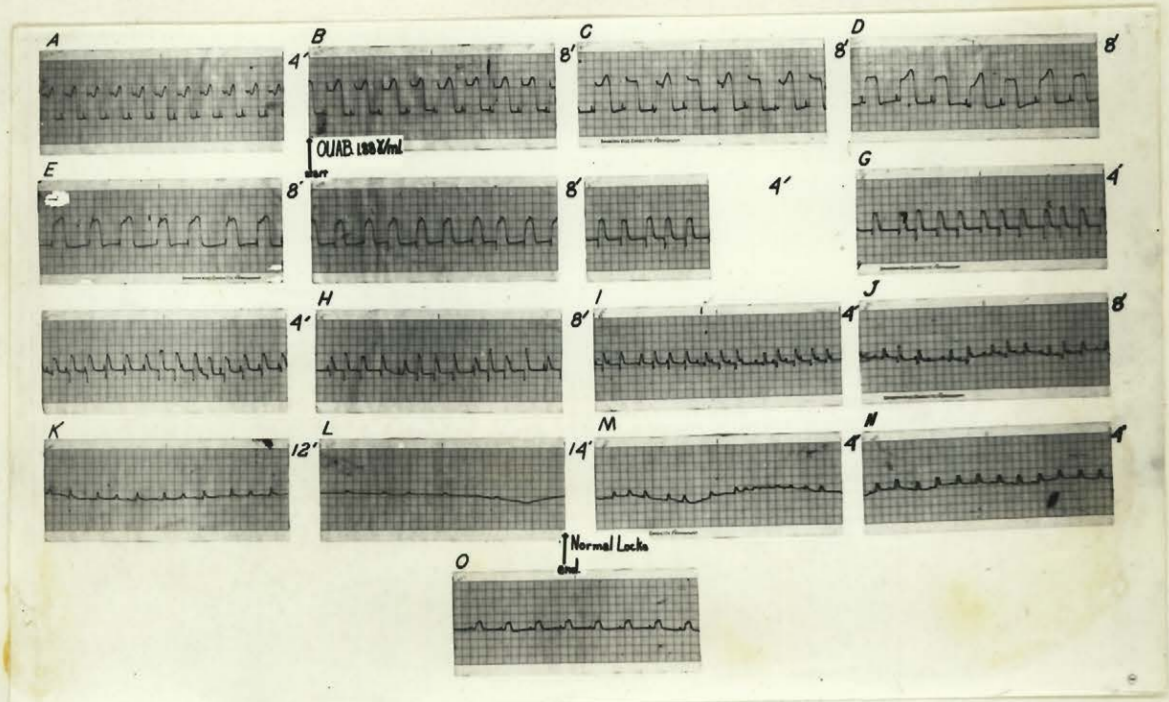


Figure 4. Isolated rabbit heart. Perfusion with ouabain (1.88 γ/ml.) see legend of Figure 2.

Upper and Lower Records: At first arrow (Start, Ouab. 7.5 γ/ml.), the perfusing fluid was changed from normal Locke to Locke containing added ouabain. At second arrow (end, normal Locke), the ouabain perfusion was changed to normal Locke.

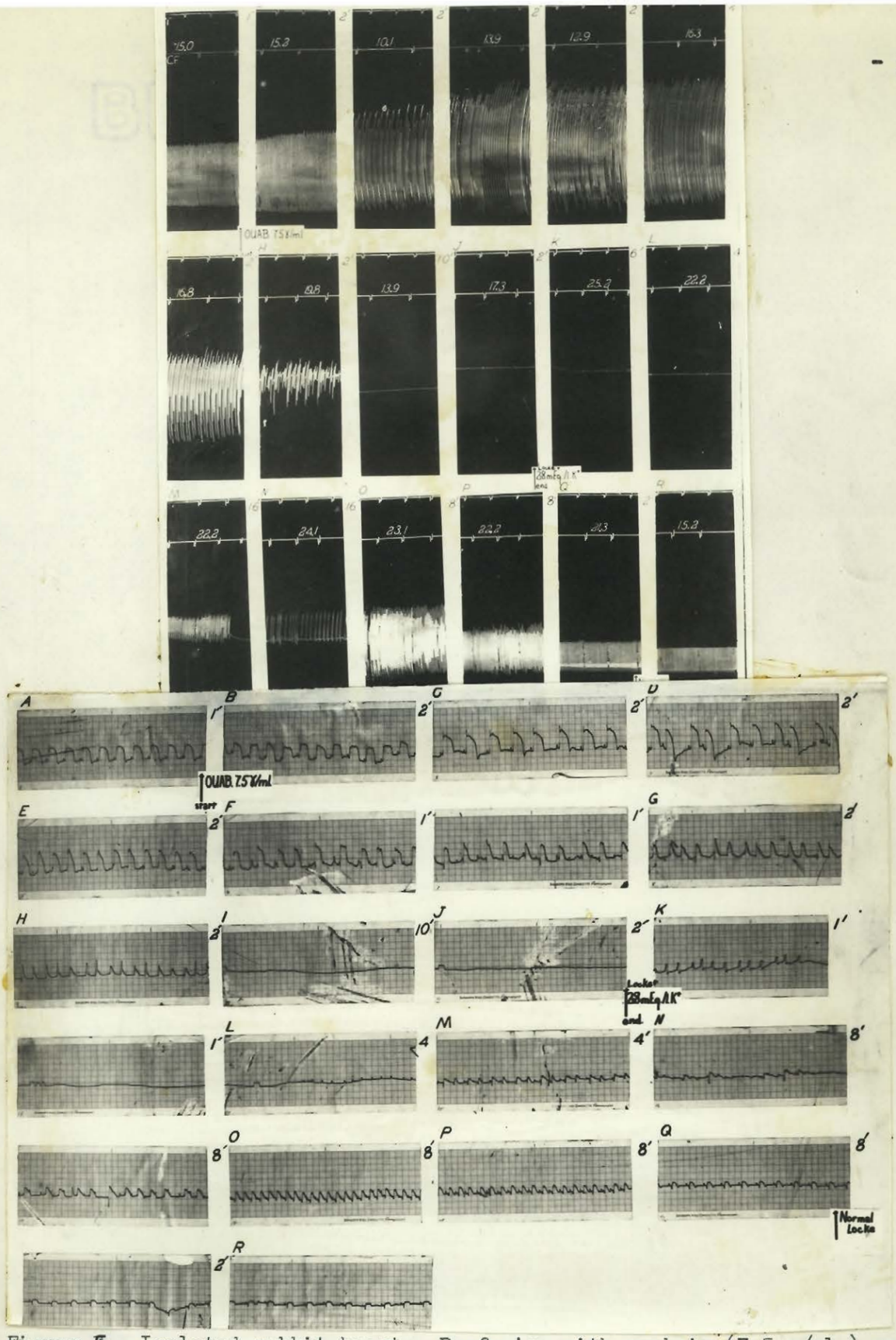


Figure 5. Isolated rabbit heart. Perfusion with ouabain (7.5 γ/ml.) see legend of Figure 2.

Upper and Lower Records: At first arrow (Start, Ouab. 7.5 γ/ml.) the perfusing fluid was changes from normal Locke to Locke containing added ouabain. At second arrow (end, Locke + 2.8 mEq./l. K^+) the ouabain perfusion was changed to Locke plus 2.8 mEq./l. potassium. At third arrow (normal Locke) perfusion fluid was changed to normal Locke.

restoration of both auricular and ventricular activity and sinus rhythm.

It is to be noted that on changing to the low potassium solution, there is immediate coronary dilatation which persisted until the end of the perfusion (Q), but on changing to normal Locke, the rate of coronary flow returned to the initial control level. Curiously enough, in similar experiments, using the perfused cats' hearts, similar coronary changes were observed, but no recovery of ventricular activity ensued. It is however evident that complete restoration of the ouabain-arrested rabbit heart can be accomplished by perfusion with either normal Locke or Locke containing fifty per cent of the normal potassium, but not with potassium-free Locke.

In Table I are summarized the preliminary findings in the experiments in which attempts were made to resuscitate the ouabain-poisoned heart, when various types of perfusing fluids were employed. In general, when normal Locke was used, there were nine complete recoveries to sinus rhythm, while when potassium-free Locke was used, there were no recoveries (two hearts). With Locke containing fifty per cent potassium, there was recovery of one rabbit heart (Figure 5 and Table I), but in the corresponding cat heart experiment there was no recovery. The addition of sodium lactate to normal Locke also led to complete recovery of four rabbit hearts, but on continued perfusion, arrhythmias recurred but were subsequently stopped by normal Locke alone. It may be concluded therefore that the addition of lactate to the perfusion fluid under these conditions did not enhance recovery of the heart.

Simple injections of potassium chloride (25 mg./ml.) or calcium chloride (25 mg./ml.) or lactate, produced no favorable effect on the ouabain-arrested hearts. In some of these recovery experiments using normal Locke solution, potassium movement was also studied, and it was observed that during the initial phase (one to eight minutes) there was a continuing "net loss"

TABLE I

Effects of Different Concentrations (γ/ml.) of Ouabain Added to Normal Locke Solution and Observations concerning Recovery** of the Heart.

Perfusion Employed		Observations During Post-Ouabain Perfusion	
Conc.	Mins.	Locke Solution Employed	Remarks
30	8 to 9	K-free (2 Exp.)	Immediate transient fibrillation, followed by arrest (5 min.), Locke perfusion (4 min.), no change. K-free re-perfusion - no change.
7.5	22	Normal	Recovery in 5 min.-sinus rhythm (12 min.) re-digitalization (7.5 /ml.) - arrest in 20 min. Second recovery with Locke (12 min.) followed by sinus rhythm.
7.5	28	Normal	Recovery after 4 min.
7.5*	27	Added 2.58 mEq./l. K.	No recovery in 16 min., massage - no effect.
7.5	27	Added 2.58 mEq./l. K.	Recovery in 12 min.
7.5*	24	Normal	No recovery in 20 min., massage - no effect.
7.5	18	Added 1% 1/6 M. Na Lactate	Sinus rhythm in 6 min., followed by arrhythmias, converted to sinus rhythm with subsequent Locke-good response to an adrenaline (100)injection.
7.5	20	Added 1% 1/6 M. Na Lactate	Recovery 2 min.- leading to sinus rhythm (10 min.) - followed by arrhythmias. Converted to sinus rhythm in 12min. by subsequent Locke perfusions.
7.5	30	Added 0.5% 1/6 M. Na Lactate	Recovery 8 min. - sinus rhythm - then arrhythmias - subsequent Locke - sinus rhythm in 14 min.
3.75	35 to 48	Normal (3 Exp.)	Recovery in 4 to 8 min.
1.88	42 to 88	Normal (3 Exp.)	No recovery in 12 to 24 min. (2 Exp.) - poor recovery (1 Exp.).

* The isolated cat's heart was used in these experiments.

** The term "Recovery" means 'initiation of visible ventricular improved contractions'.

followed during the intermediary period (eight to 16 minutes) by a "net gain", the potassium balance returning to normal equilibrium after approximately 20 minutes.

It is of some interest that the only two hearts which failed to recover when perfused with normal Locke were those submitted to prolonged (51 or 42 minutes) perfusion with the very low ouabain concentration (1.88 γ/ml.). Why this is so, is not clear. It might be however, that this prolonged exposure to the glycoside is an important factor in fixation and hence no recovery ensued. Further experiments would be necessary in order to elucidate this point.

(b). Correlated Effects of Ouabain on Coronary Flow and Potassium Balance.

In the majority of the above-described experiments, potassium studies, as previously indicated (Methods) were carried out. In Figure 6 and Figure 7 the average changes in coronary flow rate and associated "net changes" in potassium balance are shown: i.e. differences between the potassium supplied to the heart by the perfusing fluid, and that found in the perfusate collected at regular intervals.

It is clear that the initial observed decrease in coronary flow is associated with a brief net increase in potassium balance. However, both figures show that the net loss of potassium begins somewhat earlier than the onset of the coronary dilatation. Furthermore, the coronary dilatation in both instances, reached a peak value, then declined somewhat before cardiac standstill developed. In general, the potassium changes fluctuated inversely with the coronary flow changes. Indeed, the data presented emphasize again the necessity of taking into consideration the rate of coronary flow, in attempts to assess ionic movements in the heart.

In Tables II and III are presented more detailed analyses of these coronary flow and potassium changes, respectively. Again, it can be seen (TableII)

TABLE II

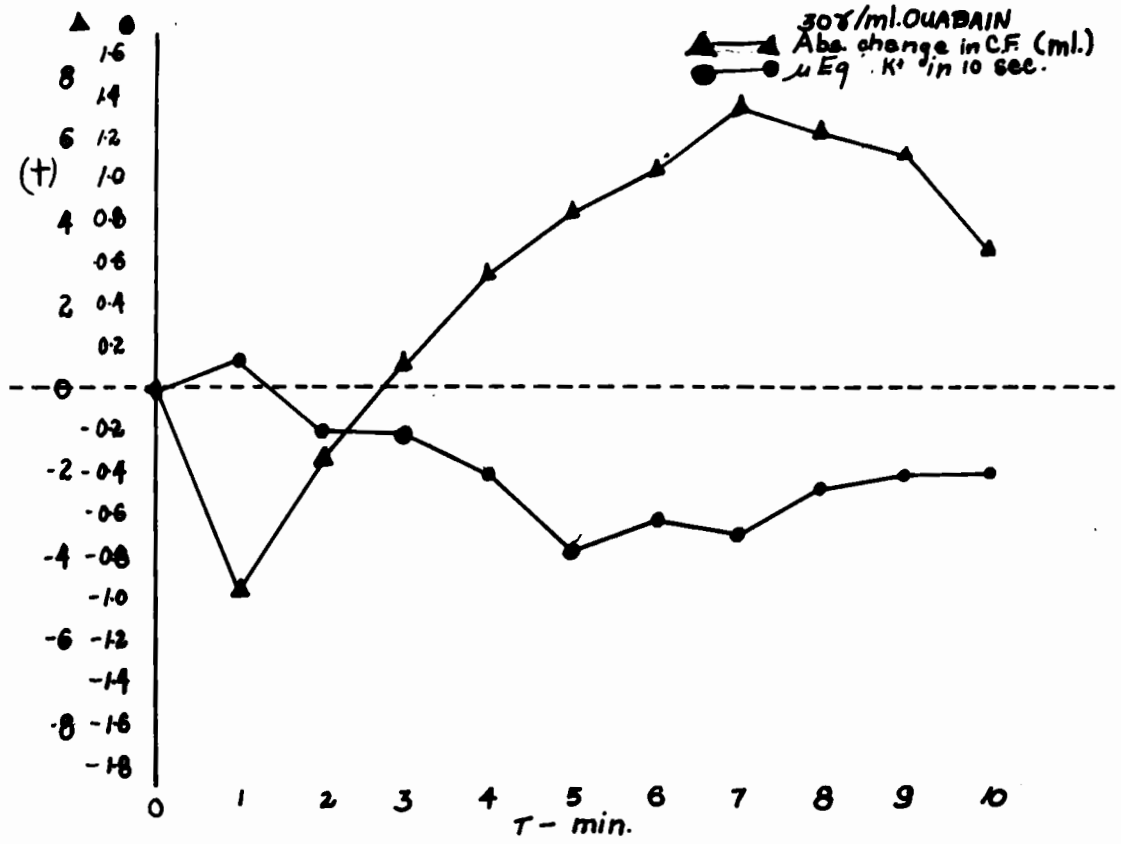
Mean Recorded Changes in Coronary Inflow Rates (ml. per min.) During Ouabain Perfusions.

Time (min.)	Concentration of Ouabain (γ/ml. in Locke Solution)				
	30	15	7.5	3.75	1.88
Control (ml/min)	12.7	19.0	19.3	12.6	16.7
1	-4.96	-4.83	-	-	-
2	-1.7	-3.55	-7.47	-1.83	-
3	+0.54	-1.44	-	-	-
4	+2.68	+0.97	-4.80	-0.90	-1.37
5	+4.14	+3.14	-	-	-
6	+5.15	+4.57	-1.03	-0.73	-
8	+6.08	+7.68	+1.79	+1.76	+0.09
9	+5.58	+7.12	-	-	-
10	+3.8	+7.80	+6.96	+1.43	-
12	-	+7.27	+7.63	+1.36	+0.87
13	-	+7.44	-	-	-
14	-	-	+7.57	+1.90	-
16	-	-	+6.55	+2.27	+1.36
18	-	-	+4.58	+4.27	-
20	-	-	-	+3.23	+0.84
22	-	--	-	+1.15	-
24	-	-	-	0.00	+0.19
26	-	-	-	-0.20	-
28	-	-	-	-	+0.76
32	-	-	-	-	+0.08
36	-	-	-	-	+0.93
40	-	-	-	-	+0.55
44	-	-	-	-	+0.55
No. Exp. per Mean	(5)	(9)	(9)	(3)	(4)

TABLE III

Mean Calculated Differences Between Potassium Inflow and Potassium Outflow, during ouabain perfusions, in Microequivalents per 10 sec.

	Concentration of Ouabain (γ/ml. in Locke Solution)				
Time (min.)	30	15	7.5	3.75	1.88
Control	-0.06	-0.02	+0.03	+0.08	-0.15
1	+0.14	+0.11	-	-	-
2	-0.21	+0.09	+0.42	+1.08	-
3	-0.22	-0.46	-	-	-
4	-0.42	-0.72	+0.08	+0.69	+1.88
5	-0.79	-0.64	-	-	-
6	-0.64	-0.55	-0.52	+1.86	
7	-0.71	-0.58	-	-	-
8	-0.49	-0.66	-1.80	-0.86	-0.56
9	-0.43	-0.58	-	-	-
10	-0.42	-0.65	-2.87	-1.89	
11		-0.51	-	-	-
12		-0.43	-2.41	-1.34	-0.19
14		-0.26	-2.55	-1.49	-
16			-2.35	-1.87	-1.27
18			-1.91	-1.98	-
20				-1.84	-0.78
24				-1.19	-0.85
26				-0.93	-
28					0.00
32					-0.72
36					+0.03
40					-0.23
44					-0.26
No. Exp. per Mean	(3)	(3)	(7)	(3)	(4)


30γ/ml. OUABAIN
Abs. change in C.F. (ml.)
μEq. K⁺ in 10 sec.
(†)
T - min.

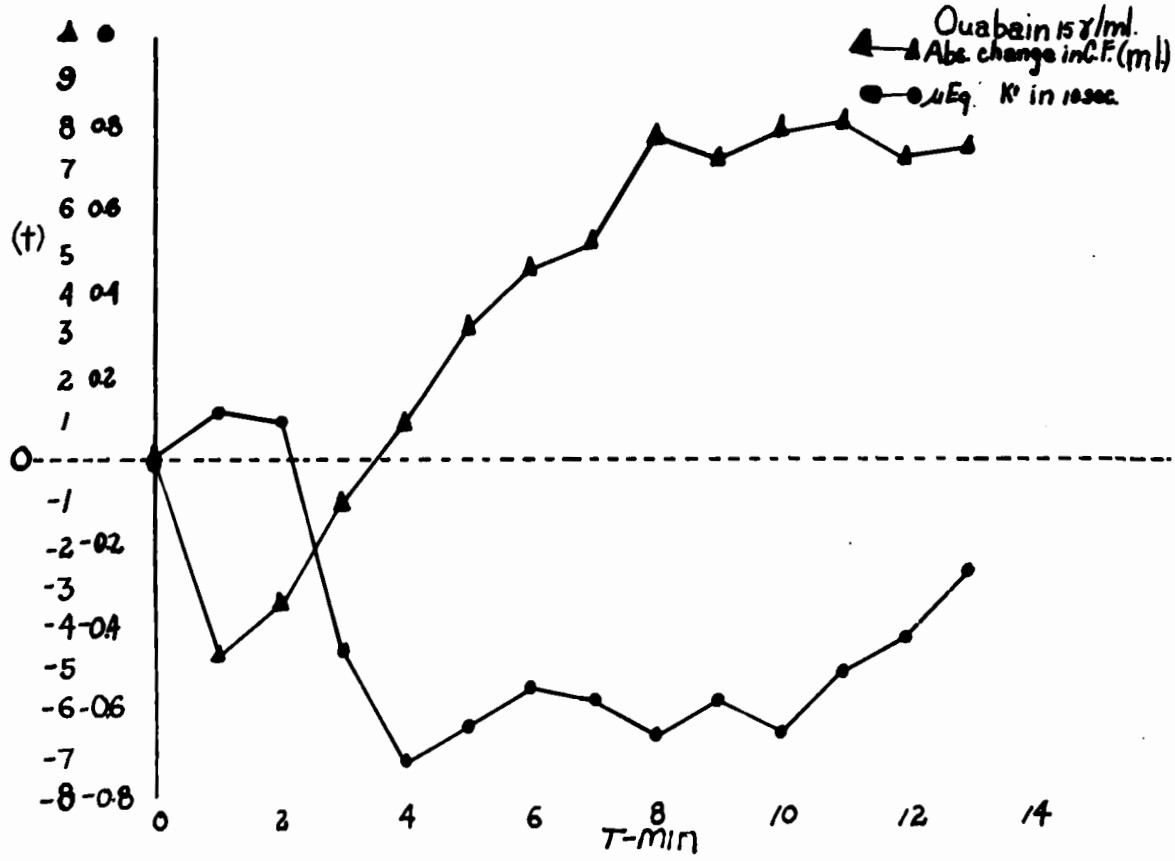

Ouabain 15γ/ml.
Abs. change in C.F. (ml)
μEq. K⁺ in 10 sec.
(†)
T-min

Figure 6.

Figure 6. Correlated average changes in coronary flow (▲-▲) and calculated "net changes" in potassium (●-●) during perfusion with ouabain. See text.

Upper and Lower Graphs: Ordinates represent both, mean changes in coronary flow (ml. per min.) marked "abs. changes in C.F. (ml.)"; and, the "net gain" or "net loss" of potassium, in microequivalents per 10 seconds, marked "γEq. K^+ in 10 sec.". Abscissa represents average duration of perfusion in minutes (T - min.). (+) indicates either increase in coronary flow or "net gain" of potassium, and (-), either decrease in coronary flow or "net loss" of potassium.

Upper Graph: Ouabain - 30 γ/ml. added to normal Locke.

Lower Graph: Ouabain - 15 γ/ml. added to normal Locke.

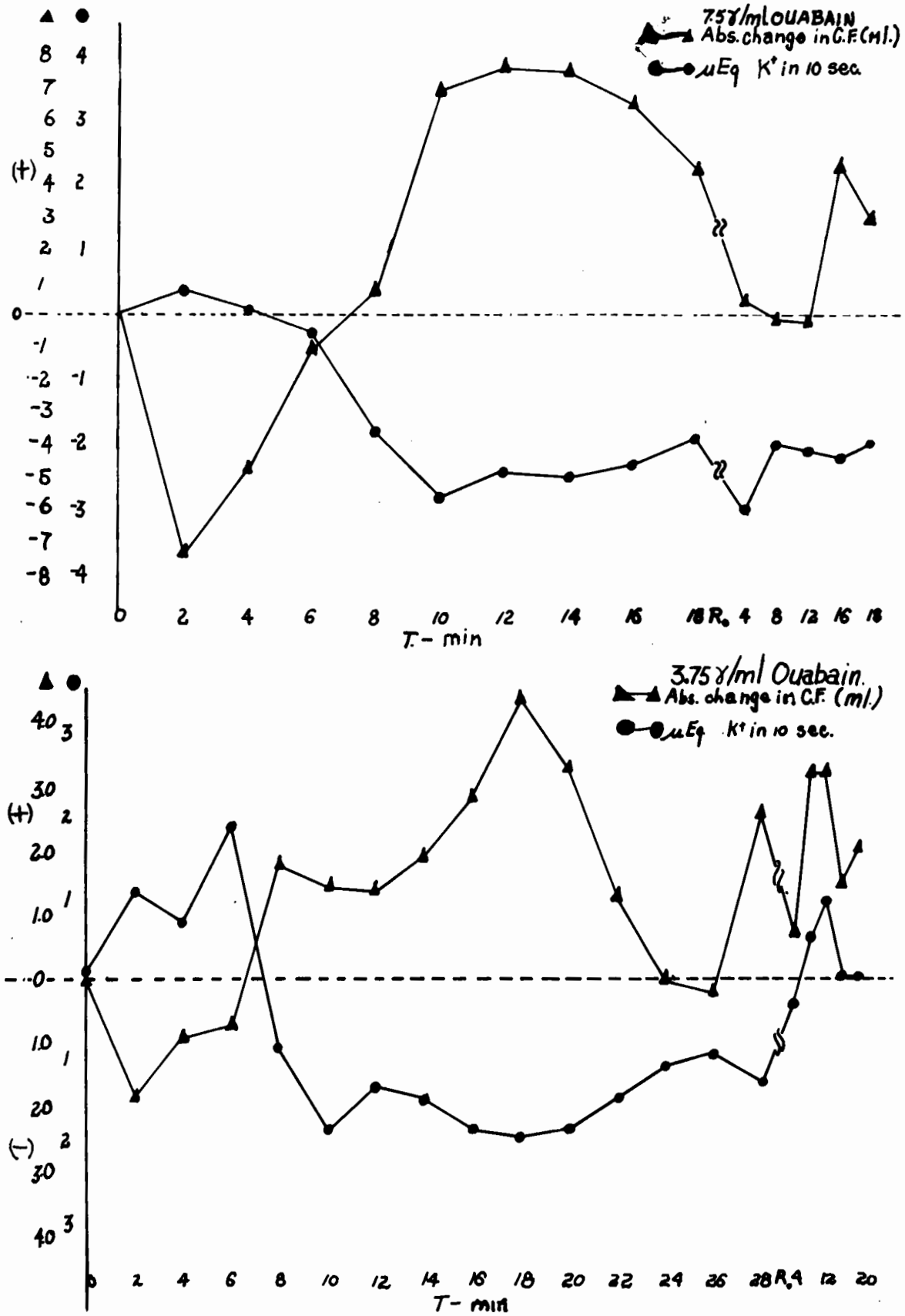


Figure 7. See legend of Figure 6. R_o - ouabain perfusion stopped and the heart perfused with normal Locke. Parallel line breaks, shown(//), indicate changes in the time scale as marked.

Upper Graph: Ouabain - 7.5 γ/ml. added to Locke.

Lower Graph: Ouabain - 3.75 γ/ml. added to Locke.

that when 30 γ of ouabain per ml. were perfused, the initial coronary constriction lasted approximately for only two minutes, and was followed by coronary dilatation which reached its height in eight minutes. This then gradually showed a decline until arrest of the heart.

A rather similar type of response was observed during perfusions with 15, 7.5 and 3.75 γ of ouabain per ml., except that the initial constriction lasted three, seven and seven minutes, respectively. The hearts perfused with the 1.88 γ per ml. of ouabain, because of the marked variations in individual response, did not lead to such striking or typical patterns of changes, as were observed with the other four dose levels studied. However, each heart in general,, showed similar slight initial coronary constriction followed by dilatation.

Table III shows the effects of the various concentrations of ouabain on the potassium balance in the heart. Again, it can be seen that when 30 γ of ouabain per ml. is perfused, there is an initial uptake of potassium for the first minute, followed by a gradual loss of potassium reaching its peak at seven minutes. Fifteen, 7.5 and 3.75 γ ouabain per ml. produced a similar pattern of potassium change, except that the initial uptake lasted for two, four and six minutes, respectively. Variable potassium balances were obtained with the 1.88 γ per ml. concentration.

It can be seen from the above that in most instances the potassium response is the mirror image of the coronary flow, that is, when the heart was taking up potassium, there was a coronary constriction and conversely, when the heart was losing potassium, there was a dilatation. Correlating these various changes, it would also appear that the potassium alterations occur slightly before the coronary flow changes. Thus, the degree of constriction or dilatation of the coronaries, seems to be dependent on the movement of potassium in or out of the myocardium. These data (Table II and III) when subjected to statistical correlation showed a highly significant inverse

dependence between coronary flow and potassium movement as shown in Table IV.

Attempts to follow sodium and calcium balances in the heart during the stages of digitalization with 30 and 15 γ ouabain per ml. in the perfusing fluid, as above, did not lead to any consistent pattern of alteration of these two ions. The calcium and sodium determinations were done in three and five experiments, respectively. There was no alteration in the concentration of calcium. The sodium balances gave inconsistent both negative and positive slight alterations. The overall negative data from these experiments have therefore been omitted. More will be said regarding calcium and sodium in regard to ouabain action later.

TABLE IV

Inverse Correlation Coefficient for Coronary Flow and Potassium Changes.

Ouabain	Correlation Coefficient (r)	Level of Significance (p)
30	-0.8884	<0.01
15	-0.7152	<0.01
7.5	-0.9799	<0.01
3.75	-0.8027	<0.01
1.88	-0.4392	>0.05

(d) Summary and Conclusions.

In the isolated perfused rabbit heart it has been observed:

1. That, increasing the concentration of ouabain (1.88 to 30 γ/ml.), in the perfusing fluid, leads to a corresponding decrease in the time required to reach cardiac standstill. The minimal concentration of ouabain, which induced the least individual variations (calculated Standard Errors) from experiment to experiment, was observed to be 7.5 γ/ml. under these conditions.

2. That, increasing similarly the concentrations of ouabain in the perfusing fluid leads to a transient initial sinus bradycardia without other significant changes in heart contractions. This is followed by a more sustained cardiac stimulation associated with increased amplitude of contractions and increased degrees of arrhythmias (shifting pacemaker, ectopic beats, paroxysmal and multifocal ventricular tachycardia and final ventricular fibrillation).

3. That, all concentrations of ouabain studied lead to an initial moderate transient decrease in mean coronary flow rate, followed by a more sustained increase. As the ouabain concentration increased, the duration of the initial constriction shortened.

4. That, all concentrations of ouabain studied lead to an initial moderate transient "net uptake" in potassium, followed by a more sustained "net loss". As the ouabain concentration increased, the duration of the initial "net uptake" shortened.

5. That, following ouabain cardiac arrest, perfusions with normal Locke lead to slight irregular coronary dilatation, followed by complete recovery. Similar perfusion with Locke containing 50 per cent of normal potassium lead to prompt and sustained coronary dilatation, and eventual restoration to normal sinus rhythm.

6. That, following ouabain cardiac arrest, prolonged perfusion with potassium-free Locke led to no recovery. Perfusion with added sodium lactate or simple

injections of potassium chloride, calcium chloride, or adrenaline did not enhance the recovery as observed with normal Locke.

It is therefore concluded that:

(a) There is an intimate relationship between changes in coronary flow, heart action and potassium balance in the beating heart which can be correlated with varying concentrations of ouabain.

(b) Ouabain leads to an initial transient coronary constriction associated with sinus bradycardia and net gain of potassium, followed by sustained coronary dilatation, cardiac stimulation and net loss of potassium.

(c) The initial coronary constriction is associated with an increased potassium uptake, while the subsequent dilatation is preceeded by and appears to be due to the observed potassium loss.

(d) Subsequent resuscitation of the ouabain-arrested heart appears to depend upon the presence of potassium in the perfusing fluid.

II. OBSERVATIONS ON THE EFFECTS OF VARYING CONCENTRATIONS OF POTASSIUM IN THE PERFUSING FLUID UPON THE RESPONSES (CORONARY FLOW, HEART ACTION, AND ELECTROLYTE MOVEMENTS) TO OUABAIN.

(a) Effects upon Ouabain Toxicity and Recovery.

In all experiments in this series, a fixed concentration (7.5 γ/ml.) of ouabain was employed. This, as already pointed out, was the concentration which led to the least variable results in different experiments under the conditions of perfusion employed.

Figure 8 shows a graphic comparison of the average duration of perfusion required to reach cardiac standstill (mechanical arrest), when the hearts of either rabbits or cats, were perfused with identical concentrations of ouabain, in the perfusing fluid. In these experiments however, the potassium concentration employed was either increased (to 16.8 mEq./l.) or decreased (to zero). A total of 35 hearts was studied.

As can be seen from Figure 8 and Table V, at the highest potassium concentration(16.8 mEq./l.)—both in the presence and in the absence of oubain—there was rapid cardiac standstill within an average of 3.33 ±0.88 minutes and 3.67 ±1.2 minutes, respectively. It is therefore clear that increasing the potassium concentration to this extent was in itself rather deleterious to the heart, and furthermore there was no apparent antagonism of the toxicity of ouabain under these conditions. Indeed, cardiac standstill ensued more rapidly than in similar ouabain experiments with normal Locke as the perfusing fluid.

Figure 8 and Table V also show that with a concentration of 11.2 mEq./l. of potassium, without added ouabain, the heart continues to beat moderately well for longer than an hour. On the other hand, addition of ouabain to Locke solution with a similar potassium concentration (11.2 mEq./l.) produced arrest in an average of 15.17 ±3.05 minutes. This is however, not significantly

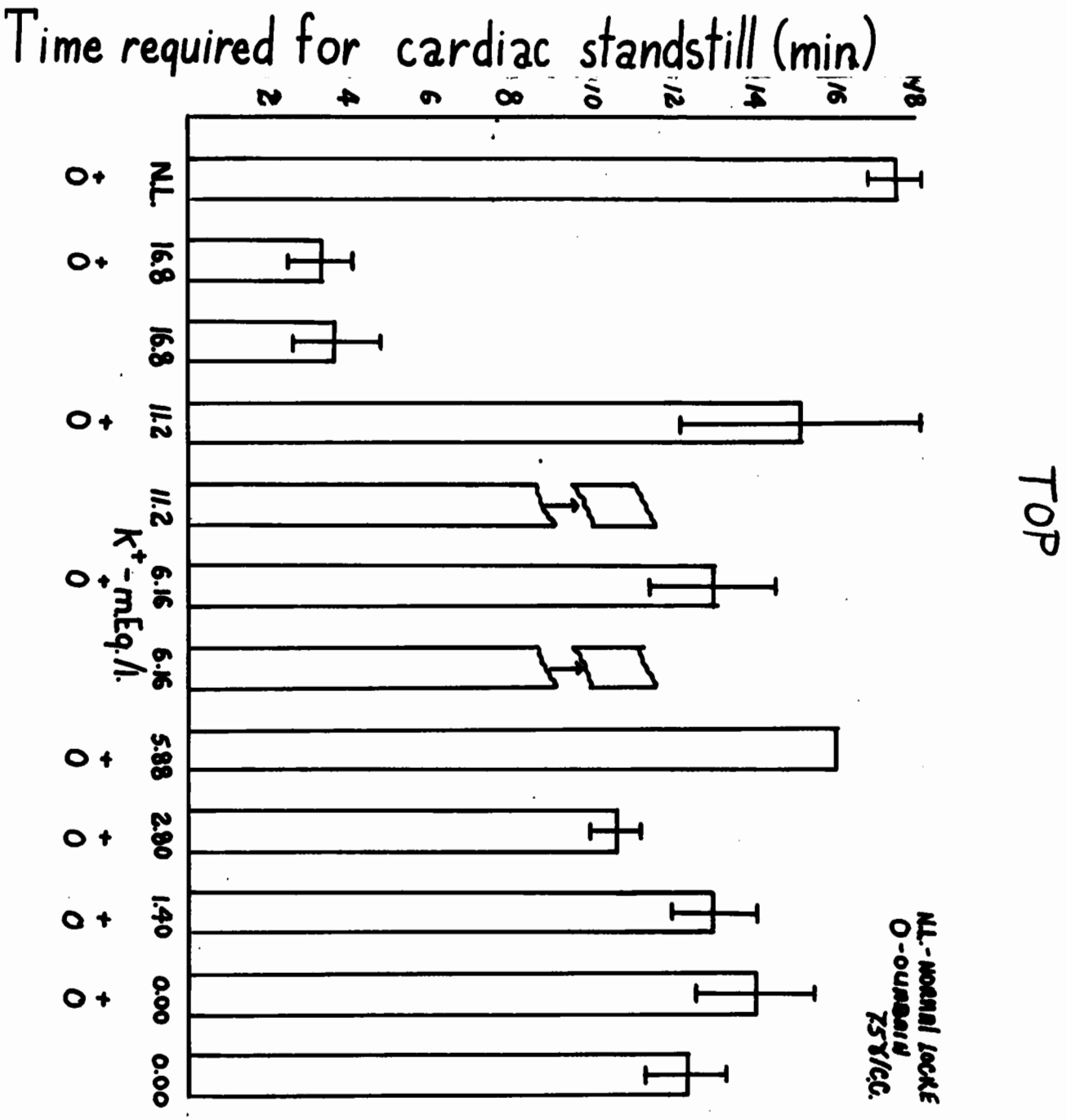


Figure 8. Graph of average times to cardiac standstill following perfusions with the same ouabain (+O) concentration in Locke solution containing either increased or decreased concentration of potassium. Standard errors also shown. See text.

TABLE V

Effects of Varying Concentrations of Potassium During Perfusion with Ouabain (7.5 γ/ml.).

Potassium Concentration (mEq./l.)	Cardiac Arrest (min.)		Recovery with Normal Locke (min.)	
	Control	Ouabain	Control	Ouabain
16.8	2.0	5.0	Immediate	0.5
16.8	6.0	2.5	0.5	2.5
16.8	3.0	2.0	2.0	2.0
Avg.	3.67 ±1.2	3.33 ±0.86		
11.2	N.A.	12	-	Immediate
11.2	N.A.	24	-	Immediate
11.2	-	25	-	Immediate
11.2*	-	9	-	0.5
11.2*	-	8	-	0.5
11.2*	-	13	-	0.5 Fibrillated after 35 min
Avg.		15.17 ±3.05		
6.16	N.A.	16	-	12
6.16	N.A.	12	-	16
6.16	-	11	-	12
Avg.		13.0 ±1.53		
5.88	-	16	-	12
2.80	-	12	-	No Recovery
2.80	-	10	-	12
2.80	-	10	-	16
Avg.		10.67 ±0.64		
1.40	-	14	-	16
1.40	-	14	-	12
1.40	-	11	-	16
Avg.		13.0 ±1.06		

TABLE V (Cont'd)

Potassium Concentration (mEq./l.)	Cardiac Arrest (min.)		Recovery with Normal Locke (min.)	
	Control	Ouabain	Control	Ouabain
0.00	9	12	-	8
0.00	16	12	-	8
0.00	12	11	-	-
0.00*	-	17	-	No Recovery
0.00*	-	12.5	-	No Recovery
0.00*	-	20.0	-	No Recovery
Avg.	12.33 ±2.03	14.09 ±1.46		

* The isolated cat's heart was used in these experiments.

The term "N.A." means 'no cardiac arrest.'

different from controls using normal Locke, in which cardiac arrest occurred in an average of 17.56 ±0.63 minutes. Thus, again this increased potassium concentration in Locke did not alter the time required for ouabain cardiac standstill.

When the potassium concentration in Locke was increased by only ten per cent (6.16 mEq./l.) with added ouabain, cardiac arrest ensued in an average of 13 ±1.53 minutes. This was significantly shorter than the average in normal Locke control experiments. Nevertheless, when only a five per cent increase in potassium concentration (5.88 mEq./l.) was added with ouabain, the time required for arrest in a single heart observed, was not significantly different from the control. It is therefore clear that increasing the potassium concentration in Locke by only ten per cent, paradoxically accelerated the onset of cardiac arrest induced by ouabain.

It can also be seen from Figure 8 that on decreasing the potassium concentrations to 2.80, 1.40 and zero mEq./l. respectively, the average durations of perfusions to cardiac arrest with ouabain were 10.67 ±0.64, 13.00 ±1.08 and 14.09 ±1.46 minutes, respectively. It had previously been shown, that with reduction of the potassium in Locke solution to 2.8 and 1.4 mEq./l., perfusion of rabbits' hearts could be continued without any apparent deleterious effects for at least one hour (Korol, 1956). In comparison with ouabain-normal Locke controls, these latter periods of arrest therefore appear to be significantly shortened. This would indicate that decreasing the potassium to these latter levels, enhances the toxicity of ouabain. However, when the heart was perfused with zero mEq./l. of potassium without ouabain, the standstill was induced on an average of 12.33 ±2.03 minutes. Thus, from results obtained in the potassium-free experiments, one cannot conclude that the complete lack of potassium enhanced digitalis toxicity.

The data concerning recovery of the ouabain arrested hearts are also shown in Table V. It is evident that with

excess potassium (16.8 and 11.2 mEq./l.) there is rapid and sustained recovery, when the perfusing fluid was changed to normal Locke, in both rabbits and cats. With ten per cent excess potassium, recovery ensued after an interval of 12 to 16 minutes. Curiously enough with reduced potassium concentrations (2.8 to zero mEq./l.) recovery in the rabbit experiments were quite delayed, and could not be achieved in the cat.

(b) Effects of Increased Potassium Concentrations on Coronary Flow and Heart Action.

As can be seen from Figure 9, when a high potassium (16.8 mEq./l.) was employed, there was a rapid, almost immediate decrease in coronary flow and associated depression of the heart contractions. These effects continued for the duration of the perfusion, which was stopped after 18 minutes (F). The electrical changes recorded concomitantly, show rapid onset of ventricular fibrillation (C), followed by sustained fine fibrillatory changes (D and E), culminating in electrical silence (F).

Figure 9 also shows that when the perfusing fluid was changed to normal Locke, there was a prompt initiation of electrical ventricular activity (G), followed by strong ventricular contractions, as shown, and associated with a relative increased coronary flow (G). As the perfusion continued, there was a period of total cardiac irregularities, shown both on the kymographic and electrical records at H to L. Finally, there was a slow (60 beats per minute) sinus rhythm (M). Concomitantly, with these changes, there was sustained good coronary dilatation, as shown.

In Figure 10 is shown an example of the responses observed in a cat's

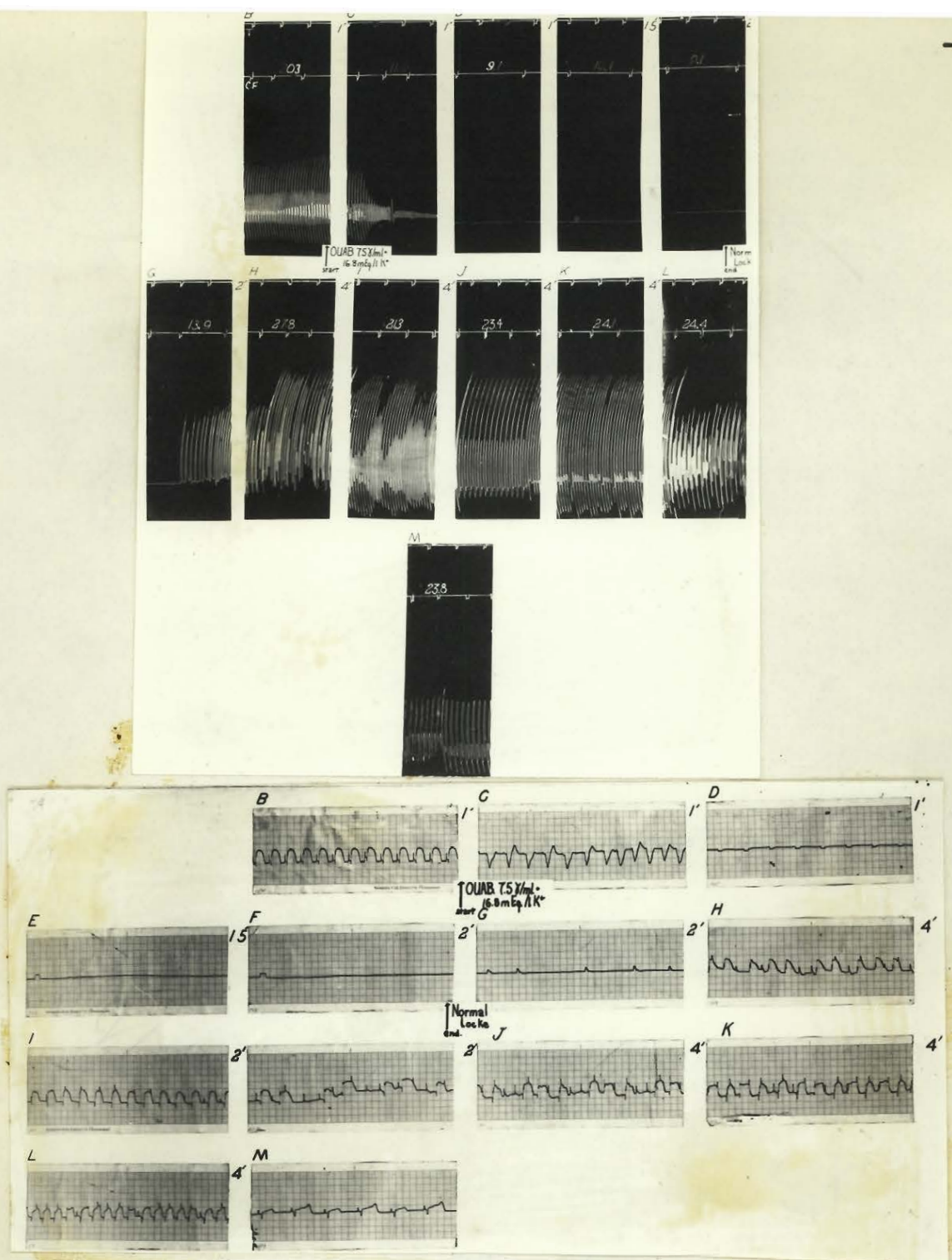
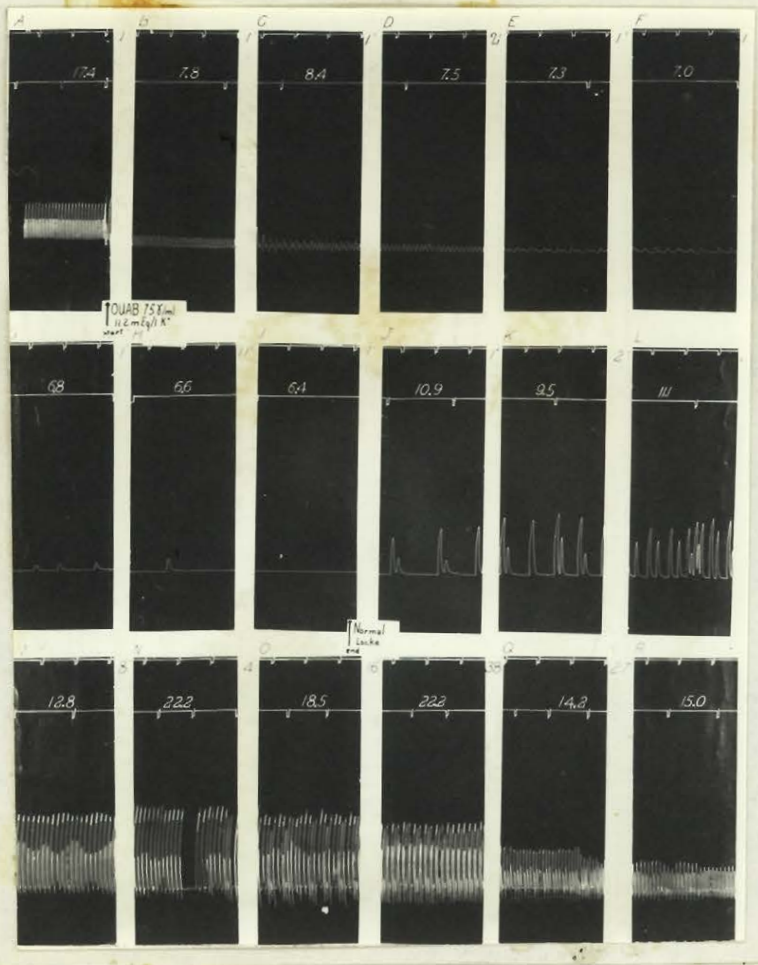


Figure 9. Isolated rabbit heart. Perfusion with Locke solution containing 16.8 mEq./l. potassium and 7.5γ /ml. ouabain. See legend of Figure 2.

Upper and Lower Record: At first arrow (Start Ouab. 7.5γ /ml. + 16.8 mEq./l. K^+) the perfusing fluid was changed from normal Locke to Locke containing added ouabain and 3 times potassium concentration. At second arrow,(end, Normal Locke) the ouabain perfusion was changed to normal Locke.



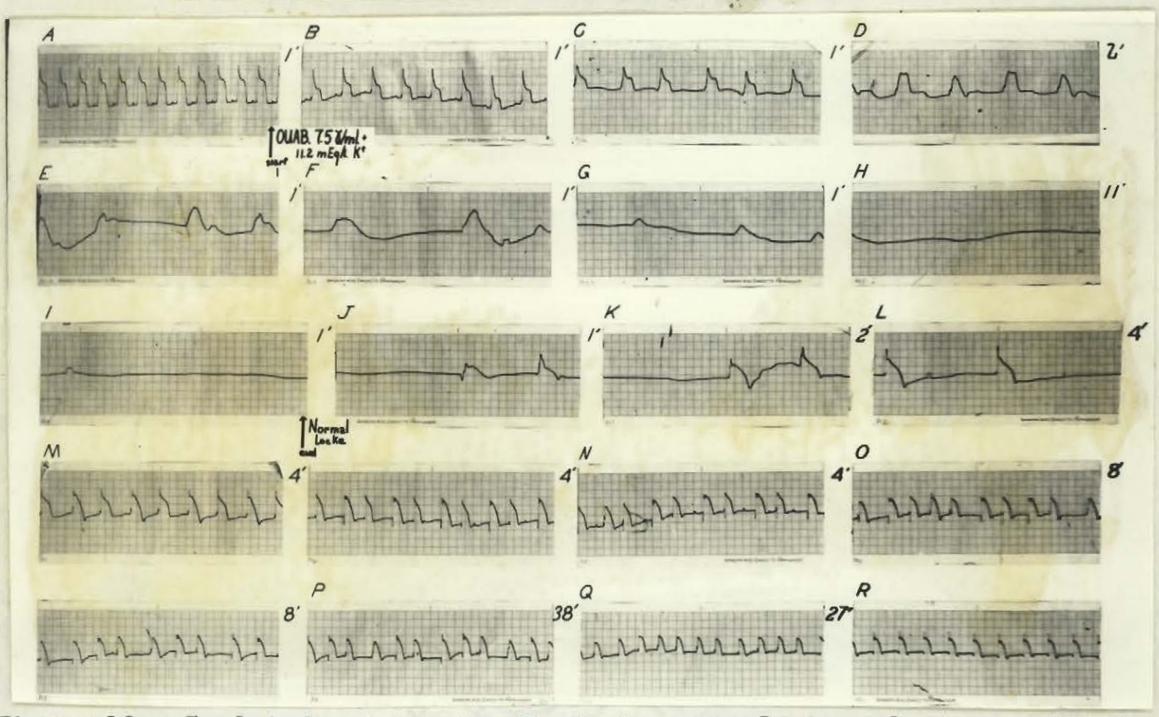
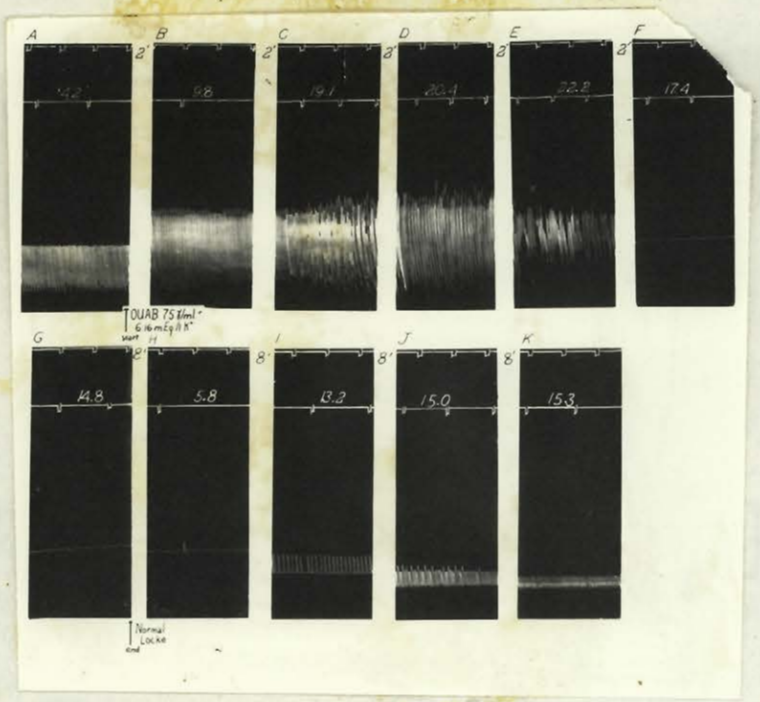


Figure 10. Isolated cat heart. Perfusion with Locke solution containing 11.2 mEq./l. potassium and 7.5 γ/ml. ouabain. See legend of Figure 2. Upper and Lower Records: At first arrow (Start Ouab. 7.5 γ/ml. + 11.2 mEq./l. K^+) the perfusing fluid was changed from normal Locke to Locke containing added ouabain and 2 times potassium concentration. At second arrow (end, normal Locke) the ouabain perfusion was changed to normal Locke.



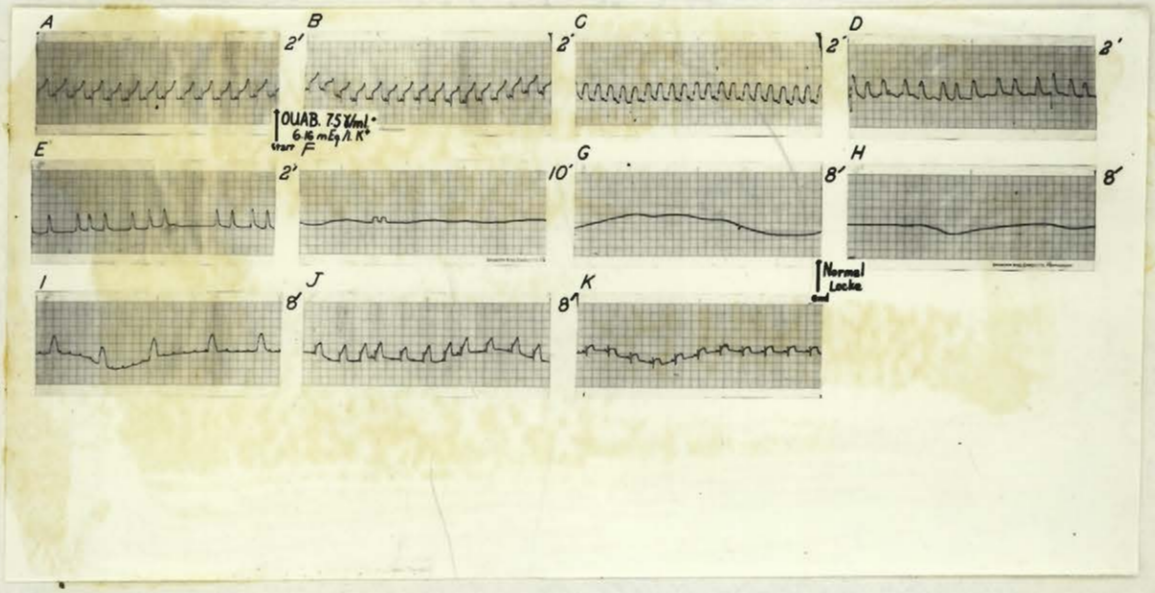


Figure 11. Isolated rabbit heart. Perfusion with Locke solution containing 6.16 mEq./l. potassium and 7.5 γ/ml. ouabain. See legend of Figure 2.

Upper and Lower Record: At first arrow (Start Ouab. 7.5 γ/ml. + 6.16 mEq./l. K^+) the perfusing fluid was changed from normal Locke to Locke containing added ouabain and a ten per cent increase in potassium. At second arrow (end, Normal Locke) the ouabain perfusion was changed to normal Locke.

heart experiment in which the potassium concentration in the perfusing fluid was high (11.2 mEq./l.). As can be seen, there was a prompt and sustained decrease in coronary flow, associated with a more slowly progressive depression of the heart contractions, culminating in complete arrest (B to I).

There was no evidence of any gross irregularities of the heart, but the concomitant electrical records showed flattening of the P waves and bradycardia (B), followed by rapid complete disorganization in the QRS complex and S-T distortion (C to G). Finally, there was complete electrical arrest (H and I).

Again, when the ouabain was discontinued and the perfusion fluid changed to normal Locke (I), there was a sudden restoration of strong but slow irregular ventricular contractions (J), followed by irregular tachycardia (M to P) and culminating in regular heart contractions (R). The associated electrograms also show the sudden onset of ventricular activity (J). Again during restoration of the heart there was a good associated coronary dilatation.

Figure 11 shows results of an experiment in which the potassium concentration in the perfusing fluid was increased by only ten per cent, i.e. to 6.16 mEq./l. As can be seen, the initial coronary constriction was of shorter duration than in the control experiment (Figure 3, ouabain in normal Locke), and the subsequent dilatation more marked. In general, the changes in the heart contractions were only quantitatively different in the two groups. Thus, sinus tachycardia developed more rapidly (B), and there was throughout, less evidence of multifocal ectopic beats (C and D). The electrocardiogram (G) showed a flattening of the P waves, and typical ST-T changes, characteristic of hyperpotassemia. However, as previously pointed out, cardiac standstill occurred more rapidly than in the control, and as can be seen when the perfusion fluid was changed to normal Locke, recovery from ouabain was more delayed (12 to 16 minutes) than in the controls (4 to 8 minutes). The electrograms also

show initiation of auricular activity with sporadic ventricular activity (I), and gradual restoration to normal sinus rhythm (K).

In one experiment, perfusing the heart similarly with only a five per cent excess of potassium, i.e. 5.88 mEq./l., with added ouabain, led to no significant difference from the effect observed in the control.

In comparison with the coronary flow and heart contraction changes induced by the same concentration of ouabain (7.5 γ/ml.), when perfused in normal Locke solution (Figures 3 and 7), it is apparent that in the presence of excessive potassium (11.2 to 16.8 mEq./l.) ouabain perfusion leads to (1) more marked prolonged coronary constriction with no evidence of coronary dilatation, (2) no multifocal ectopic beats, and (3) no stimulation of heart contractions. However, in the presence or absence of ouabain in the perfusing fluids with excess potassium, there occurred an initial and sustained sinus bradycardia but this developed much more rapidly in the hearts perfused with ouabain. This might indicate that the observed digitalis bradycardia, under these conditions, is due to the increased potassium intake. More will be said about this later.

On the other hand, during the "recovery phase" with normal Locke perfusion, after the heart was previously ouabainized in the presence of excess potassium, the recovery is characterized by a rapid onset of ventricular activity and stimulation with multifocal ectopic beats rather suggesting the usual type of ouabain action, although the drug had previously been discontinued. This might indicate some change in ouabain fixation in the presence of excess potassium. In addition, when the potassium concentration is only slightly increased to 6.16 mEq./l. or ten per cent above that of normal Locke, the initial bradycardia is not clearly seen, and tachycardia also develops more rapidly. This might again indicate more rapid fixations of the glycoside with the higher potassium concentration.

The toxicity of the digitalis is also clearly accentuated under these

conditions. It is therefore clear that excess potassium (per se) does not counteract or inhibit the toxic action of digitalis, although the occurrence of multifocal ectopic beats is definitely lessened. Why this is so, is not clear.

(c) Effects of Decreased Potassium Concentrations on Coronary Flow and Heart Action.

Figures 12, 13 and 14 show some typical examples of the responses observed when the potassium concentration in the ouabain perfused Locke solution was decreased. As can be seen from Figure 12, when the potassium concentration was reduced to 75 per cent of the normal, the initial coronary constriction and sinus bradycardia are generally reduced, while the latter coronary dilatation and tachycardia are greatly increased. There were associated ventricular tachycardia and multifocal ectopic beats, and finally cardiac arrest. As already pointed out, with reduced concentrations of potassium, cardiac standstill developed earlier than during similar ouabain perfusion in normal Locke solution.

During the recovery, as shown at P (Normal Locke), again there is a greater delay (eight minutes) in the initiation of electrical ventricular action (Q), although no visible changes were seen in the kymographic record (Q). After a further eight minutes, relatively normal auricular and ventricular action ensued (R), and finally a sinus bradycardia with curiously widened complexes. The coronary flow concomitantly was decreased, and the existing slow flow over a prolonged period of time (24 minutes) at S might be responsible for these T wave changes. It should also be added, that when the rabbit heart is perfused with either 50 per cent or 25 per cent of the normal potassium concentration in Locke solution, relatively normal heart action can be maintained for at least 60 minutes (Korol, 1956).

Figures 13 and 14 show examples of the responses observed when either

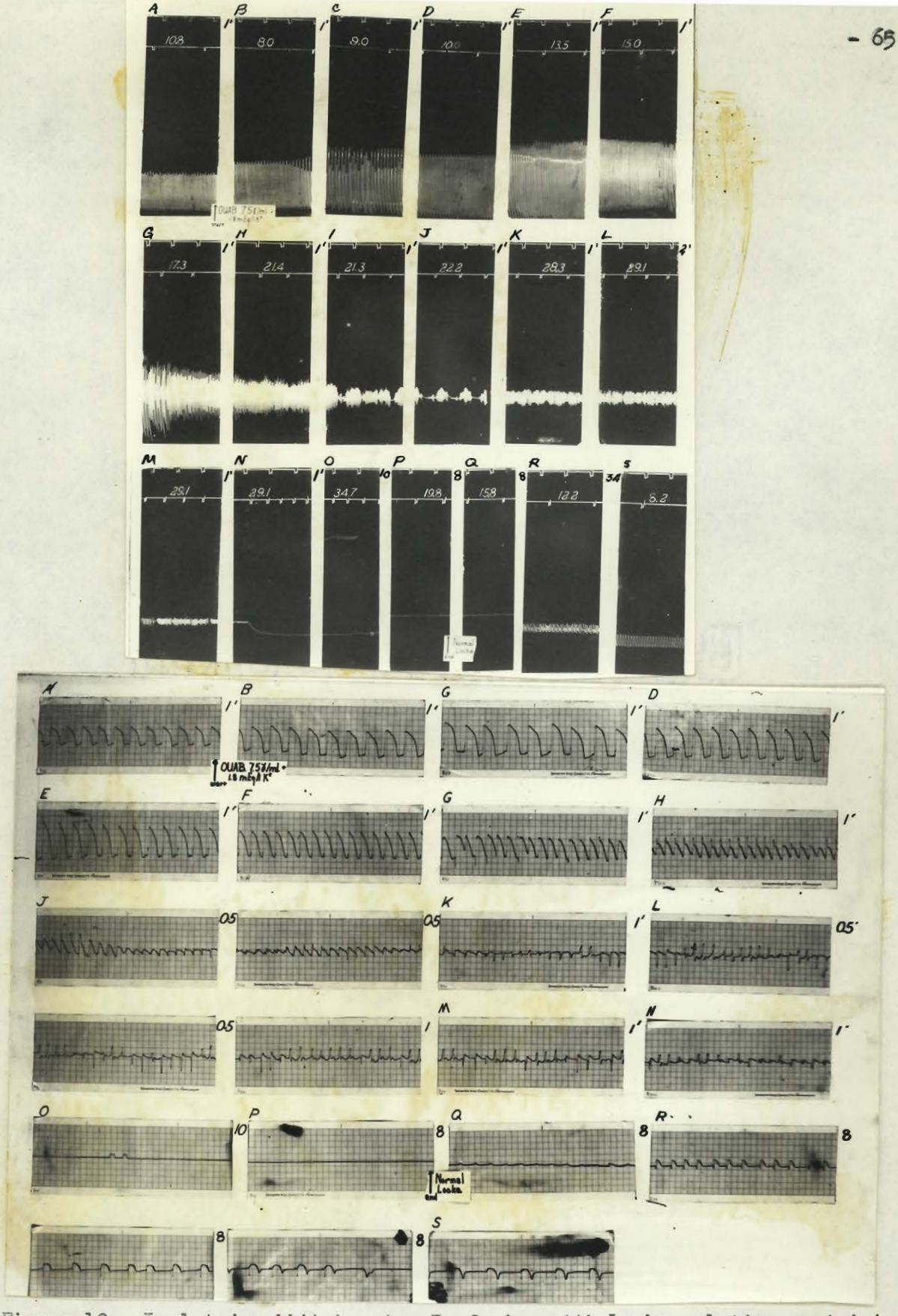
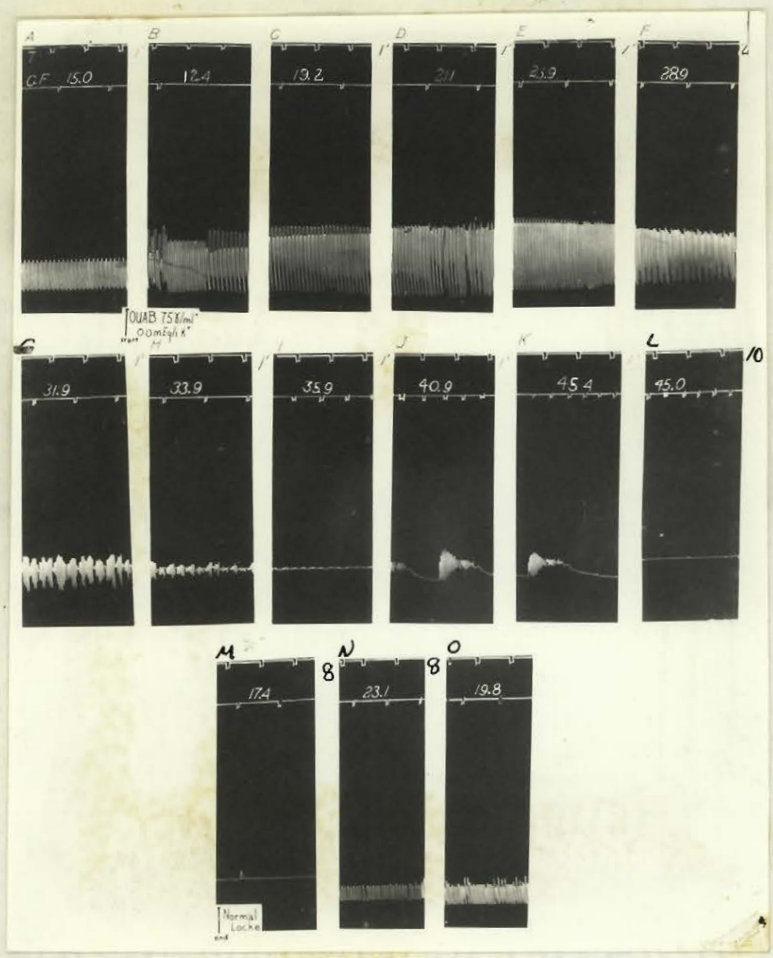


Figure 12. Isolated rabbit heart. Perfusion with Locke solution containing only 1.8 mEq./l. potassium and 7.5 γ/ml. ouabain. See legend of Figure 2. Upper and Lower Record: At first arrow (Start, Ouab. 7.5 γ/ml. + 1.8 mEq./l. K^+) the perfusing fluid was changed from normal Locke to Locke containing ouabain and a reduced amount of potassium (1.8 mEq./l.). At second arrow (end, Normal Locke) the modified ouabain solution was changed to normal Locke.



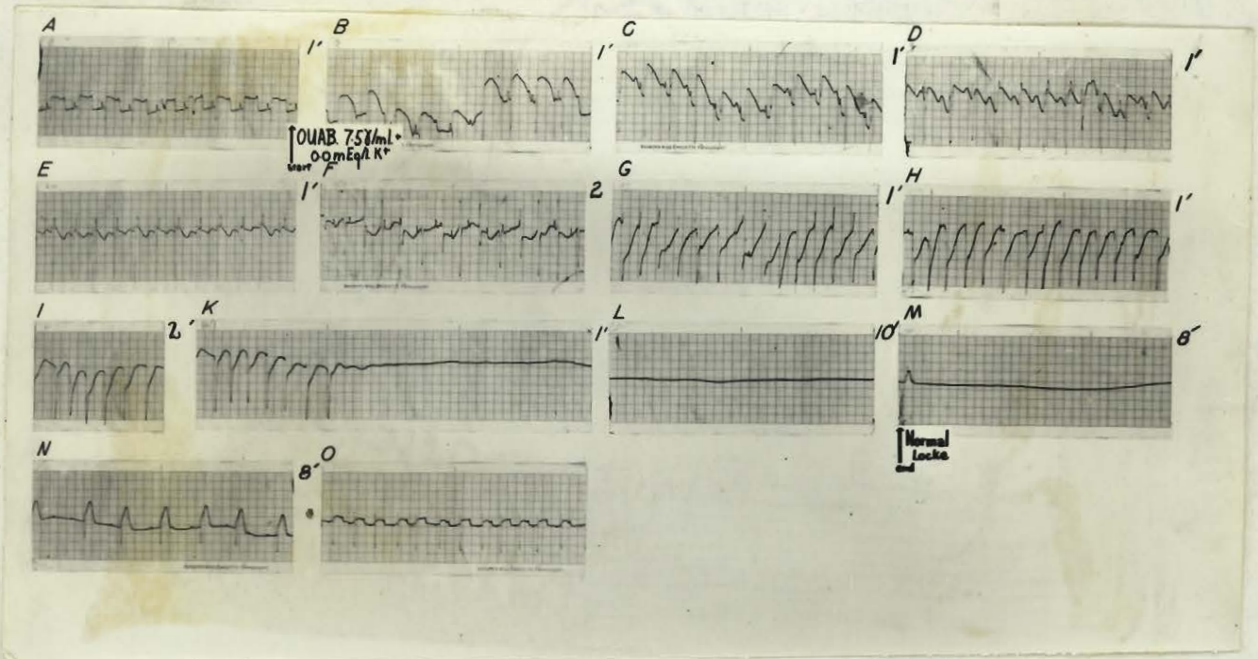


Figure 13. Isolated Rabbit Heart. Perfusion with potassium-free Locke solution with added ouabain (7.58 γ/ml.). See legend of Figure 2.
Upper and Lower Record: At first arrow (Start, Ouab. 7.5 γ/ml. + 0.0 mEq./l. K^+) the perfusion fluid was changed from normal Locke to Locke solution containing ouabain and no potassium. At the second arrow (end, Normal Locke) the modified ouabain solution was changed to normal Locke.

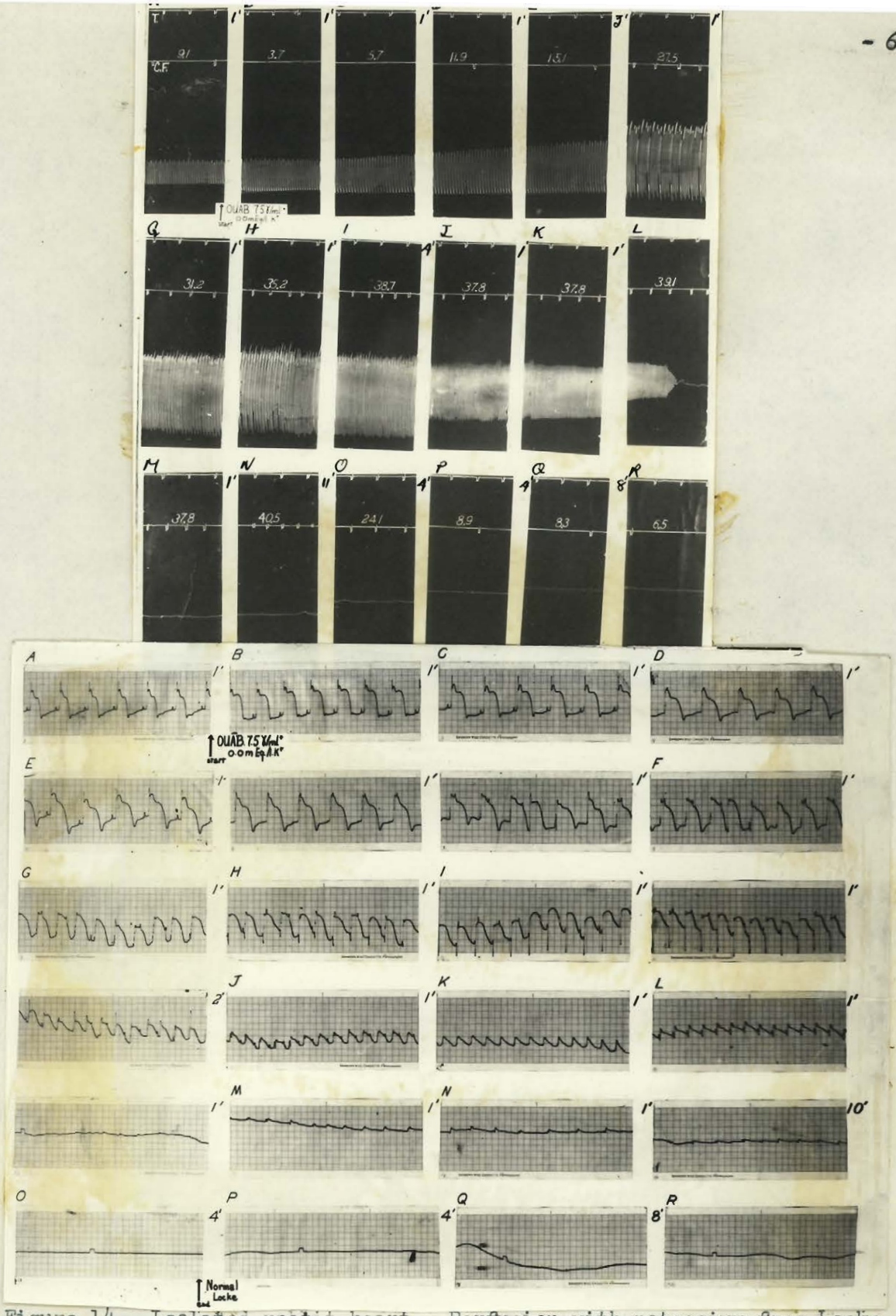


Figure 14. Isolated rabbit heart. Perfusion with potassium-free Locke solution with added ouabain (7.58 γ/ml.). See legend of Figure 2. Upper and Lower Record: At first arrow (Start, Ouab. 7.5 γ/ml. + 0.0 mEq./l. K^+) the perfusion fluid was changed from normal Locke to Locke solutation containing ouabain and no potassium. At second arrow (end, Normal Locke) the modified ouabain solution was changed to normal Locke.

the cat's heart (Figure 14) or the rabbit's heart (Figure 13) is perfused with ouabain in potassium-free Locke solution. In both instances, it is evident that there was only a brief initial coronary constriction followed by intense coronary dilatation, associated with increasing degrees of cardiac stimulation—ventricular extrasystoles and tachycardia and finally ventricular fibrillation (Figure 13 **K** and Figure 14 **L**).

When the perfusing fluid was then changed to normal Locke, in the case of the cat's heart (Figure **14**), there was no evidence of restitution of heart activity (P to R), although the coronary flow decreased to the normal rate. On the other hand, in the case of the rabbit's heart, (Figure **13**), following an initial delay (eight minutes), there was a restoration of the P-waves (N) and finally a shifting sinus pacemaker (O).

In comparison with the changes observed when ouabain is perfused in normal Locke's solution, it would appear that, in the presence of a <u>decreased potassium</u> concentration (a) there is less of the initial bradycardia and associated coronary constriction, (b) the subsequent coronary dilatation is greatly enhanced, and (c) recovery of the heart by perfusion with normal Locke solution, occurred more slowly in the rabbit's heart, and could not be achieved in the cat's heart.

These findings combined with the fact that the rapidity of the development of cardiac standstill is increased, would rather suggest that decreasing the potassium concentration in the perfusing fluid, enchances the cardiac effects and toxicity of ouabain under these conditions. However, in the case of the potassium-free experiments, no definite conclusions can be drawn, since, as already pointed out, perfusion with potassium-free Locke's without ouabain, leads to ventricular fibrillation in an average of 13 minutes (three experiments).

<u>(d) Effects on Cardiac Potassium Balance.</u>

Average changes in coronary flow and potassium movement during ouabain

perfusion in the presence of increased or decreased potassium concentrations are illustrated in Figures 15 to 19 and the data summarized in Tables VI and VII. In most instances, the corresponding changes induced by the altered potassium concentrations (without ouabain) are also indicated.

Figure 15 shows that in the presence of an excessive concentration (16.8 mEq./l.) of potassium, there is an immediate increased uptake of potassium concomitant with a decreased coronary flow, in both the "ouabain" and "control" experiments. It would appear that there is a less marked uptake in the presence of ouabain (upper graph) as compared with the control (lower graph). However, due to the rapid changes involved, leading to cardiac arrest within three to four minutes, coupled with the fact that only three experiments were done in each case, the exact significance of this is not clear.

When the perfusing fluid was then changed to normal Locke solution in the ouabain arrested heart, there was a rapid coronary dilatation associated with a net gain of potassium. In the control experiment, however, there was only a slight increase in the coronary flow and no net difference in potassium balance occurred.

In Figure 16, one sees the results of similar experiments in which the potassium concentration employed was 11.2 mEq./l., i.e. twice the normal potassium in Locke solution. It is clear that in the presence of ouabain (upper record), there is an initial net uptake of potassium associated with a marked and sustained decrease in coronary flow during the first six minutes of perfusion. Although the coronary constriction persisted, there was a subsequent slight net loss of potassium until arrest ensued. It is to be noted that this net loss of potassium occurred despite the high concentration in the perfusing fluid. In the absence of the ouabain (lower record), there was a rapid equilibrium of the potassium exchange but this

TABLE VI

Mean Recorded Changes in Coronary Inflow Rate (ml./min.) During Perfusion with Ouabain (7.5 γ/ml.) in Varying Concentrations of Potassium.

Time (min.)	Potassium Concentration (mEq./l.)									
	16.8 Ouab.	16.8 Cont.	11.2 Ouab.	11.2 Cont.	6.16 Ouab.	6.16 Cont.	2.80 Ouab.	1.40 Ouab.	0.00 Ouab.	0.00 Cont.
Control (ml/min)	22.4	19.6	19.0	15.2	13.8	14.6	15.6	16.9	13.6	17.0
1	-5.68	-10.95	-8.88	-	+5.57	-	-6.00	-3.57	-4.03	+0.60
2	-0.87	-13.05	-9.30	-	-	-	-1.87	-2.40	+2.20	+2.80
3	-8.89	-12.95	-9.07	-	-	-	+0.93	+4.63	+4.76	+2.70
4	-10.36		-8.83	-1.40	+3.53	0.00	+2.13	+4.97	+8.28	+3.20
5	-10.97		-8.55	-	+4.67	-	+7.13	+7.70	+12.37	+4.80
6			-8.87	-	+5.43	-	+8.53	+10.00	+15.00	+11.80
7			-9.00	-	+6.93	-	+11.43	+14.30	+17.80	+20.10
8			-9.18	-1.10	+7.57	+0.95	+11.97	+16.97	+19.70	+20.00
9			-8.62	-	+7.37	-	+15.73	+18.23	+24.28	+20.10
10			-8.50	-	+7.87	-	+15.50	+16.03	+27.90	+21.90
11			-8.83	-	+10.60	-	+15.57	+18.53	+27.87	+22.10
12			-9.35	-2.85	+10.55	-		+18.53	+27.34	+24.10
13			-9.87	-	+11.10	-		+18.67	+24.93	+24.10
14			-7.65	-	+10.30	-			+24.73	
15			-6.95	-		-			+28.70	
16				-4.55		+0.75				
20				-5.60		-				
24				-6.35		+0.60				
32				-7.25		+0.70				
40				-8.60		-0.30				
44				-8.75		-				
48				-9.15		+0.30				
52				-8.30						
No.Exp. per Mean	3	3	6	2	3	2	3	3	6	3

TABLE VII

Mean Calculated Differences (Microequivalent per 10 sec.) Between Potassium Inflow and Potassium Outflow During Perfusion with Ouabain (7.5 γ/ml.) in Varying Concentrations of Potassium.

Time (min.)	Potassium Concentration (mEq./l.)									
	16.8 Ouab.	16.8 Cont.	11.2 Ouab.	11.2 Cont.	6.16 Ouab.	6.16 Cont.	2.80 Ouab.	1.40 Ouab.	0.00 Ouab.	0.00 Cont.
Control (ml/min)	-0.17	+0.58	+0.13	-0.22	-0.08	-0.04	-0.39	-0.01	-0.05	+0.15
1	+8.07	+18.36	-	-	-	-	-3.16	-	-	-4.26
2	+2.49	+3.92	+1.20	-	+1.11	-	-	-1.99	-1.68	-1.51
3	+1.15	-	-	-	-	-	-2.78	-	-	-
4		+1.29	+0.74	-0.57	+1.28	0.00	-	-1.93	-1.64	-0.88
6		+1.58	+0.38	-	+1.48	-	-1.67	-0.96	-0.52	-0.98
8			-0.20	-	-1.44	-0.17	-1.78	-1.35	-0.76	-0.15
10			-0.13	-	-1.27	-	-1.17	-0.88	-0.77	-0.19
12			-0.23	+0.38	-1.46	-	-2.08	-0.04	-0.20	-1.00
14			-0.09	-	-2.09	-		-0.60	-0.20	
16				-	-1.53	-0.08		+0.02		
20				+0.13		-				
24				-		-0.11				
28				-0.19		-				
32				-		-0.15				
36				-0.07		-				
40				-		-0.13				
44				+0.01		-				
48				-		-0.19				
52				-0.22		-				
No. Exp. per Mean	1	1	6	2	3	2	3	3	6	3

Cont. = Perfusion with varying potassium without added ouabain.
Ouab. = Added ouabain.

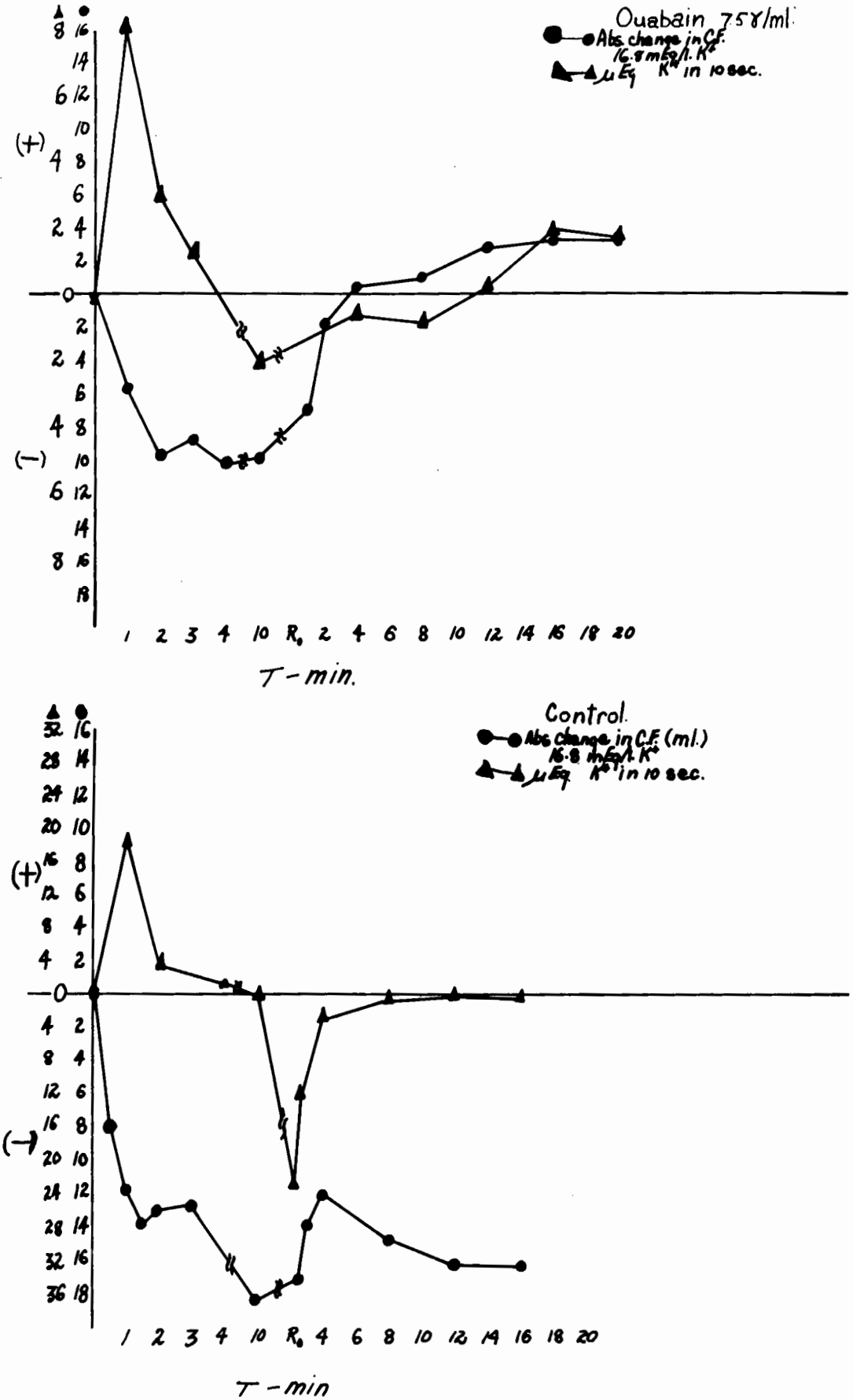


Figure 15. See legends of Figures 6 and 7.

Upper Graph: Ouabain - 7.5 γ/ml. added to Locke containing excess potassium (16.8 mEq./l.).

Lower Graph: Control (no ouabain added), perfused with Locke and excess potassium (16.8 mEq./l.).

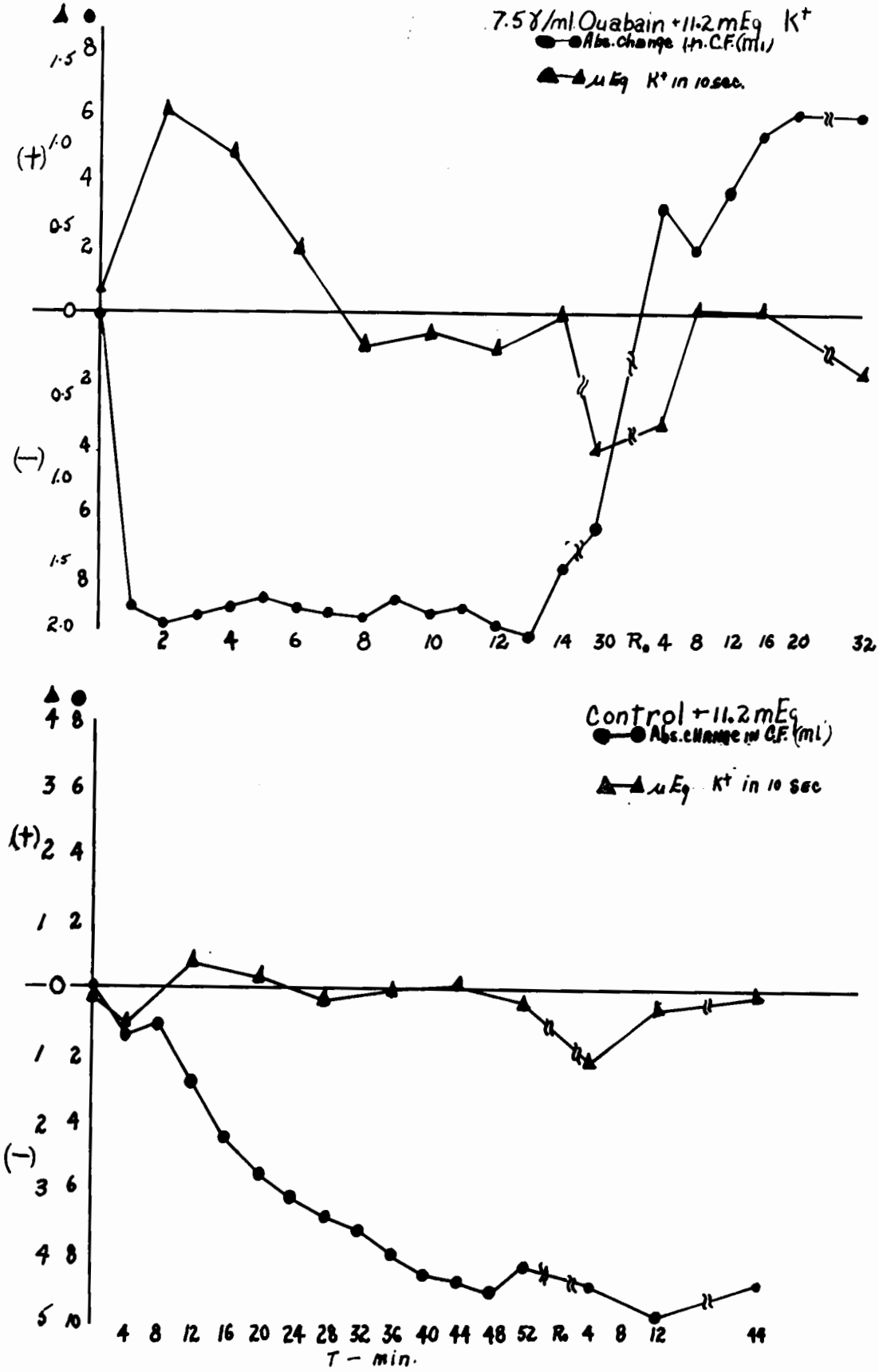


Figure 16. See legends of Figure 6 and 7.
Upper Graph: Ouabain - 7.5 γ/ml. added to Locke containing excess potassium (11.2 mEq./l.).
Lower Graph: Control (no ouabain added), perfused with Locke and excess potassium (11.2 mEq./l.).

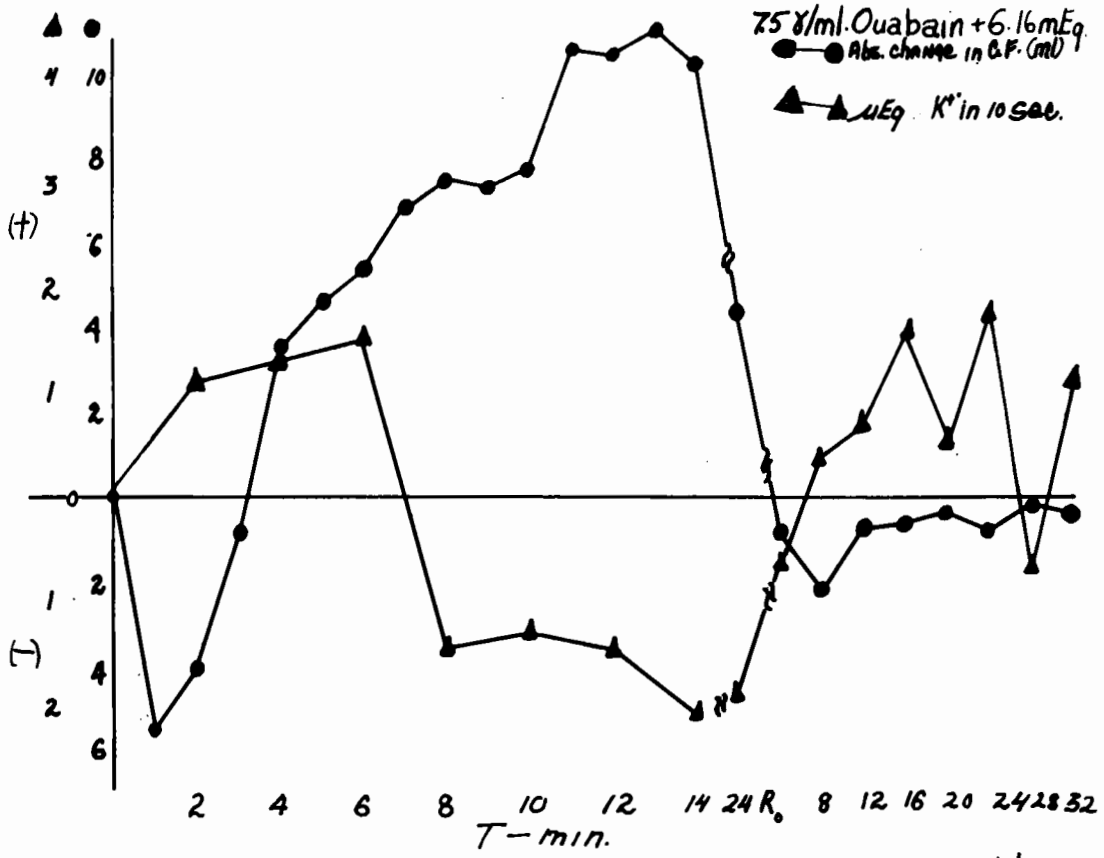


Figure 17. See legends of Figure 6 and 7.
Upper Graph: Ouabain - 7.5 γ/ml. added to Locke containing excess potassium (6.16 mEq./l.).
Lower Graph: Control (no ouabain added), perfused with Locke and excess potassium (6.16 mEq./l.).

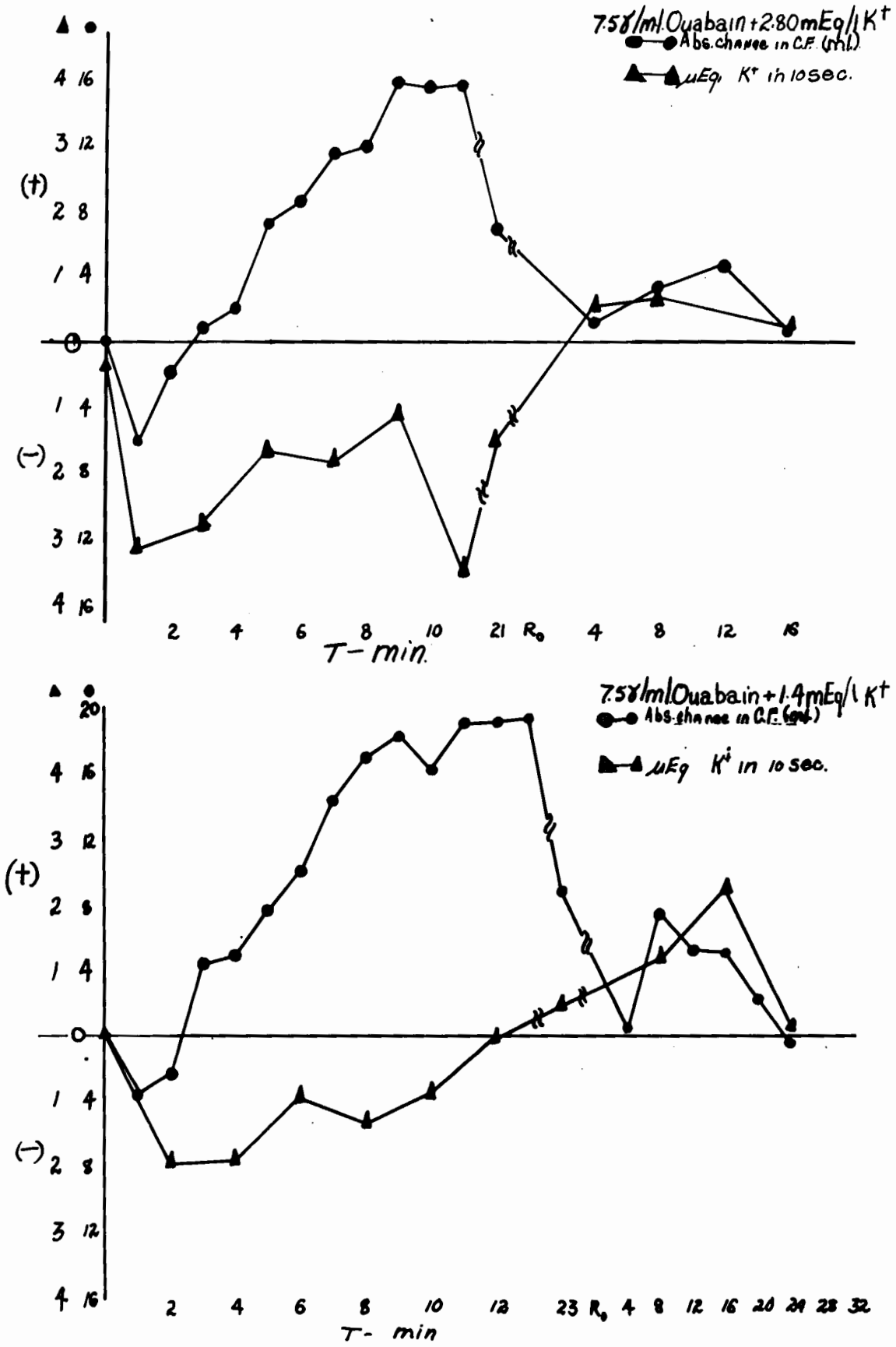


Figure 13. See legend of Figure 6 and 7.
Upper Graph: Ouabain - 7.5 γ/ml. added to Locke containing decreased potassium (2.80 mEq./l.).
Lower Graph: Control (no ouabain added), perfused with Locke containing decreased potassium (1.4 mEq./l.).

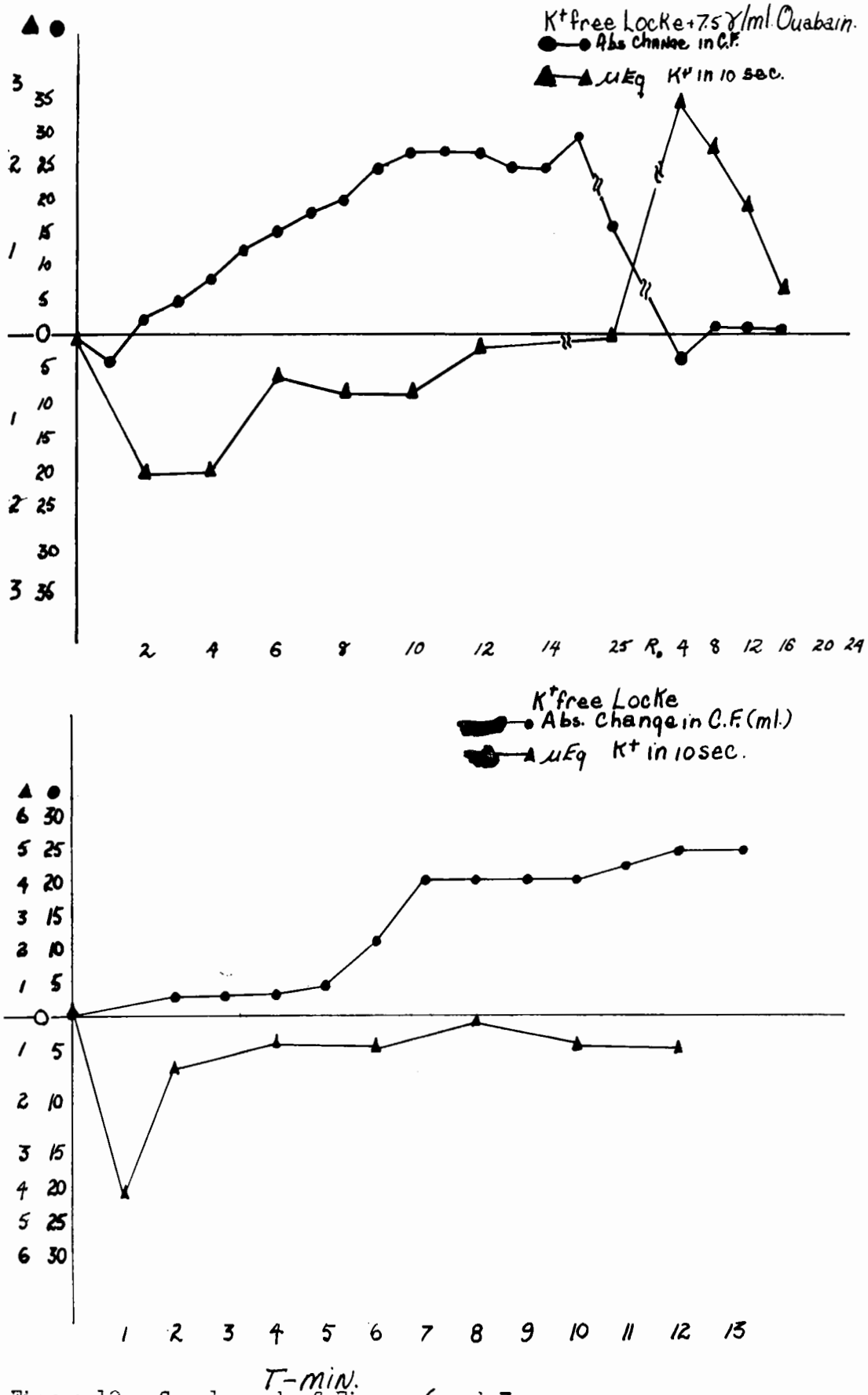


Figure 19. See legend of Figure 6 and 7.
Upper Graph: Ouabain - 7.5 γ/ml. added to potassium-free Locke.
Lower Graph: Control - no ouabain added - perfused with potassium-free Locke.

was associated with a slowly progressive coronary constriction, in contrast to the ouabain experiment (upper record).

When the perfusing fluid was then changed to normal Locke, there was again a marked and sustained coronary dilatation, associated with a lesser loss of potassium, with restoration almost to equilibrium.

The potassium changes, associated with ouabain are strikingly seen in Figure 17, when the potassium concentration was only slightly increased (ten per cent) to 6.16 mEq./l. As can be seen, during the ouabain perfusion (upper record) there was a net uptake of potassium for six minutes, followed by a marked sustained net loss, until cardiac arrest ensued. There was an associated initial coronary constriction (three minutes) followed by progressively increasing coronary dilatation until cardiac standstill developed. Whereupon changing the perfusing fluid to normal Locke solution led to a slow progressive positive potassium balance with some striking fluctuations, as shown in the figure. These latter changes were associated with coronary constriction. In the comparative control experiments (lower record), these changes in potassium movement and coronary flow were both almost neglegible.

It appears from the above observations that ouabain in the presence of an increased potassium concentration leads to an exaggerated coronary constriction, associated with a rapid initial increase in net uptake of potassium, followed in general, by a net loss <u>despite the high extracellular potassium concentrations</u>. Conversely, during recovery of the ouabainized heart under these conditions, there is a marked coronary dilatation, associated with a net increased potassium uptake. These findings would again indicate that under the influence of ouabain, there is a close correlation between the changes in potassium movement and those occurring in the coronary vascular bed.

Figure 18 shows concomitant changes in potassium movement and coronary flow during perfusion with ouabain and Locke solution containing 50 per cent of the normal potassium or 2.8 mEq./l. (above) and Locke solution containing 25 per cent of normal potassium or 1.4 mEq./l. (below). As can be seen in both groups, there was a sustained"net loss"of potassium associated with a brief initial coronary constriction followed by sustained coronary dilatation. These changes continued until cardiac standstill.

When the perfusion fluids were changed to normal Locke solution without ouabain, in both instances, again there was a rapid decrease in coronary flow associated with a positive potassium balance in the heart.

Finally, in Figure 19, one sees again, during perfusion with ouabain in potassium-free Locke (upper record), there is a brief initial coronary constriction followed by progressive and intense coronary dilatation. Throughout the experiment, also, there was a moderate negative potassium balance, reaching a maximum of minus 1.68 γ-equivalents per ten seconds, followed by progressive restoration to equilibrium. When the perfusing fluid was then changed to normal Locke (without ouabain) again one sees the sharp increase in potassium balance, associated with a decreased coronary flow prior to restoration to the initial equilibrium.

The lower section of the figure demonstrates that in the absence of ouabain, rather similar changes in potassium movement and coronary flow were obtained. Moreover, there was no significant difference in the duration of perfusion leading to cardiac arrest in the two series of experiments.

It would appear therefore, that in the presence of <u>decreased</u> potassium in the perfusing fluid, ouabain produces relatively less initial coronary constriction but greater subsequent coronary dilatation, and concomitantly no evidence of the usual initial uptake of potassium, but rather a continuous and sustained net loss.

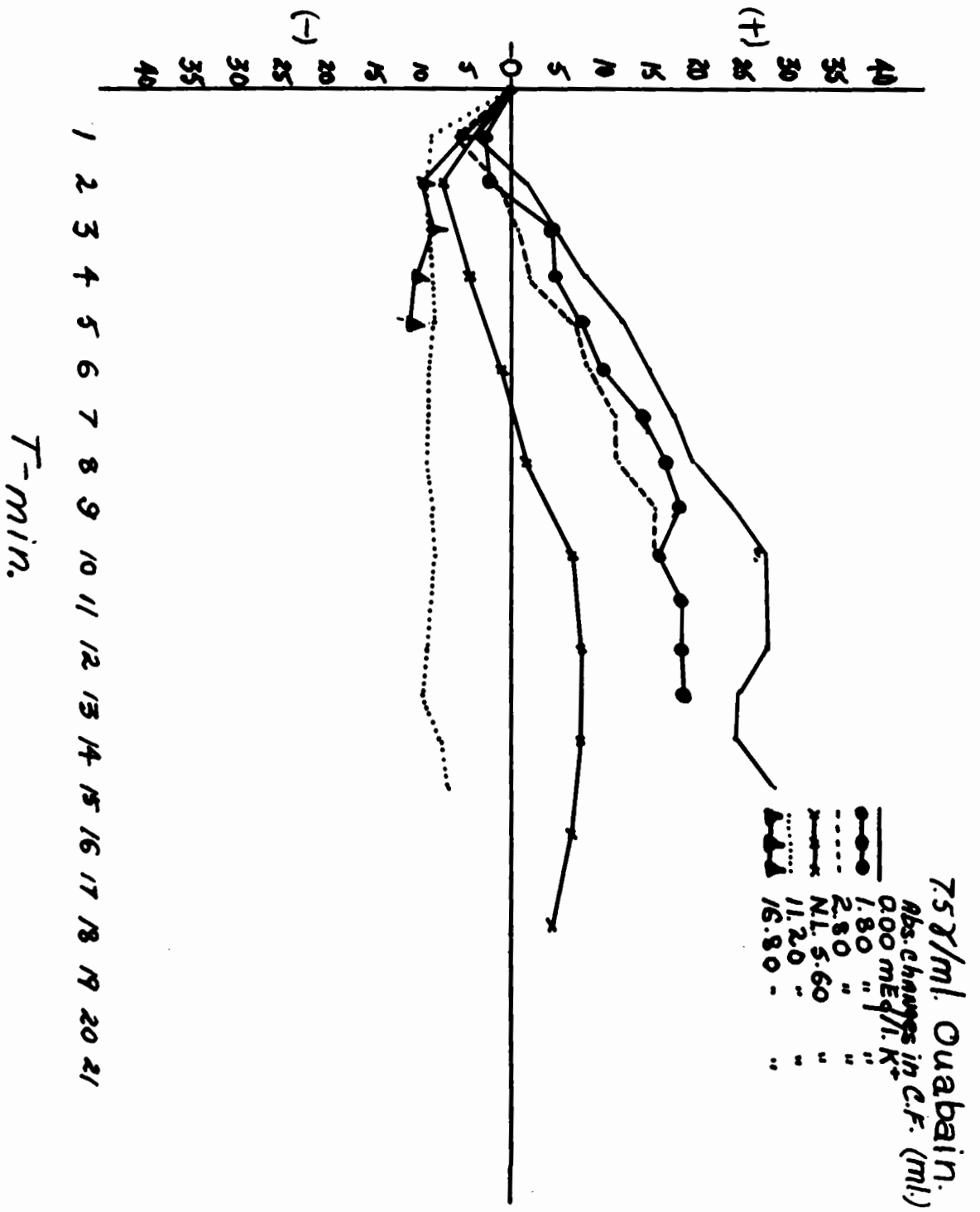


Figure 20. Graph summarizing coronary flow changes during perfusion with ouabain (7.5 γ/ml.) in varying concentrations of potassium. N.L. = normal Locke. mEq./l. K+ = concentration of potassium in the perfusing solution, as shown.

In Figure 20 are graphically summarized the relative changes in coronary flow during perfusions with Locke solution containing different potassium concentrations ranging from 16.8 mEq./l. (three times normal) to zero potassium (potassium-free). It is clear from this comparison that in the presence of ouabain, high concentrations of potassium in the perfusing fluid lead to only a coronary constriction, while with normal Locke (N.L. 5.6 mEq./l.) there is an initial transient constriction followed by dilatation. Finally, with progressively lower concentrations of potassium, the duration of the initial constriction was reduced, and subsequent coronary dilatation enhanced. These findings again demonstrate a close relationship between the effects of ouabain upon coronary flow and potassium equilibrium in the heart.

(e) Summary and Conclusions.

In the isolated rabbit and cat heart perfused with a fixed concentration (7.5 γ/ml.) of ouabain, **it was observed:**

1. That, during perfusion with excessive potassium (16.8 mEq./l.) in Locke, there is no significant difference between the average times required to lead to cardiac arrest, as compared with similar "control" experiments without ouabain. In both groups of experiments there was a rapid and sustained coronary constriction associated with a net uptake of potassium, but no coronary dilatation. Rapid ventricular fibrillation ensued in both groups.

2. That, with twice the normal potassium concentration (11.2 mEq./l.) added to Locke, the average period for cardiac arrest was 15.17 ±3.05 minutes, while in similar experiments without ouabain, the heart continued to beat relatively well, for at least 60 minutes of observation. During perfusions with ouabain (7.5 γ/ml.) in normal Locke, cardiac standstill occurred in 17.56 ±0.63 minutes.

3. That, during perfusion with twice normal potassium (11.2 mEq./l.), there is a prompt and sustained coronary constriction associated with initial marked increase in "net uptake" of potassium, followed by a slight "net loss". In the controls (without ouabain) there was a more gradual coronary constriction associated with little change in potassium equilibrium.

4. That, during perfusion with ten per cent excess potassium (6.16 mEq./l.) the toxicity of ouabain was enhanced, as judged by the average time required for cardiac arrest (13.0 ±1.53 minutes), and as compared with that required when normal Locke was the perfusion fluid (17.56 ±0.63 minutes). There was a brief coronary constriction followed by marked and sustained dilatation, and associated with an average "net uptake" of potassium, followed by a "net loss".

5. That, with all concentrations of potassium above that in normal Locke, there was a definite diminution in the tendency to the development of ventricular ectopic beats, although there was no significant increase in the perfusion times required for cardiac arrest.

6. That, with decreased potassium in the perfusing fluid, ouabain induced relatively less initial coronary constriction followed by greater coronary dilatation. Concomitantly, there was no evidence of the usual initial "net uptake" of potassium, but rather a continuous and sustained "net loss".

7. That, following cardiac arrest during perfusion with high potassium (16.8 and 11.2 mEq./l.) normal Locke perfusion led to rapid recovery, that is, initiation of ventricular activity associated with striking coronary dilatation. Conversely, after previous cardiac arrest with decreased potassium, recovery with normal Locke was definitely more delayed in the rabbit and absent in the cat.

It is therefore concluded that:

(a) Excess potassium in the perfusing fluid can antagonize cardiac

irregularties induced by ouabain, but no mutual antagonism appears to exist between the two agents in respect to the onset of cardiac standstill.

(b) Decreased potassium, but not complete absence of potassium, augmented cardiac irregularities and enhanced cardiac standstill in comparison with controls.

(c) The findings substantiate the view that under the influence of ouabain, there is an increased uptake of potassium, which is responsible for the initial coronary constriction, while the coronary dilatation appears to depend upon the concomitant potassium loss.

III. OBSERVATIONS ON THE EFFECTS OF VARYING CONCENTRATIONS OF CALCIUM IN THE PERFUSING FLUID UPON THE RESPONSES (CORONARY FLOW, HEART ACTION AND ELECTROLYTE MOVEMENTS) TO OUABAIN.

(a) Effects Upon Ouabain Toxicity and Recovery.

In all experiments in this series, a fixed concentration (7.5 γ/ml.) of ouabain was again employed. Figure 21 and Table VIII show comparisons of the average durations of perfusion required to reach cardiac standstill (mechanical arrest), when the hearts of rabbits were perfused with this concentration of ouabain in Locke solution. In these experiments, the calcium concentration was either increased (as high as 16.8 mEq./l.) or decreased (to zero). The figure and table also show the findings in a similar group of experiments from which both the calcium and potassium were removed from the perfusing fluid. Three hearts were studied in each group in which the altered Locke solution was perfused.

These data show clearly that there is no significant difference between the toxicity of ouabain (7.5 γ/ml.), whether perfused (a) in normal Locke (N.L.),or (b) in calcium-free Locke, or (c) in Locke containing four times the normal calcium, i.e. 16.8 mEq./l., or (d) in both calcium-free and potassium-free Locke. It is therefore evident that even wide changes in the calcium concentration in the perfusing fluid, do not significantly potentiate or antagonize digitalis toxicity.

In regard to the question of recovery, when the perfusing fluid was changed to normal Locke (without ouabain) following cardiac arrest, as can be seen in Table VIII, there were however some differences noted. Thus, in the absence of calcium during the ouabain perfusion, changing to normal Locke led to restoration of regular auricular activity after a period of 36 minutes in one experiment, but there was no evidence of ventricular restoration during a subsequent 15 minutes of perfusion, when the experiment

TABLE VIII

Observed Effects During Perfusion with Ouabain (7.5 γ/ml.) in Varying Concentrations of Calcium.

Concentration (mEq./l.)	Cardiac Arrest (min.)	Recovery* with Normal Locke
0 Ca^{++}	14	No Recovery (auricular beat after 36 min.)
0 Ca^{++}	14	No Recovery (54 min.)
0 Ca^{++}	16 Avg. 14.67 ±0.22	No Recovery (30 min.)
16.8 Ca^{++}	12	Recovery in 16 min.
16.8 Ca^{++}	16	Recovery in 12 min.
16.8 Ca^{++}	17 Avg. 15.00 ±1.53	Recovery in 12 min.
0 Ca^{++}, 0 K^{+}	17	No Recovery (53 min.)
0 Ca^{++}, 0 K^{+}	18	No Recovery (16 min.)
0 Ca^{++}, 0 K^{+}	12 Avg. 15.67 ±1.86	Atypical coronary flow, no attempted recovery.

* The term "Recovery" means 'initiation of visible ventricular improved contractions'.

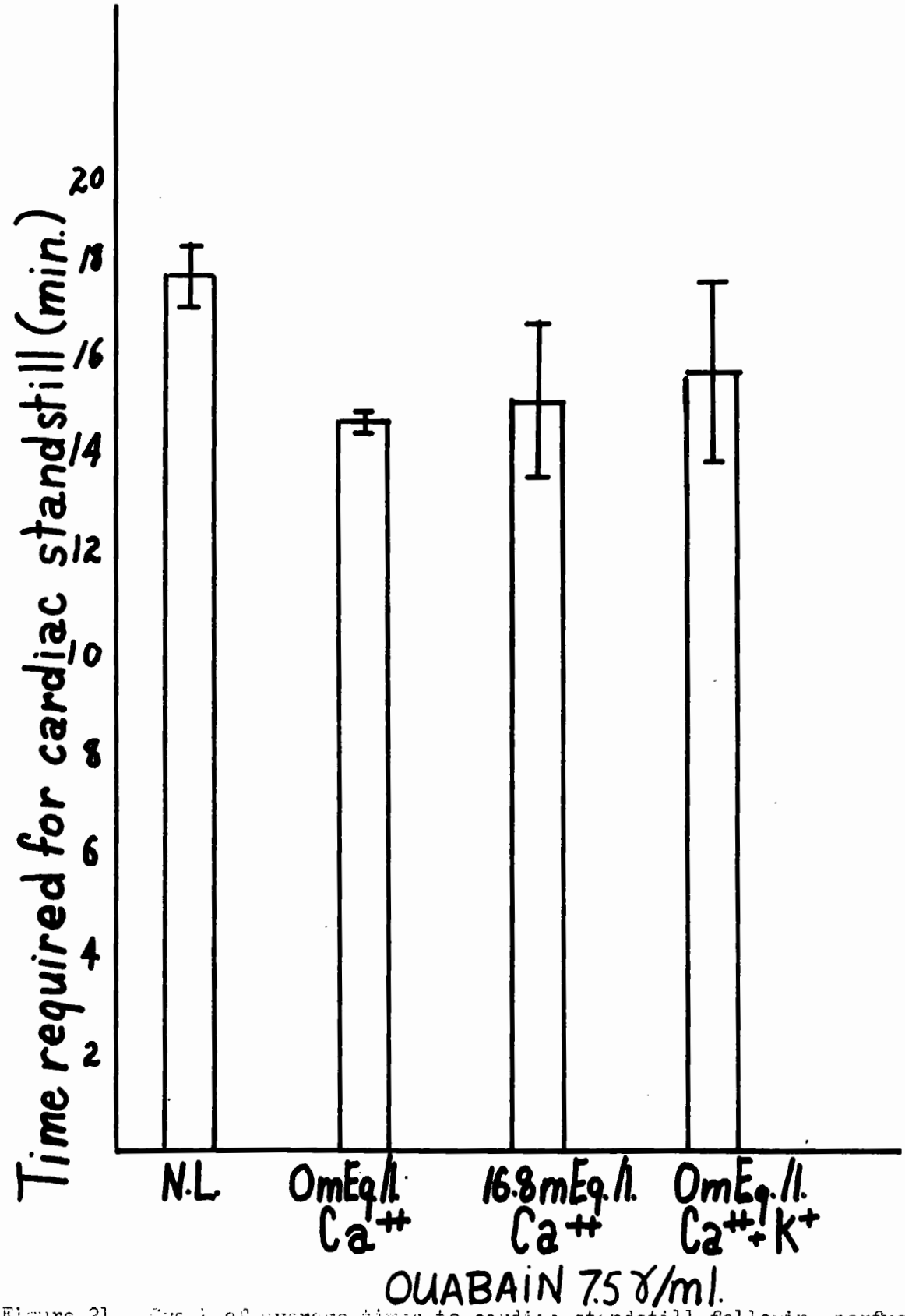


Figure 21. Graph of average times to cardiac standstill following perfusions with the same ouabain concentration in Locke solution containing either increased calcium (16.8 mEq./l. Ca^{++}) or decreased calcium (0 mEq./l. Ca^{++}) or decreased calcium and potassium (0 mEq./l. Ca^{++} + K^{+}). Standard errors also shown. See Text.

was terminated (see Figure 23, below). In addition with the two other hearts in this group, as well as those in the group in which both calcium and potassium were omitted from the ouabain perfusion fluid, there was no evidence whatsoever of recovery under normal Locke, even after continuing perfusion for as long as 50 - 60 minutes.

On the other hand, following perfusion with ouabain in Locke with excess calcium, recovery of the hearts in 12 to 16 minutes occurred when the perfusing fluid was changed to normal Locke. It has been previously shown (Mazurkiewicz, 1955) that following similar perfusions of isolated rabbit hearts with both calcium-free Locke or excess calcium for 30 - 50 minutes, that changing the perfusing fluid to normal Locke led to rapid restoration of the heart. Since, as previously pointed out (Section II), recovery also occurred in all experiments in which the rabbit heart was perfused with ouabain containing either high or low potassium concentrations, it is clear that digitalization of the heart in the absence of calcium leads to an irreversible type of toxicity.

Typical examples of the records obtained in the above experiments are shown in Figures 22 and 23. As can be seen from Figure 22, during ouabain perfusion in a high calcium concentration (16.8 mEq./l.), there is the usual initial coronary constriction (lasting for eight minutes) followed by coronary dilatation, continuing until cardiac standstill. Concomitantly the heart contractions were progressively stimulated with the development of gross irregularities and tachycardia, followed by standstill (L and M). The associated electrograms show the usual initial sinus bradycardia, with disappearance of P-waves and ST-T changes (D), followed by ventricular tachycardia (E to G) with multifocal ectopic beats (H and I). It is to be noted that in these experiments, there were frequent intervals of transient fibrillation before final cardiac arrest (I and J).

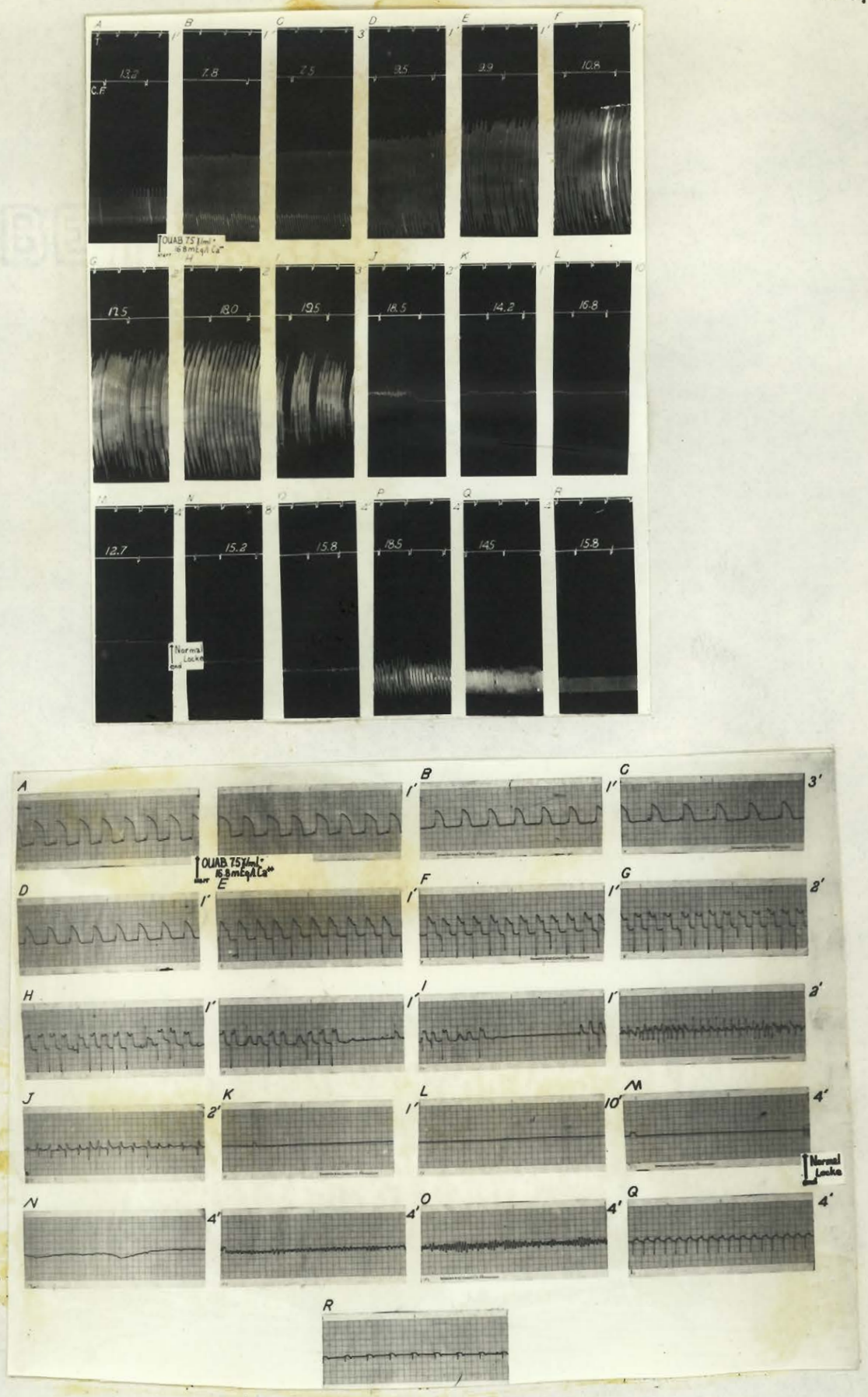
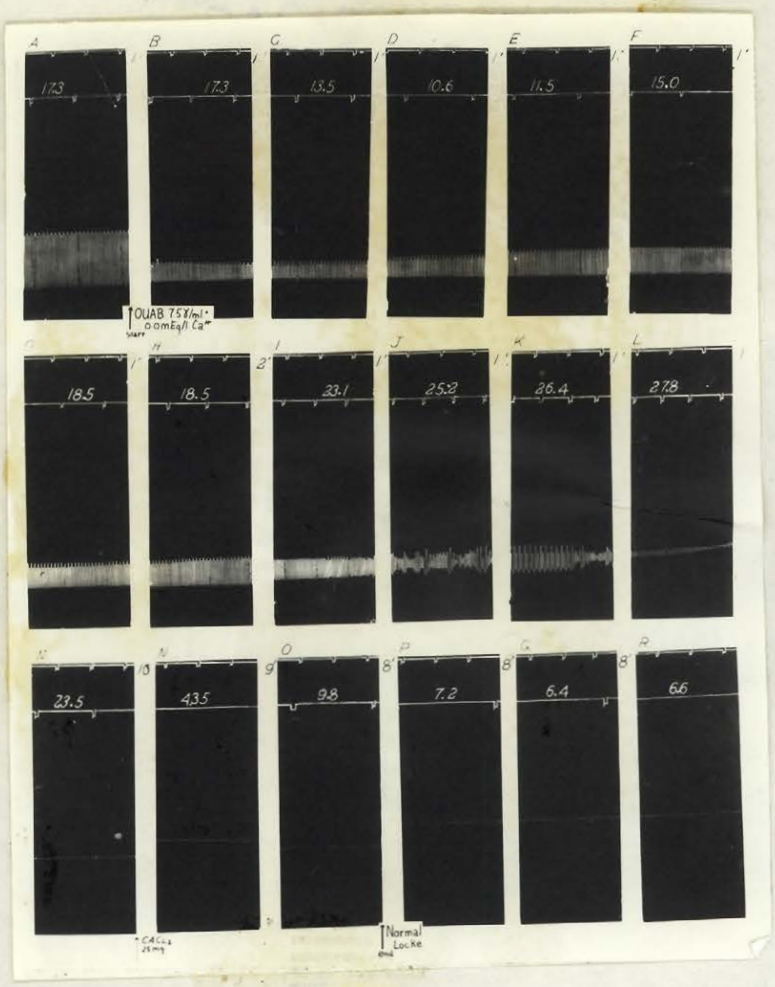


Figure 22. Isolated rabbit heart. Perfusion with Locke solution containing 16.8 mEq./l. calcium and 7.5 γ/ml. ouabain. See legend of Figure 2. Upper and Lower Record: At first arrow (Start, Ouab. 7.5 γ/ml. + 16.8 mEq./l. Ca^{++}) the perfusing fluid was changed from normal Locke to Locke containing added ouabain plus excess calcium. At the second arrow (end, normal Locke) the ouabain perfusion was changed to normal Locke.



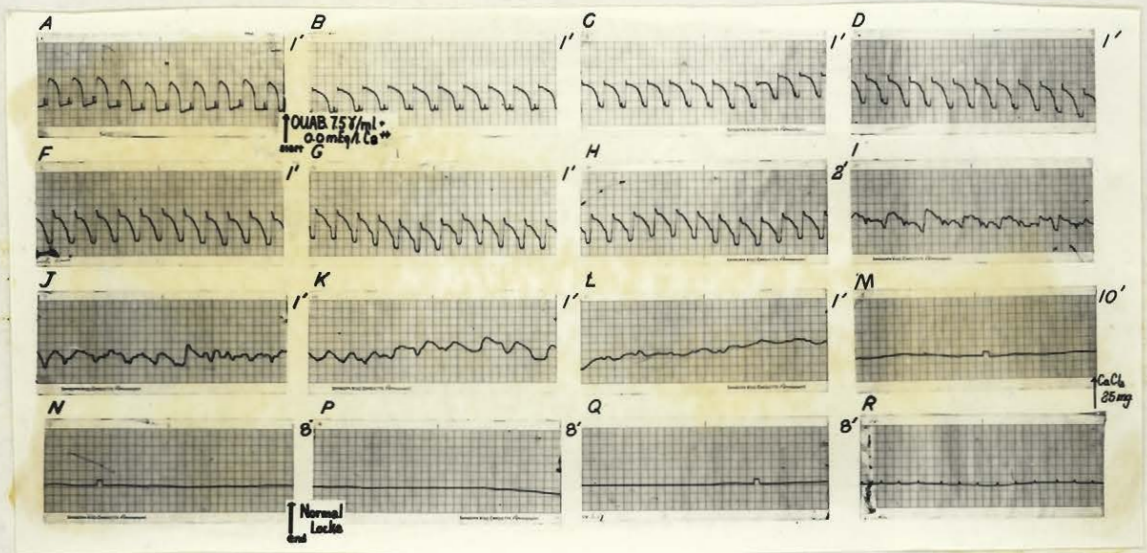


Figure 23. Isolated rabbit heart. Perfusion with calcium-free Locke with added ouabain (7.5 γ/ml.). See legend of Figure 2.
Upper and Lower Record: At first arrow (Start, Ouab. 7.5 γ/ml. + 0.00 mEq./l. Ca^{++}) the perfusing fluid was changed from normal Locke to calcium-free Locke and ouabain. At second arrow ($CaCl_2$, 25 mg.) an injection of 25 mg. of calcium chloride was administered. At third arrow (end, Normal Locke) the ouabain perfusion was changed to normal Locke.

When the heart was subsequently perfused with normal Locke (Figure 22) there was gradual development and intensification of ventricular fibrillation (N and O), followed by ventricular tachycardia (Q) and restoration to normal sinus rhythm with slowing. Associated with this recovery, there was a return of normal coronary flow.

In contrast (Figure 23), when the heart is perfused with ouabain in calcium-free Locke, there was again initial progressive coronary constriction (six minutes) followed by progressive dilatation with cardiac standstill. However, associated with these changes, there was neither the initial sinus bradycardia, nor the usual subsequent augmentation of the amplitude of the heart contraction. The electrograms show disappearance of the P waves at F, with progressive tachycardia and fibrillation (I to M). It should also be noted that an injection of a large dose of calcium chloride (25 mg.), as shown on the record at M, led to no effect. Subsequent perfusion with normal Locke resulted in the appearance of **small contractions at regular intervals (either P waves or feeble ventricular contractions).**

It would appear from these findings that in the presence of excess calcium, ouabain leads to accentuated cardiac stimulation with increased arrhythmias. The tendency to the development of ventricular fibrillation of a transitory type also occurred. On the other hand, in the presence of decreased calcium, ouabain leads to no significant sustained stimulation of the heart but only curious preterminal arrhythmias and fibrillation. However, under both conditions the effects were associated with the usual brief coronary constriction followed by sustained coronary dilatation.

(b) Effects on Cardiac Potassium Balance.

The average changes in potassium movement and coronary flow in the above groups of experiments are summarized in Tables IX and X, and the correlated changes in Figure 24. As is evident from the data under these

TABLE IX

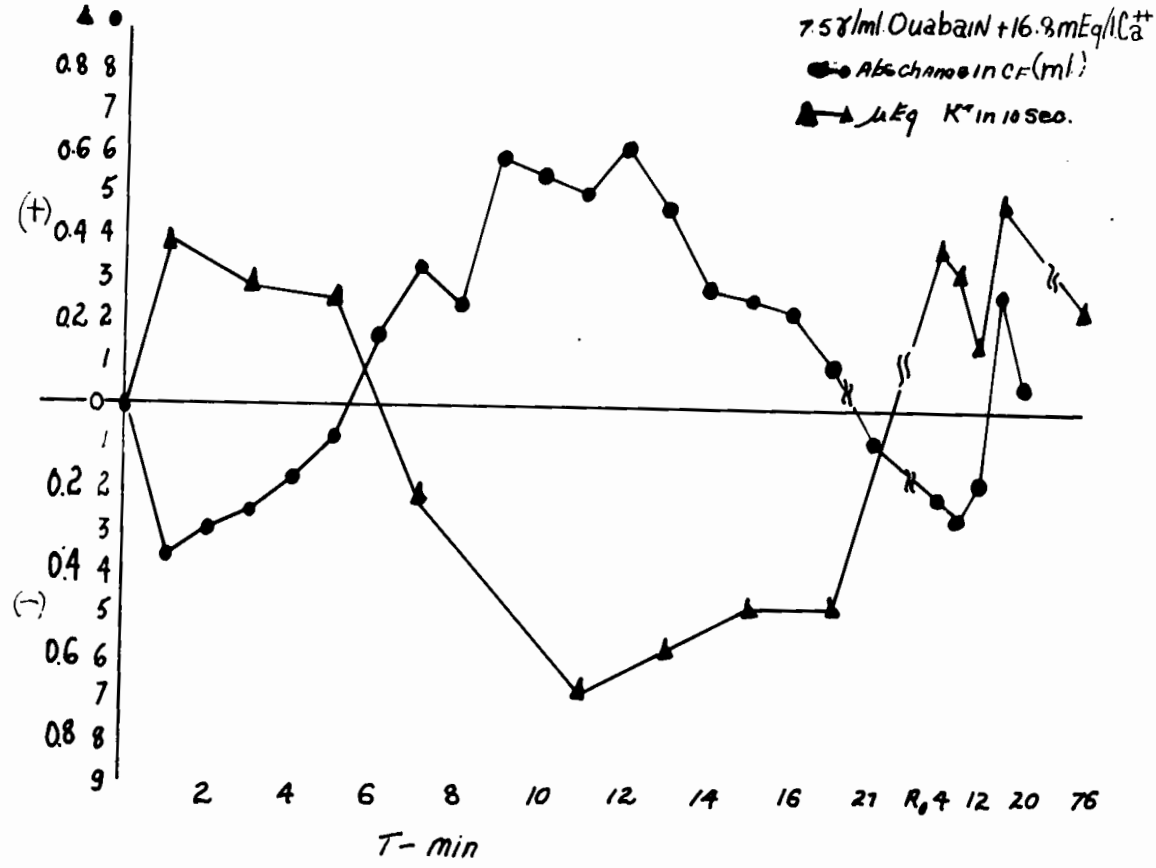
Mean Recorded Changes in Coronary Inflow Rate (ml./min.) During Perfusion with Ouabain (7.5 γ/ml.) in Varying Concentrations of Calcium.

Time (min.)	Locke with 16.8 mEq./l. Ca^{++}	Locke with Zero Ca^{++}	Locke with Zero Ca^{++} and Zero K^{+}
Control (ml./min.)	13.9	14.4	13.3
1	-3.60	-2.53	-2.18
2	-3.00	-4.24	-4.12
3	-2.57	-5.87	+3.52
4	-1.80	-5.46	+10.68
5	-0.77	-4.19	+13.78
6	+1.70	-1.63	+16.23
7	+3.30	-1.92	+18.29
8	+2.40	+1.79	+19.43
9	+5.97	+3.59	+18.89
10	+5.50	+4.75	+20.30
11	+5.07	+6.16	+20.65
12	+6.20	+6.83	+22.20
13	+4.77	+9.15	+22.00
14	+2.80	+3.56	+22.00
15	+2.55	+3.09	+22.00
16	+2.35		+22.00
No. Hearts per mean	(3)	(3)	(2)

TABLE X

Mean Calculated Differences (Microequivalents per Liter per 10 sec.) Between Potassium Inflow and Potassium Outflow During Perfusion with Ouabain (7.5 γ/ml.) in Varying Concentrations of Calcium.

Time (min.)	Locke with 16.8 mEq./l. Ca^{++}	Locke with Zero Ca^{++}	Zero with Zero Ca^{++} and Zero K^{+}
Control (ml./min.)	0.00	-0.12	-0.11
1	+0.38	+0.54	-1.82
3	+0.28	+0.24	-1.43
5	-0.25	+0.06	-1.13
7	-1.35	-0.58	-0.68
9	-1.75	-1.19	-0.49
11	-1.95	-2.78	-0.65
13	-2.15	-2.38	-0.80
15	-1.80	-2.08	-0.56
No. Hearts per Mean	2	2	1



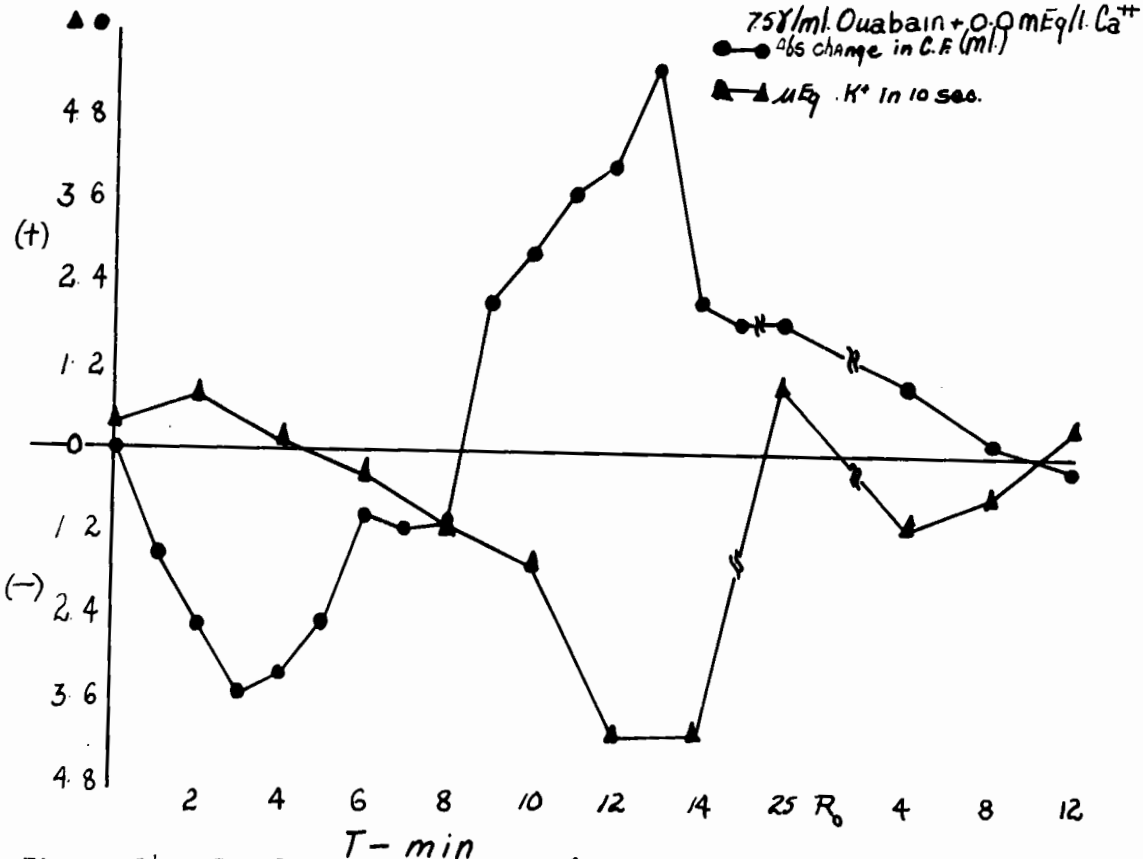


Figure 8: See legends of Figures 6 and 7.
Upper Graph: Ouabain - 7.5 γ/ml. added to Locke containing excess calcium (16.8 mEq./l.).
Lower Graph: Ouabain - 7.5 γ/ml. added to calcium-free Locke (0.0 mEq./l. Ca^{++}).

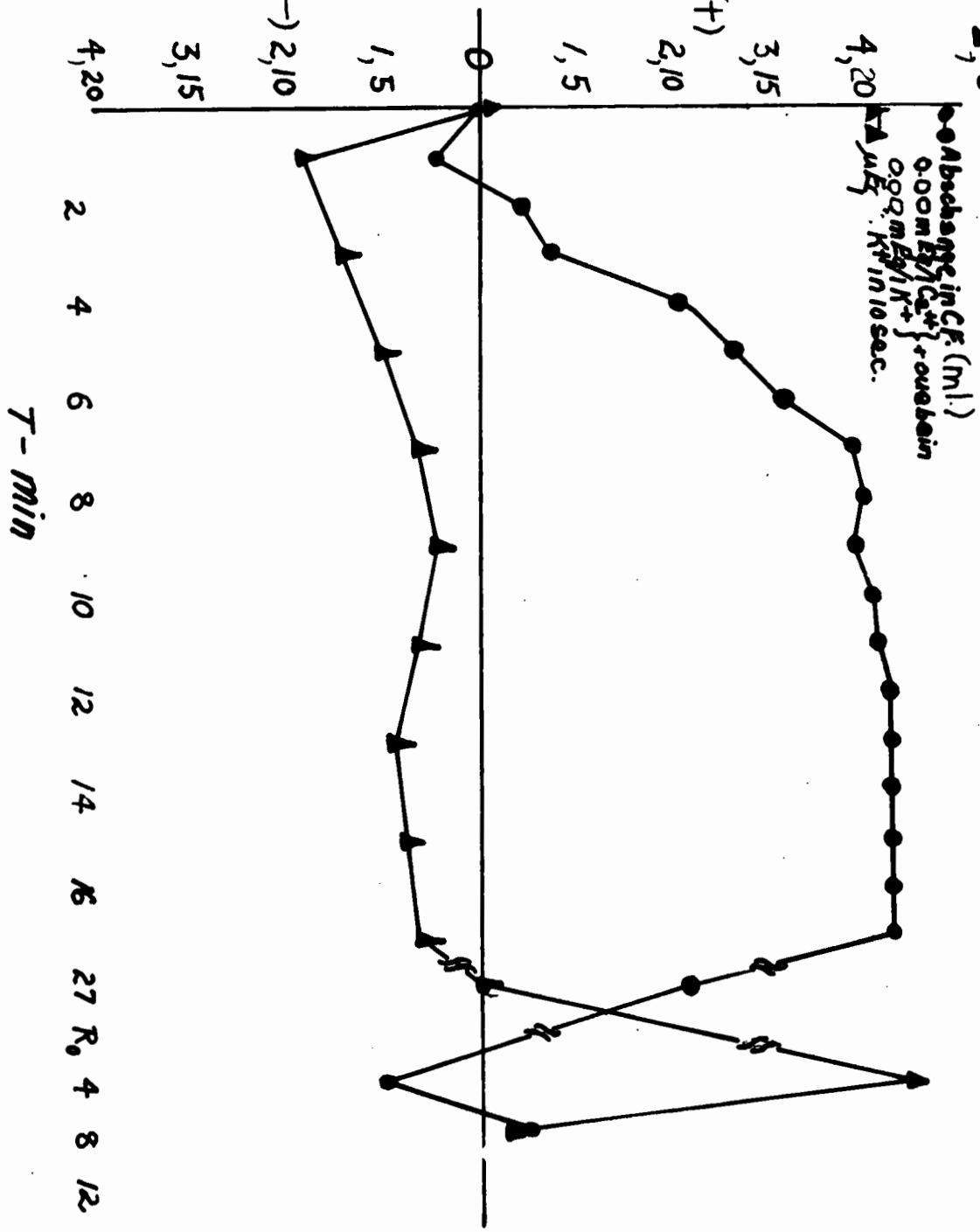


Figure 25. See legends of Figure 6 and 7.
Graph: Ouabain - 7.5 γ/ml. added to calcium-free, potassium-free Locke.

conditions, that is, with high and low calcium perfusions, ouabain induced the usual initial coronary constriction followed by coronary dilatation and concomitantly, a net gain of potassium followed by a net loss. Following arrest, perfusion with normal Locke again induced rapid decrease in coronary flow and a relative increased potassium uptake in both types of experiments. It is therefore obvious that these coronary and potassium changes induced by ouabain are not dependent on the presence or absence of calcium in the perfusing fluid.

Figure 25 shows a typical example of the effect of ouabain during perfusion with Locke solution from which both the calcium and potassium were omitted. It is again evident that after removal of both of these ions, there is a marked sustained coronary dilatation associated with a sustained negative potassium balance. Both of these effects were reversed, on perfusion with normal Locke during a period of eight minutes, although there was no evidence of subsequent recovery of either auricular or ventricular contractions as previously stated.

(c) Summary and Conclusions.

In the isolated rabbit heart, perfused with a fixed concentration (7.5 γ/ml.) of ouabain; it was observed:

1. That, during perfusion with either increased (16.8 mEq./l.) or decreased (zero) calcium, there was no significant difference between the average times required to lead to cardiac arrest.

2. That, perfusion with normal Locke led to recovery and resuscitation of the hearts, when previously arrested by ouabain in excess calcium; but, that no recovery ensued after similar arrest by ouabain in calcium-free or calcium-potassium-free Locke.

3. That, in the presence of excess calcium, accentuated cardiac stimulation and arrhythmias ensued, and conversely, in zero calcium, there was no sustained stimulation but only a short period of preterminal arrhythmias.

4. That, during perfusion with either increased calcium or calcium-free solutions, there was no significant deviation from the usually observed ouabain effects on coronary flow or potassium balance.

. It is therefore concluded that:

(a) The onset of ouabain cardiac standstill is not influenced by either calcium concentration per se, or by the calcium-potassium ratio in the perfusing fluid, although the appearance of arrhythmias was clearly modified by the presence or absence of calcium.

(b) With excess calcium, there was normal recovery of the heart but in the absence of calcium, ouabain toxicity was irreversible, suggesting either an enhanced fixation or retention of the glycosides in the absence of calcium.

(c) The effects of ouabain on the coronary flow and on the cardiac potassium equilibrium are not dependent on calcium.

IV. OBSERVATIONS ON THE EFFECTS OF OTHER TYPES OF IONIC ALTERATIONS (MAGNESIUM, LACTATE AND SODIUM) OF THE PERFUSING FLUID UPON THE CARDIAC RESPONSES TO OUABAIN.

(a) Effects of Added Magnesium.

In the first series of experiments, a comparison was made of the cardiac responses to relatively low concentrations of magnesium chloride (ranging from 0.009 to 0.364 mg. magnesium per ml. of Locke solution) perfused in four different groups of experiments. The average (five experiments) per cent changes in the recorded heights of amplitude of contractions, heart rates (per minute) and mean coronary inflow (ml. per minute), as were observed after five minutes of perfusion of isolated rabbit hearts with each of these solutions, are graphically shown in Figure 26.

As can be readily seen, with increasing concentrations of magnesium, there were increasing degrees of coronary dilatation. The observed changes in both the amplitude of contraction and the heart rate were not striking, although there was a definite trend towards increasing degrees of depression of contractions.

In order to determine the significance of the above responses, particularly those in amplitude and heart rate, the data from the different groups have been summarized and statistically analysed (Table XI). As can be seen from the table, neither the changes in amplitude nor those in heart rate were significant at the 0.05 level. On the other hand, the observed striking changes in coronary flow were highly significant.

It should be added that in all of these experiments, coronary dilatation occurred promptly after the perfusion fluid was changed from normal Locke solution to the magnesium-containing Locke, and this continued as long as the magnesium perfusion was maintained. However, depression in heart action developed more slowly, and the five minute observation period was arbitrarily

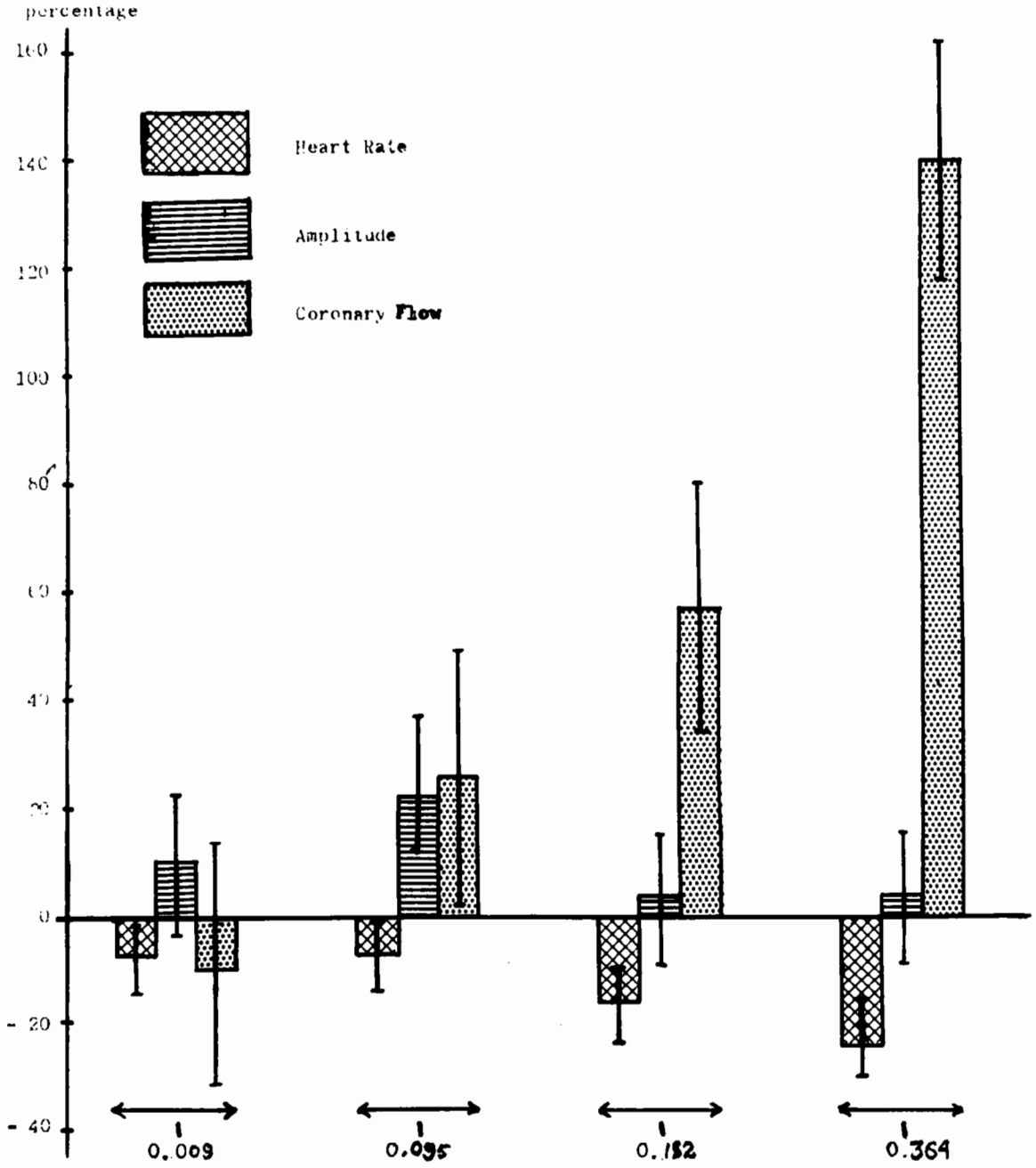


Figure 26. Varying concentrations (0.009 - 0.364 mg./ml.) magnesium added to Locke.

TABLE XI

Average Percent Changes after 5 Minutes Perfusion with Locke Solution Containing Added Magnesium.

Mg. conc. mg./ml.	0.009	0.095	0.182	0.364
Coronary Flow (ml./min.)	-10.06*	25.60	56.70	139.90
Amplitude (mm.)	9.98	21.90	3.62	3.74
Heart Rate (per min.)	-7.78	-7.30	-16.12	-24.12

* Five hearts were used in each case.

Analysis of Variance.

	Source of Variation	Mean Squares	Variance Ratio
Coronary Flow	Treatments	30487.70	11.28*
	Individuals	2701.92	
Amplitude	Treatment	368.93	0.45
	Individuals	823.85	
Heart Rate	Treatment	317.28	1.25
	Individuals	257.87	

Degrees of Freedom: Treatment (3); Individuals (16); Distribution of F; 0.05 (3.24); 0.01 (5.29) (Snedecor 1955).

chosen, as an adequate time to permit comparisons under stabilized conditions.

In Figure 27 and Table XII are shown respectively, a graph and summaries of comparisons between the time required for cardiac standstill during ouabain perfusions in 23 different experiments in which either, normal Locke (N.L.); or Locke with added sodium lactate (Lact.); or added magnesium (Mg.); or Locke solution from which either sodium (Na.) or dextrose (Dex.) was omitted.

In reference to the effects of magnesium, it can be seen from the data that in the presence of added magnesium alone (0.36 mg./ml.), the heart continued to beat for as long as the perfusion continued (approximately 60 minutes). On the other hand, under similar conditions, with added ouabain, cardiac standstill developed within an average of 16.00 $\pm$3.61 minutes. These latter figures were however, not statistically different from the time required to cardiac standstill with ouabain in normal Locke (17.5 $\pm$0.63 minutes). These findings would suggest that magnesium in this concentration, does not prevent the cardiac toxicity of ouabain. Higher concentrations of magnesium were not studied in this connection, since these in themselves hastened depression of heart action.

In the magnesium experiments, during the recovery perfusion with normal Locke, it was observed that complete recovery ensued in five out of six hearts arrested by the ouabain-magnesium combination. The table (Table XII) shows that there were some differences between the responses in rabbits' and cats' hearts, both in the onset of cardiac standstill and in recovery, but not enough experiments have been performed to draw any definite conclusions on this point.

A typical example of the coronary flow and heart contraction changes observed in a ouabain-magnesium experiment on a rabbit heart is shown in Figure 28. As can be seen, in the presence of the magnesium, there is no

TABLE XII

Observed Effects During Perfusion with Ouabain (7.5 γ/ml.) in Varying Concentrations of Magnesium, Sodium, Dextrose and Lactate.

Concentration (mg./ml.)	Cardiac Arrest (min.)		Recovery** with Normal Locke
	Control	Ouabain	
0.36 Mg^{++}	N.A.	9	8
0.36 Mg^{++}	N.A.	7	12
0.36 Mg^{++}	-	13	28
0.36 Mg^{++}*	-	12	8 min., maintained for 24 min. followed by sudden fibrillation.
0.36 Mg^{++}*	-	30	No Recovery in 30 min.
0.36 Mg^{++}*	-	25 Avg.16.0 ±3.61	Massage induced recovery in 3 min. Maintained for 26 min., followed by sudden fibrillation.
0 Na^{+}	4	7	Immediate recovery in both experiments.
0 Na^{+}	7	5	Immediate recovery in both experiments.
0 Na^{+}	- Avg.5.5	7 6.34 ±0.21	Immediate recovery
0 Dextrose	-	12	No Recovery - fibrillation continued for several minutes.
0 Dextrose	-	16	No Recovery observed after 50 min.
0 Dextrose	-	14 Avg.14.0 ±1.16	Partial Recovery of right ventricle after 32 min.
1 Lactate	N.A.	12	16
1 Lactate	-	13 Avg.12.5	24
10 Lactate*	-	7	No Recovery after 22 min.
10 Lactate*	-	7	No Recovery after 76 min.
10 Lactate	-	11	No Recovery after 20 min.
10 Lactate	-	12 Avg.10.0 ±1.64	No Recovery after 36 min.

The term "N.A." means 'no cardiac arrest'.

* The isolated cat heart was used in these experiments.

** The term "Recovery" means 'initiation of visible ventricular improved contractions'.

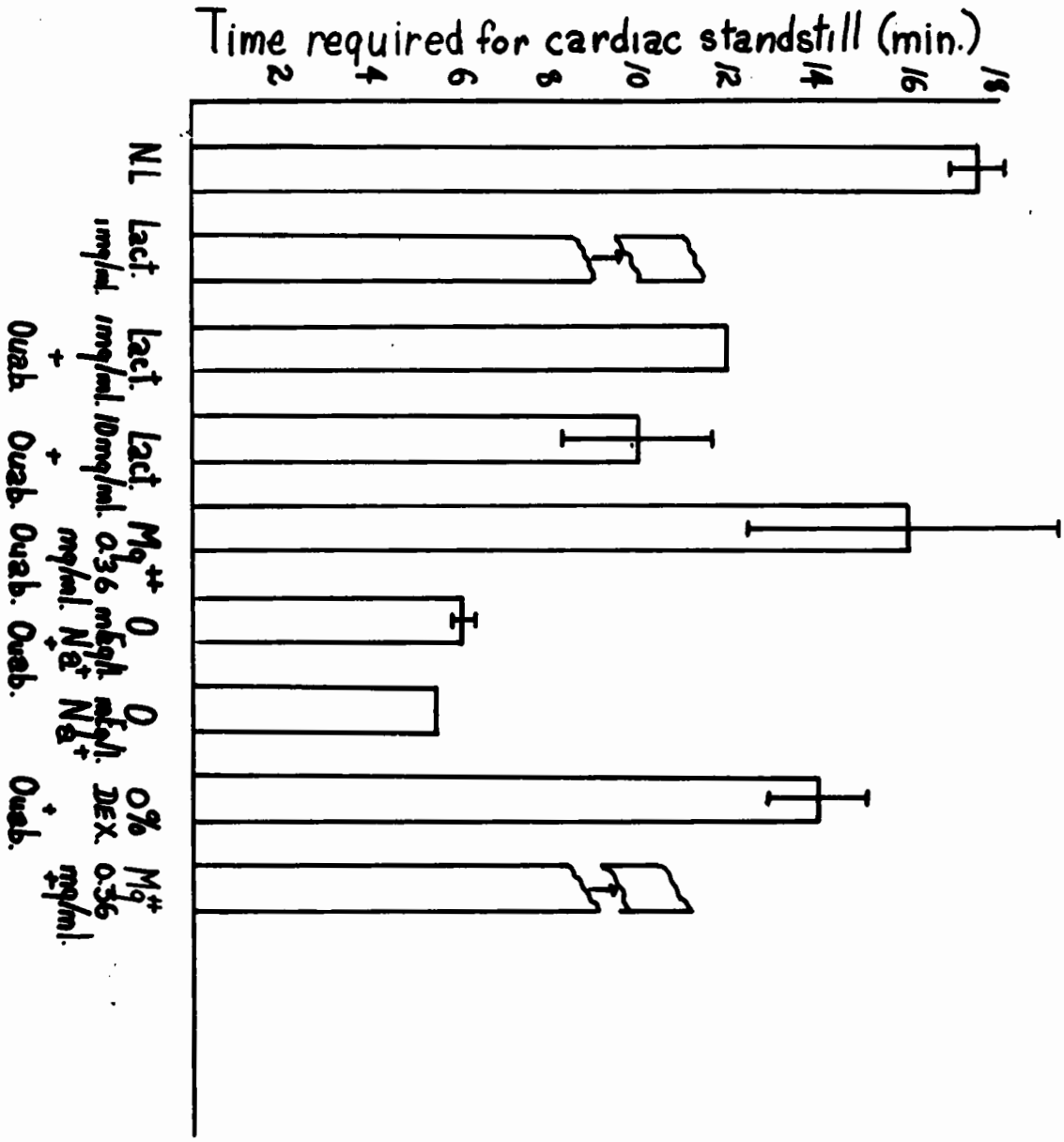
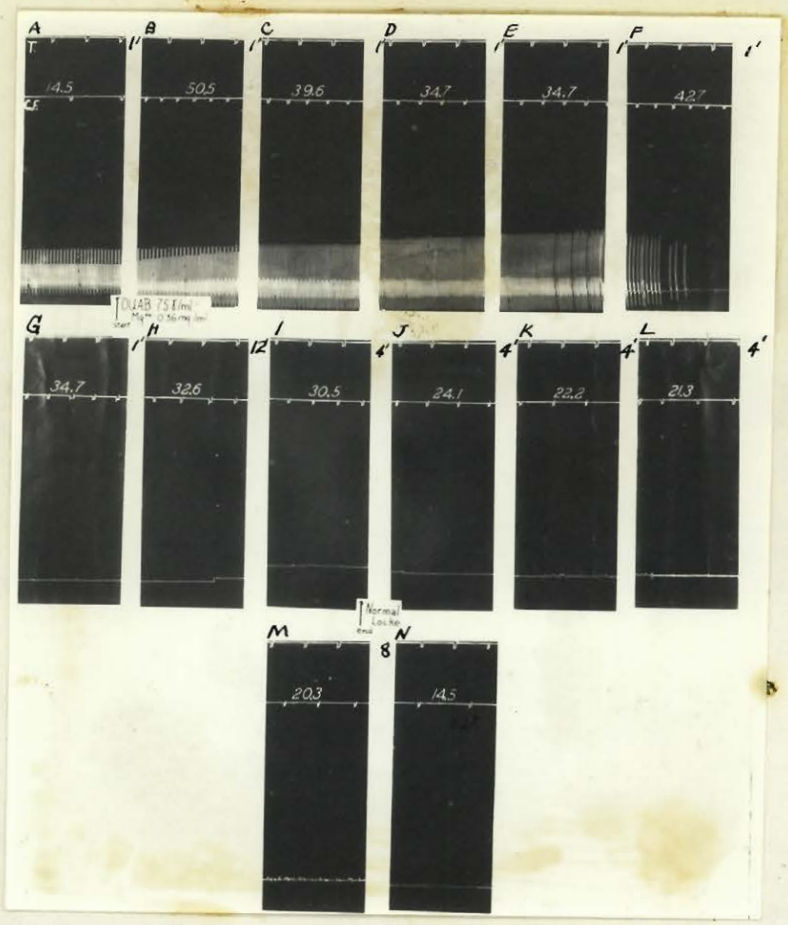


Figure 27. Graph of average times to cardiac standstill following perfusions with ouabain in varying concentrations of lactate, magnesium, sodium and dextrose. N.L. = normal Locke; Lact. = lactate; Mg^{++} = magnesium; Na^{+} = sodium; Dex. = dextrose; Ouab. = ouabain, 7.5 γ/ml. which was added to the perfusing fluid. See text.



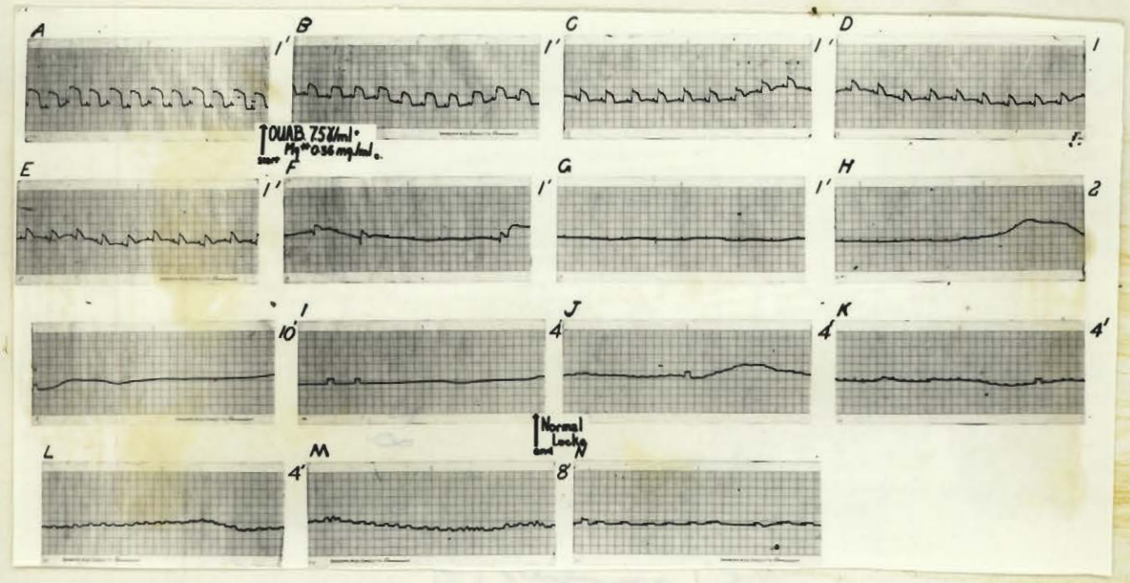


Figure 28. Isolated rabbit heart. Perfusion with Locke solution containing 0.36 mg./ml. of magnesium and added ouabain (7.5 γ/ml.).

Record: At first arrow (Start, Ouab. 7.5 γ/ml. + $Mg.^{++}$ 0.36 mg./ml.), the perfusing fluid was changed from normal Locke to Locke containing 0.36 mg./ml. of magnesium and added ouabain (7.5 γ/ml.). At the second arrow (end, Normal Locke), perfusion solution was changed to normal Locke.

initial coronary vasoconstriction but rather an immediate intense dilatation which becomes less marked, although well sustained throughout the whole duration of the perfusion. Concomitantly, there was also less marked stimulation in the amplitude of the heart contractions with only slight bradycardia, and no gross irregularities until almost complete standstill. The associated electrograms show the slight progressive sinus slowing (B - D), associated with ST-T changes (C to E). As the effects continued, the ventricular complexes appear less and less frequent (F), and are completely absent at G, with only irregular auricular activity seen. Ultimately, complete arrest occurred at H and I.

During subsequent perfusion with normal Locke, the reverse changes were observed on the electrograms, with final restoration to sinus rhythm (J to N), although the restoration of the mechanical contraction was rather poor.

Figure 29 demonstrates the typical changes observed during ouabain-magnesium perfusion in a cat's heart. As can be seen, there is sustained coronary dilatation, associated with slight initial slowing, followed by some stimulation and increased heart rate. These changes were found less marked than in the absence of magnesium, and there were clearly fewer irregularities. At J one sees the rather characteristic sudden cessation of heart action and again at O, during the recovery period the characteristic sudden restoration of heart function, as was generally observed in these magnesium experiments.

It is also noteworthy that during the recovery period, there were some irregularities observed (P and Q) and a sudden sustained ventricular fibrillation (R).

In Figure 30 and Tables XIII and XIV, are compared the coronary flow and potassium movements in this group of experiments. In both the control

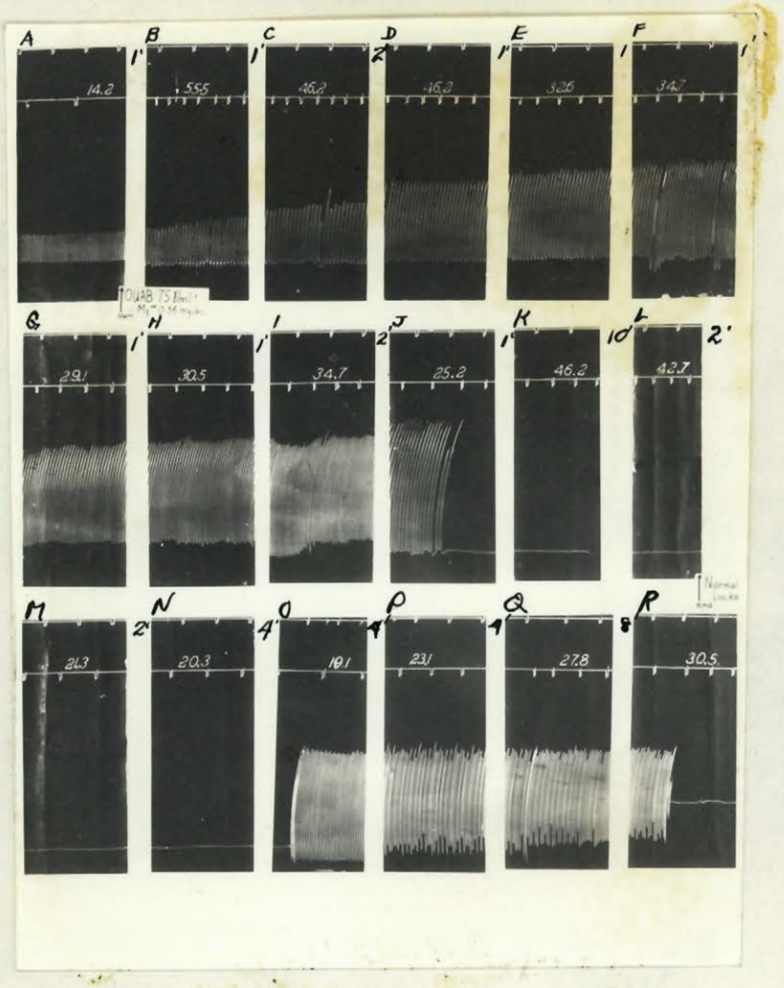


Figure 29. Isolated cat heart. Perfusion with Locke solution containing 0.36 mg./ml. of magnesium and added ouabain (7.5 γ/ml.).

Record: At first arrow (Start, Ouab. 7.5 γ/ml. + $Mg.^{++}$ 0.36 mg./ml.) the perfusing fluid was changed from normal Locke to Locke containing 0.36 mg./ml. of magnesium and added ouabain (7.5 γ/ml.). At the second arrow (end, Normal Locke), perfusion solution was changed to normal Locke.

TABLE XIII

Mean Recorded Changes in Coronary Inflow Rate (ml./min.) during Perfusion with Ouabain (7.5 γ/ml.) in Varying Concentrations of Magnesium, Sodium, Dextrose and Lactate.

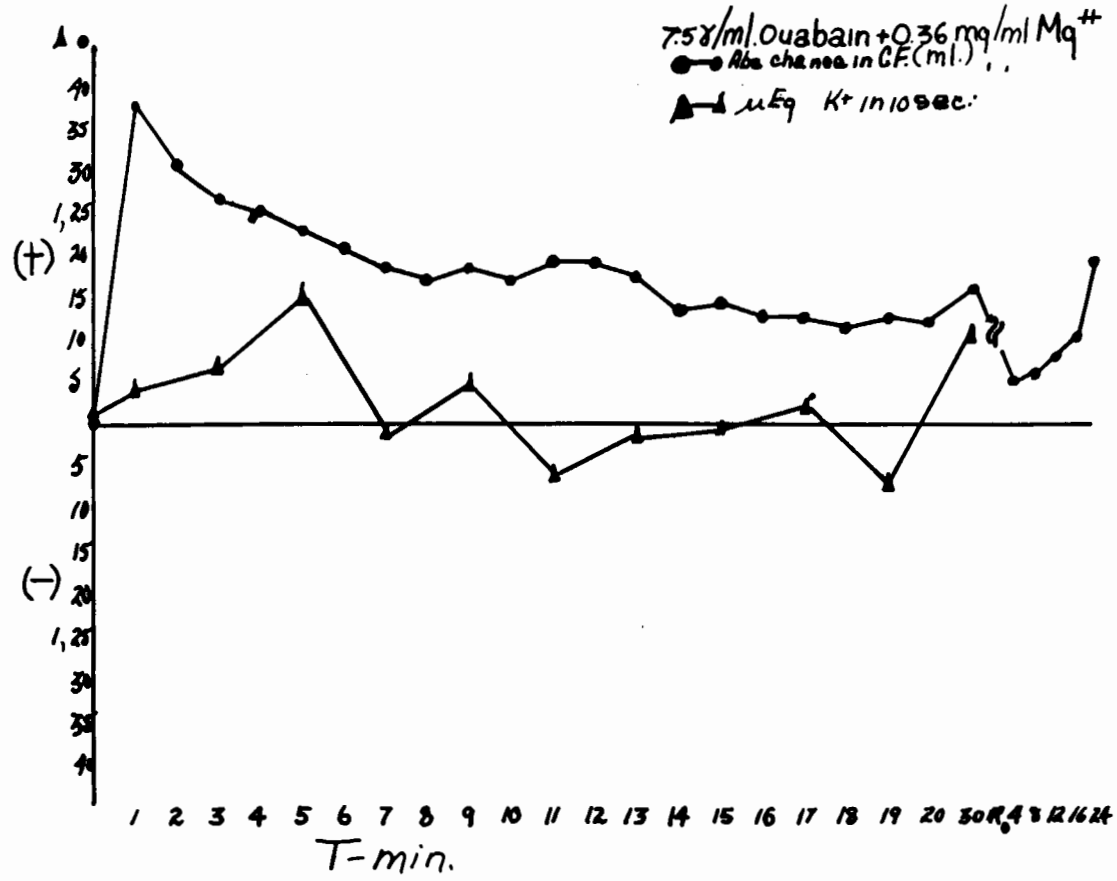
Time (min.)	Concentrations (mg./ml.) and Agents							
	0.36 Mg^{++} Ouab.	0.36 Mg^{++} Cont.	1 Lactate Ouab.	1 Lactate Cont.	10 Lactate Ouab.	0 Na^{+} Ouab.	0 Na^{+} Cont.	0 Dex. Ouab.
Control (ml/min)	15.1	16.3	-	-	15.3	11.0	24.09	24.2
0.17	-	-	-	-	+7.65	-	-	-
0.5	-	-	-	-	+13.10	-	-	-
1	+38.05	-	-	-	+5.65	-4.80	-17.95	-11.97
2	+31.33	-	-8.3	-0.8	+2.10	-4.54	-14.52	-9.92
4	+25.71	+26.44	-3.5	+0.2	+3.40	-5.68	-13.93	-6.44
6	+21.22	-	+3.3	+0.3	+11.22	-4.99	-11.29	+2.25
8	+17.12	+21.28	+4.8	-1.1	+12.16	-	-	+8.57
10	+17.72	-	+4.3	-	+12.43			+7.75
12	+19.03	+17.58	+0.4	-2.0	+23.90			+9.77
14	+13.80	-		-				+10.56
16	+13.25	+11.41		-1.7				+5.00
20		+9.32		-2.5				
24		+8.25		-2.6				
28		+9.05		-3.2				
32		+7.43						
36		+7.55						
40		+7.81						
44		+5.35						
70				-5.2				
No. Hearts Per Mean	6	2	1	1	5	3	2	3

Ouab. = ouabain; Cont. = Control; Dex. = Dextrose.

TABLE XIV

Mean Calculated Differences (Microequivalent per 10 sec.) Between Potassium Inflow and Potassium Outflow During Perfusion with Ouabain (7.5 γ/ml.) in Varying Concentrations of Magnesium, Lactate, Sodium and Dextrose.

Time (min.)	Concentrations (mg./ml.) and Agents				
	0.36 Mg^{++} Ouabain	0.36 Mg^{++} Control	10 Lactate Ouabain	0 Na^{+} Ouabain	0 Dextrose Ouabain
Control (ml/min.)	+0.02	-0.10	-0.27	+0.56	+0.30
0.17	-	-	-0.75	-	-
0.5	-	-	-0.68	-	-
1	+0.15	+0.13	-1.54	+0.32	-
2	-	-	-0.87	+0.23	+0.10
3	+0.27	-	-	+0.07	-
4	-	-0.06	-0.96	+0.35	+0.18
5	+0.60	-	-	+0.11	-
6	-	-	-2.77		-0.44
8	-0.04	+0.24	-0.57		-0.29
10	+0.18	-	+0.42		-0.55
12	-0.24	-			-1.58
14	-0.06	-			-2.56
16	+0.03	-0.13			-1.60
24		+0.39			
32		-0.12			
40		-0.12			
44		-1.33			
96		-0.04			
No. Hearts per Mean	4	2	3	2	2



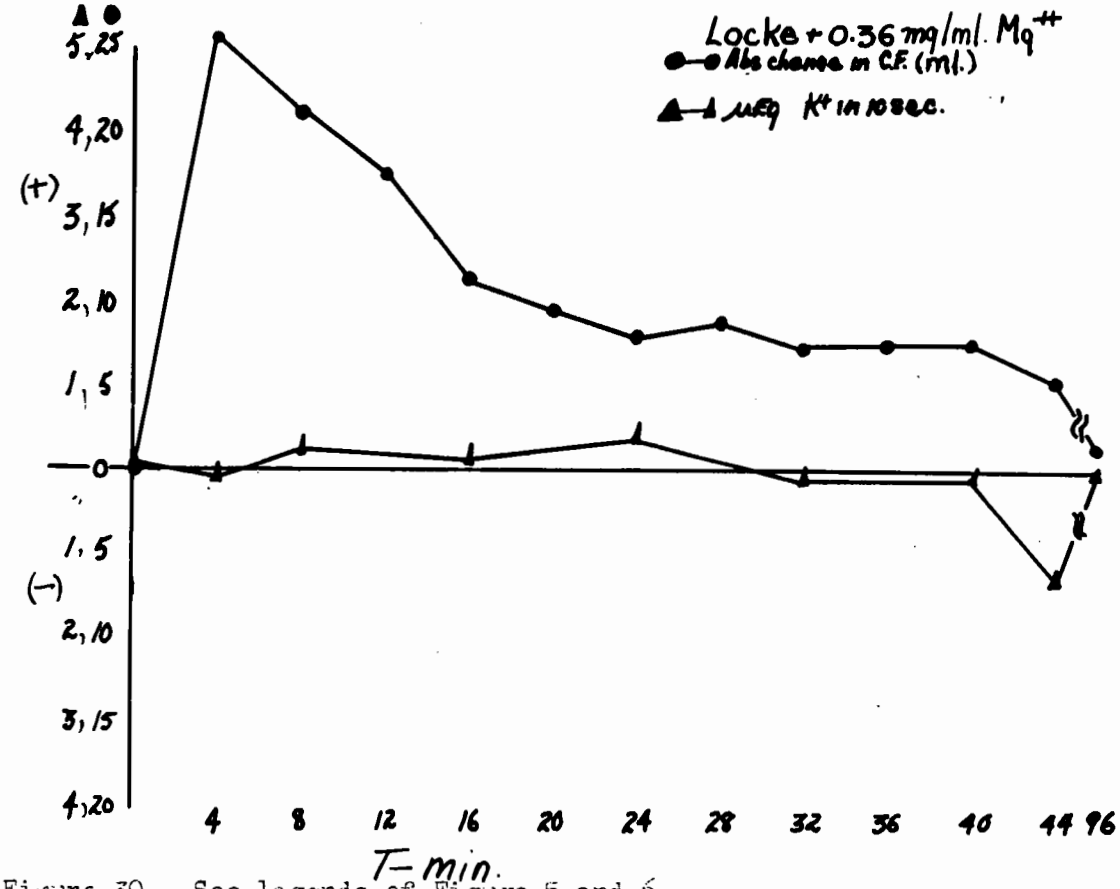


Figure 30. See legends of Figure 5 and 6.
Upper Graph: Ouabain - 7.5 γ/ml. added to Locke containing added magnesium (0.36 mg./ml. Mg^{++}).
Lower Graph: Control (no ouabain added), perfused with Locke containing added magnesium (0.36 mg./ml. Mg^{++}).

(lower section) and ouabain experiment (upper section) there were no observed changes in potassium equilibrium, associated with the marked increased coronary flow.

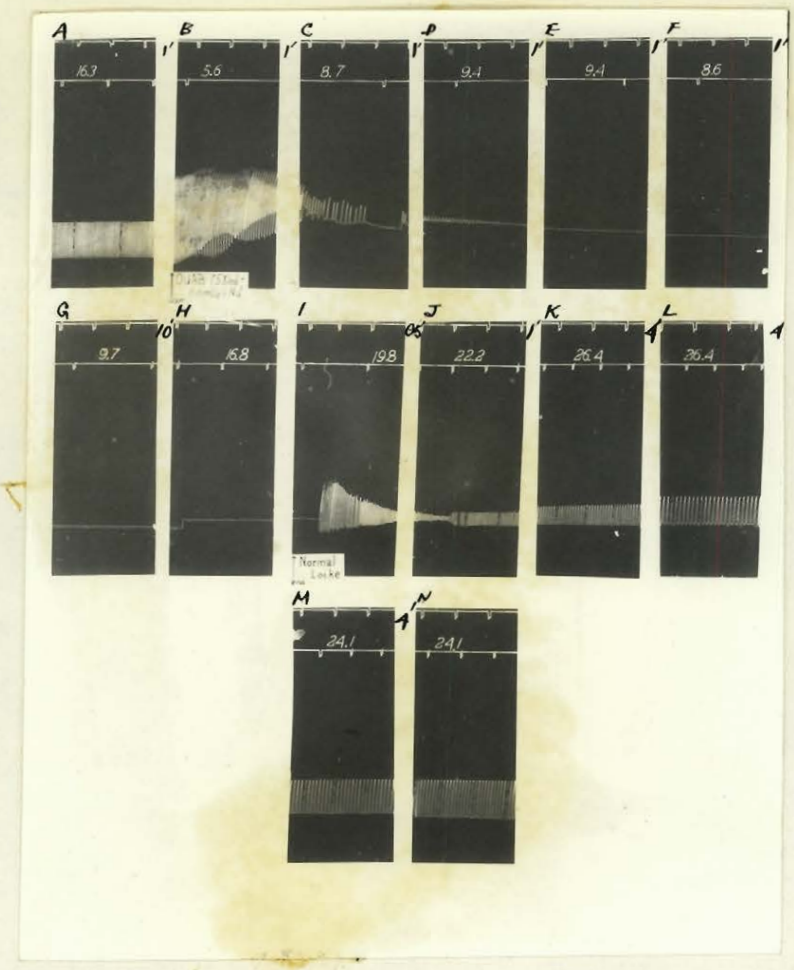
(b) Effect of Locke Solution from which Either Sodium Chloride or Dextrose Has Been Omitted.

Figure 27 and Table XII also contain data from the experiments in which the heart was perfused with ouabain in Locke solution from which either sodium, chloride or dextrose was omitted. The osmotic pressure of the sodium-free Locke was maintained by the addition of sucrose. It is clear from the findings, that cardiac standstill occurred equally rapidly in the controls and in the ouabain experiments under both of these conditions. Table XII show that the durations for standstill in the sodium-free experiments were 5.5 and 6.34 $\pm$0.21 minutes, respectively, while from the dextrose-free experiments performed, the average was 14.0 $\pm$1.16 minutes. This latter does not differ significantly from the average time (17.56 $\pm$0.63 minutes) required when normal Locke is used.

The latter also shows that immediate recovery ensued in the sodium-free experiments, when the perfusing solution was changed to normal Locke, while in the dextrose-free, there was no recovery in two and only partial recovery in one instance.

Some examples of the records obtained in the sodium-free experiments are presented in Figures 31 and 32. In both instances, there is prompt and sustained coronary constriction, associated with brief myocardial stimulation (tachycardia) and gross irregularities, leading to standstill. The records also show, in both instances, prompt restoration of heart contractions, associated with marked coronary dilatation, when the perfusion fluid was changed to normal Locke.

The associated electrograms demonstrate that during the ouabain perfusion (Figure 31), there was an initial sinus bradycardia with prominent



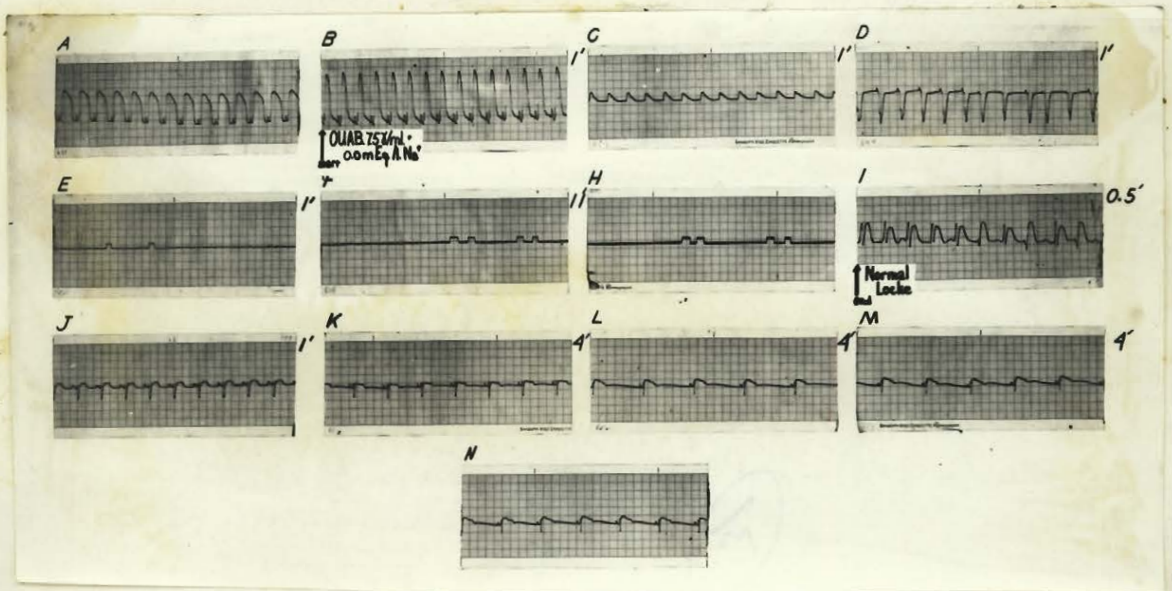
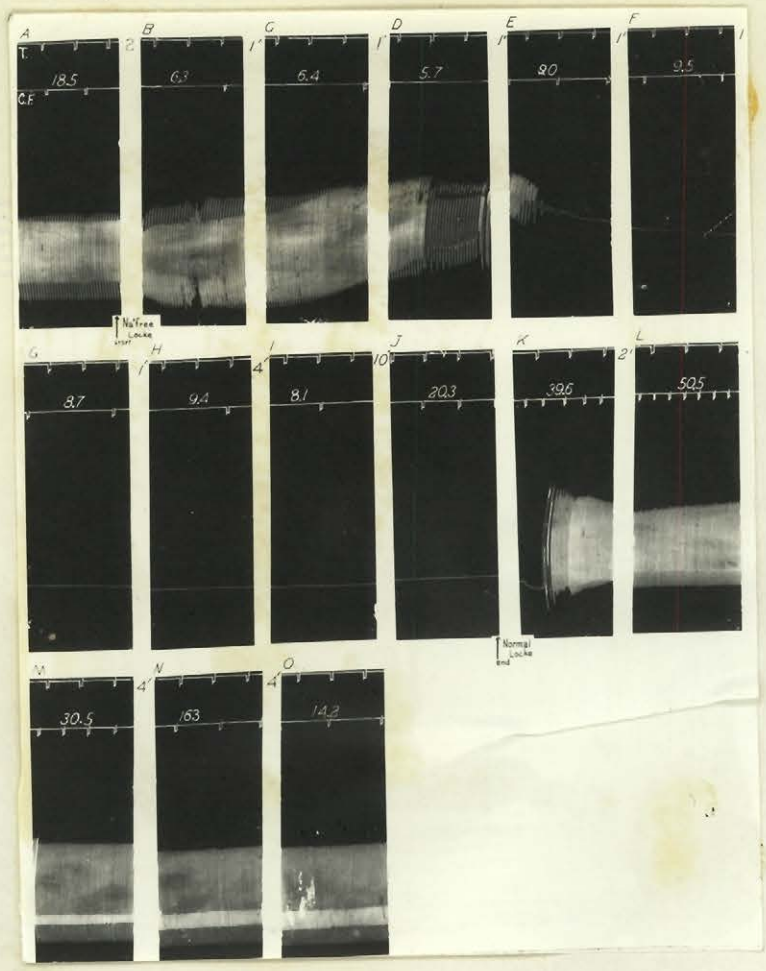


Figure 31. Isolated rabbit heart. Perfusion with sodium-free Locke with added ouabain (7.5 γ/ml.). See legend of Figure 2.
Upper and Lower Record: At first arrow (Start, Ouab. 7.5 γ/ml. + 0.00 mEq./l. Na^+) the perfusing fluid was changed from normal Locke to sodium-free Locke and ouabain. At second arrow (end, Normal Locke) the ouabain perfusion was changed to normal Locke.



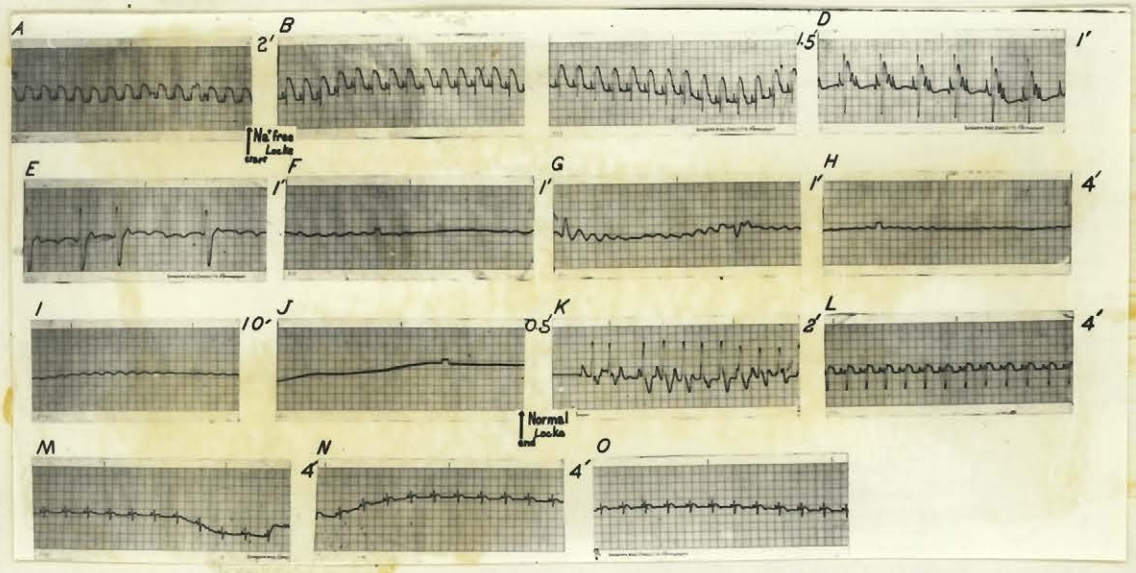


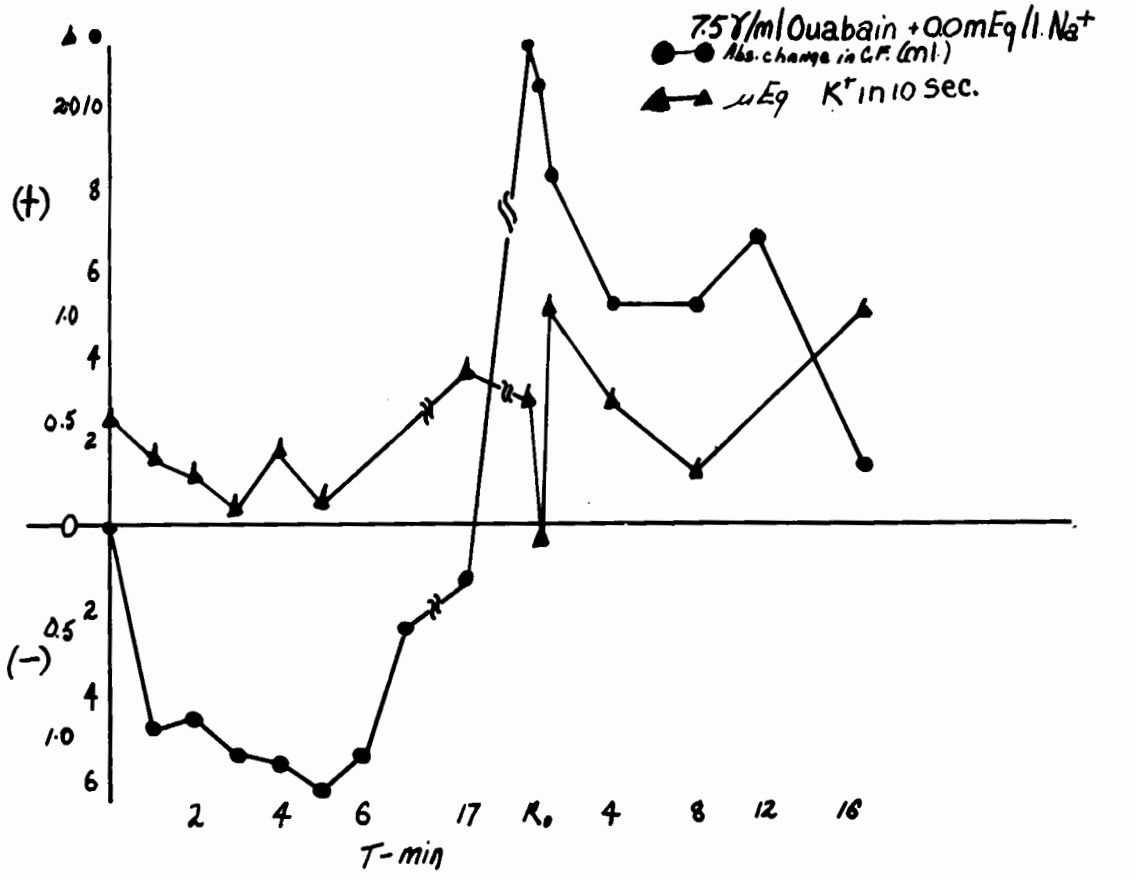
Figure 32. Isolated rabbit heart. Perfusion with sodium-free Locke. See legend of Figure 2.

Upper and Lower Record: At first arrow (Start, Na^+ free Locke) the perfusing fluid was changed from normal Locke to sodium-free Locke. At second arrow (end, Normal Locke) the sodium-free solution was changed to normal Locke.

P waves and increased height of the R waves, within two minutes (Section B, time interval not shown on record). Within the next minute transitory ventricular fibrillation ensued (C) followed by ventricular tachycardia (D), with only auricular activity seen at E, and finally cardiac arrest at H. During the recovery period, there was rapid restoration to normal sinus rhythm. The changes observed in the electrograms in the sodium-free experiments without added ouabain (Figure 32) were also rather similar, except that following the initial tachycardia, there was a brief 2:1 block (D) followed by auricular fibrillation with scattered ventricular beats (E), proceeding to ventricular fibrillation (F to I), and final cardiac arrest (J). It appeared that ventricular fibrillation ensued somewhat more rapidly in the ouabain experiment, than in the control (sodium-free alone). The recovery changes in these latter were however rather similar to those observed in the former.

Figure 34 shows an example of the results obtained in a dextrose-free experiment, and needs little comment. In general, the changes in both coronary flow and heart contractions following the ouabain, were similar in every respect to those observed when the perfusing fluid was normal Locke (compare Figure 3). In respect to the recovery, however, under these conditions the usual restoration with normal Locke perfusion, either could not be accomplished (after 50 minutes of perfusion) or was only partial.

The associated coronary flow and potassium changes in the above experiments are summarized in Tables XIII and XIV, and graphically correlated in Figure 33. As can be seen in this sodium-free experiment (upper record), there is sustained coronary constriction, not associated with any marked changed in potassium equilibrium, and conversely, during the recovery period, there is intense coronary dilatation, again without any consistent change in potassium movement. On the contrary, when the dextrose-free perfusing



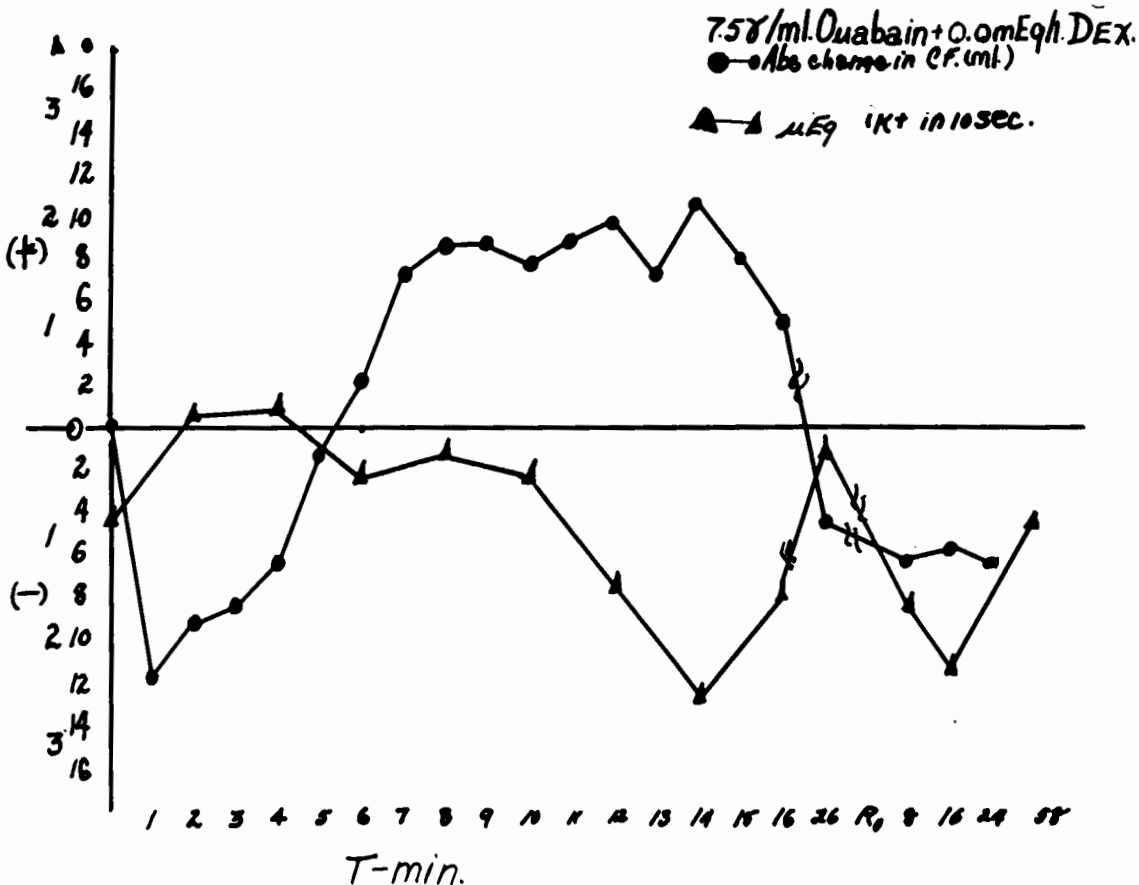
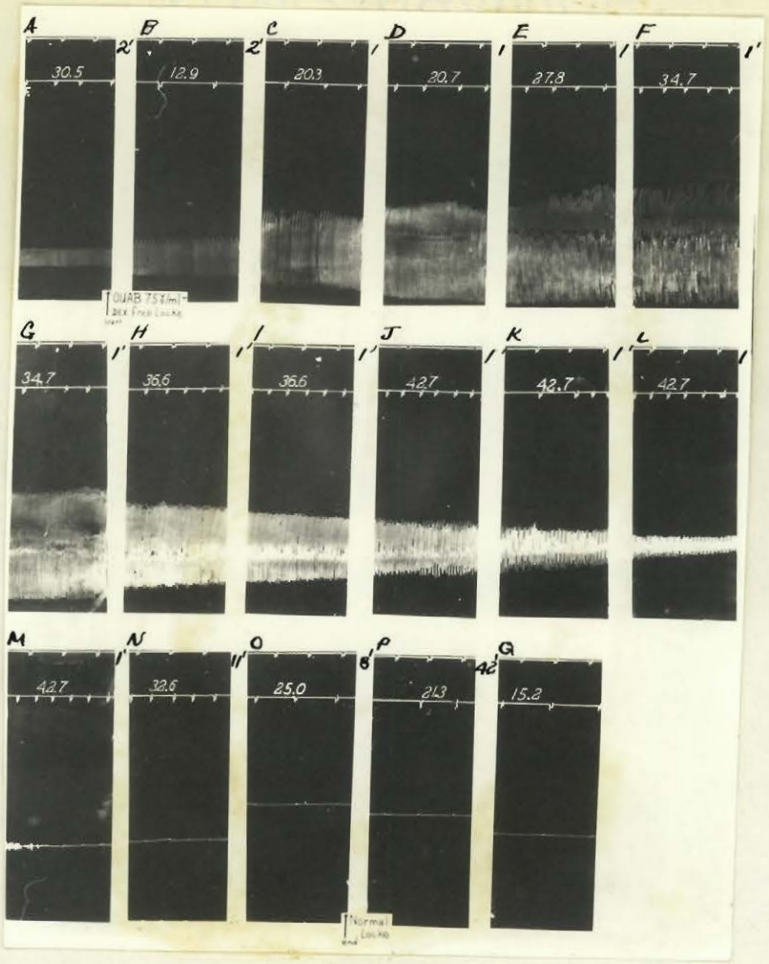


Figure 33. See legend of Figure 32 C.
Upper Graph: Ouabain - 7.5 γ/ml. added to sodium-free Locke.
Lower Graph: Ouabain - 7.5 γ/ml. added to dextrose-free Locke.



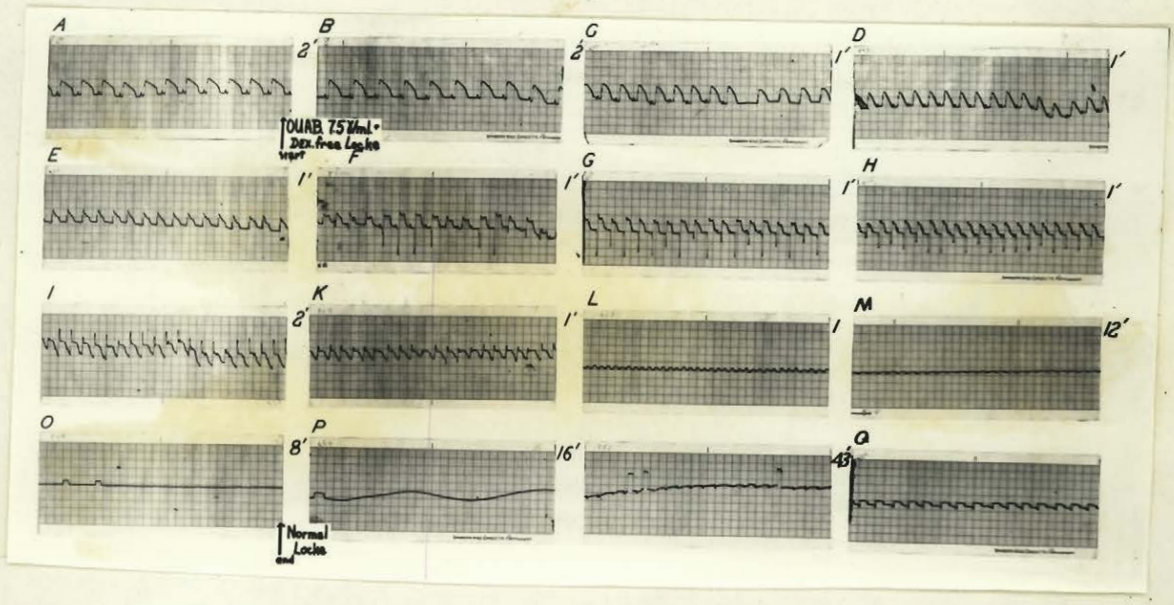


Figure 34. Isolated rabbit heart. Perfusion with dextrose-free Locke with added ouabain (7.5 γ/ml.). See legend of Figure 2.
Upper and Lower Record: At first arrow (Start, Ouab. 7.5 γ/ml. + Dex. free Locke) the perfusing fluid was changed from normal Locke to dextrose-free Locke and ouabain. At second arrow (end, Normal Locke) the ouabain perfusion was changed to normal Locke.

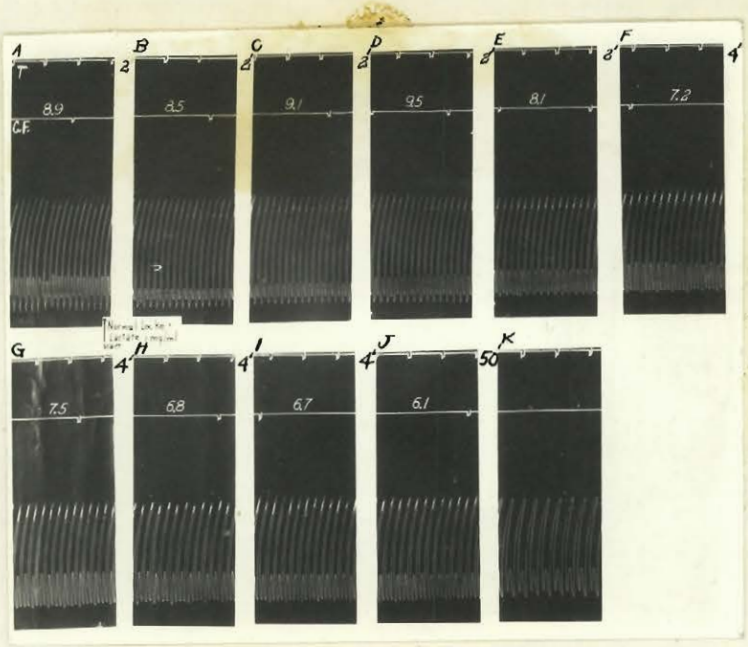
medium was used, the lower section of Figure 33 shows the usual ouabain response—initial coronary constriction followed by dilatation and associated initial net uptake of potassium, followed by a net loss. During the recovery period the coronary flow was also decreased and there appeared to be a sustained negative potassium balance.

(c) Preliminary Effects of Added Lactate.

Again, in Figure 27 and Table XII, are presented some data concerning the effects of ouabain perfusion in Locke solution containing added lactate. With a low concentration of lactate (Lact. 1 mg./ml.), without added ouabain, in one experiment, relative normal heart action was maintained for as long as 60 minutes of perfusion. On the other hand, when a similar perfusing fluid was used with added ouabain (Lact. 1 mg./ml. + Ouab.) in two experiments, cardiac standstill occurred somewhat more rapidly than normal, that is in 12.5 minutes. Additional experiments would be needed before a definite conclusion can be drawn on this point. However, when the concentration of lactate was increased to 10 mg./ml., in the presence of ouabain (four experiments), the time to cardiac standstill was definitely reduced (average 10.0, ±1.64 minutes), in comparison with the ouabain responses in normal Locke.

It can also be seen in Table XII, that following cardiac arrest with the lower concentrations of lactate, recovery was rather delayed (16 to 24 minutes), after the perfusion fluid was changed to normal Locke. Indeed, with the high concentrations of lactate, no recovery could be achieved under these conditons, as long as 76 minutes of perfusion.

Typical examples of the coronary flow and heart contraction changes observed in the above experiments are shown in Figures 35, 36 and 37. Thus, with a low concentration of lactate (Figure 35), there is a progressive moderate coronary constriction and gradual slowing of the heart during a 90-minute period of perfusion (from B to K). The corresponding electrograms show gradual sinus bradycardia associated with progressive ST-T changes.



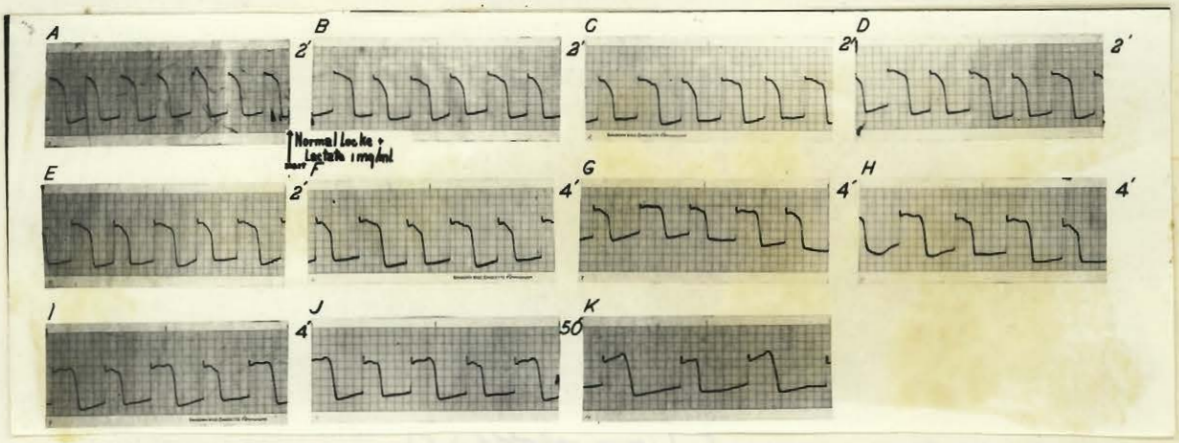
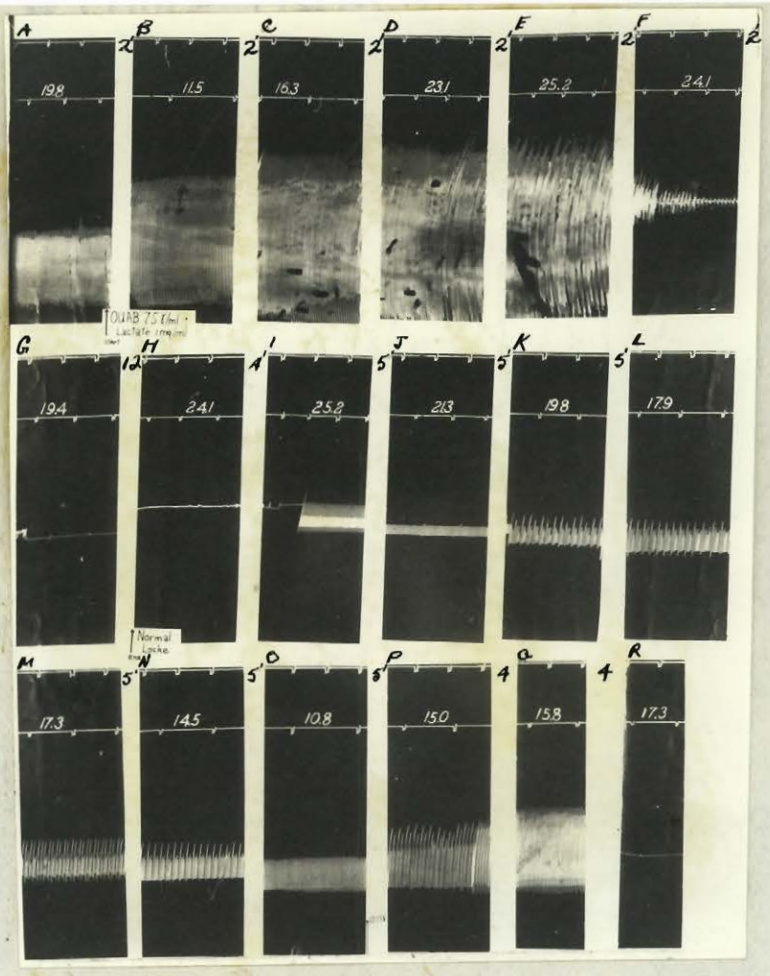


Figure 35. Isolated rabbit heart. Perfusion with Locke solution containing added lactate (1 mg./ml.).
Upper and Lower Record: At arrow (Start, Normal Locke + Lactate 1 mg./ml.) the perfusing fluid was changed from normal Locke to Locke containing 1 mg./ml. lactate.



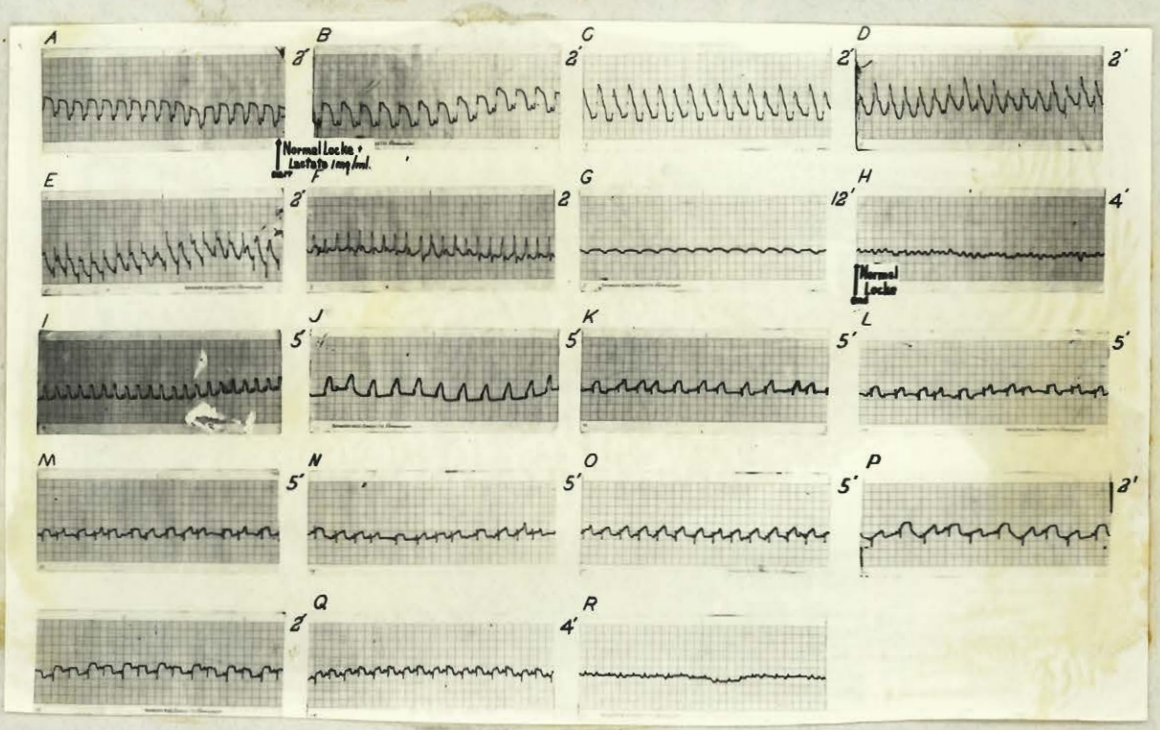
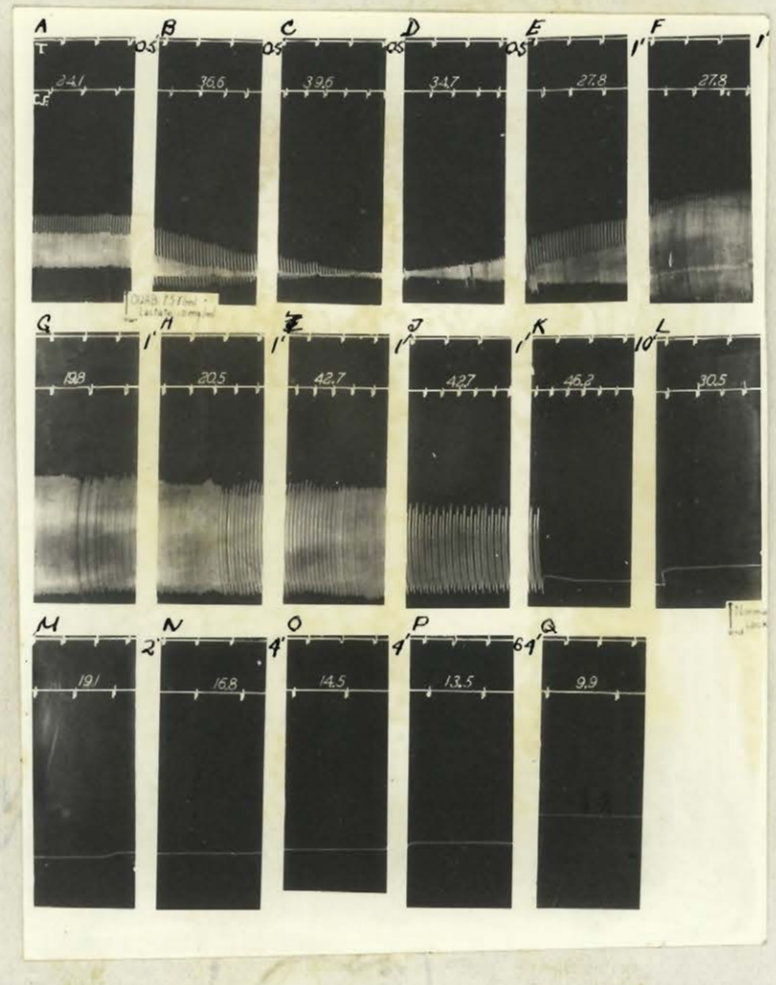


Figure 36. Isolated rabbit heart. Perfusion with Locke solution containing 1 mg./ml. lactate and added ouabain (7.5 γ/ml.).
Upper and Lower Record: At first arrow (Start, Ouab. 7.5 γ/ml. + Lactate 1 mg./ml.) perfusing fluid was changed from normal Locke to Locke containing 1 mg./ml. of lactate and added ouabain (7.5 γ/ml.). At second arrow (end, normal Locke) perfusing fluid was changed to normal Locke. At third arrow (O. 7.5 γ) perfusing fluid was changed from normal Locke to normal Locke containing added ouabain.



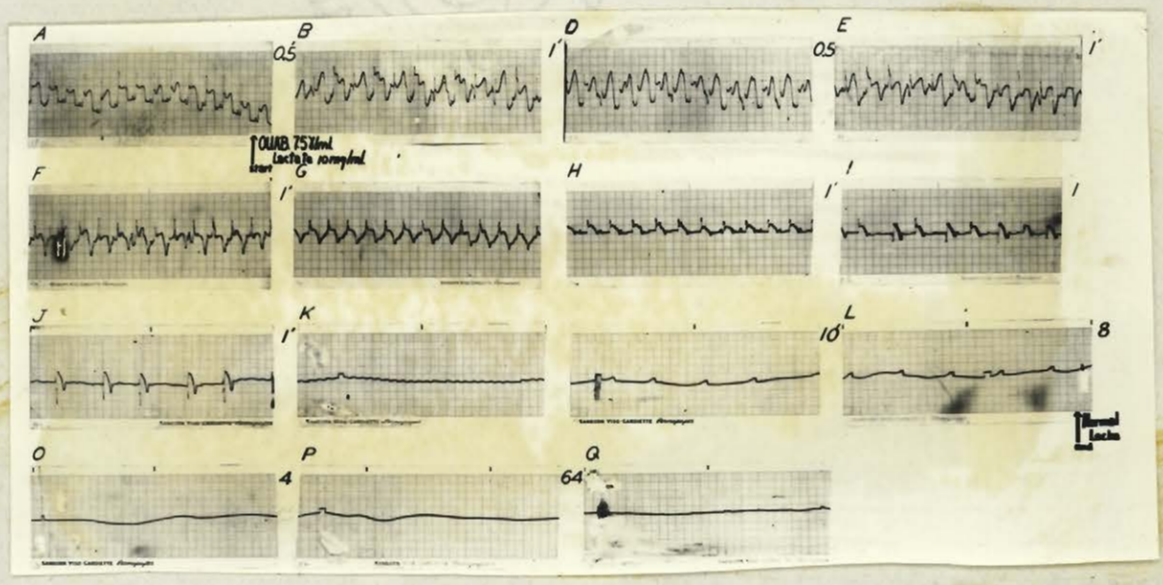


Figure 37. Isolated rabbit heart. Perfusion with Locke solution containing 10 mg./ml. lactate and added ouabain (7.5 γ/ml.).
Upper and Lower Record: At first arrow (Start, Ouab. 7.5 γ/ml. + Lactate 10 mg./ml.) perfusing fluid was changed from normal Locke to Locke containing 10 mg./ml. of lactate and added ouabain (7.5 γ/ml.). At second arrow (end, Normal Locke) perfusing fluid was changed to normal Locke.

In the presence of ouabain, a low concentration of lactate (Figure 36) led to the usual initial coronary constriction followed by coronary dilatation, associated with progressive cardiac stimulation (amplitude and rate) with gross irregularities and final arrest. The associated electrical changes B to G, were not significantly different from those shown during ouabain perfusion in normal Locke, and the record at G shows terminal ventricular fibrillation.

On changing the perfusing fluid to normal Locke (without ouabain), there was, after a delay of approximately 16 minutes, a sudden restoration to normal sinus rhythm, which persisted for 30 minutes (O), when relatively normal heart action was restored. The records also show that reperfusion with ouabain(7.5 γ/ml.) alone in normal Locke, precipitated irregular ventricular actions (R) in approximately 14 minutes. The kymographic records at P and Q show the associated stimulated heart contractions and cardiac standstill at R. These changes were not essentially different from those observed in other experiments in which the heart was similarly ouabainized after previous arrest following perfusion in normal Locke.

With the high lactate concentration (Figure 37), the usual initial ouabain coronary constriction was not observed, but rather immediate coronary dilatation, associated with depression rather than stimulation of the heart contractions (C and D). On continued perfusion, however, with the mixture, there was a progressive reduction in coronary flow, associated with improved heart contractions, followed by marked coronary dilatation and cardiac stimulation (H and I). There were no arrhythmias observed in these experiments, but a terminal slowing (J) with sudden complete arrest (K). When the perfusion fluid was changed to normal Locke, no recovery of the heart contractions was observed, and the coronary flow showed a progressive constriction.

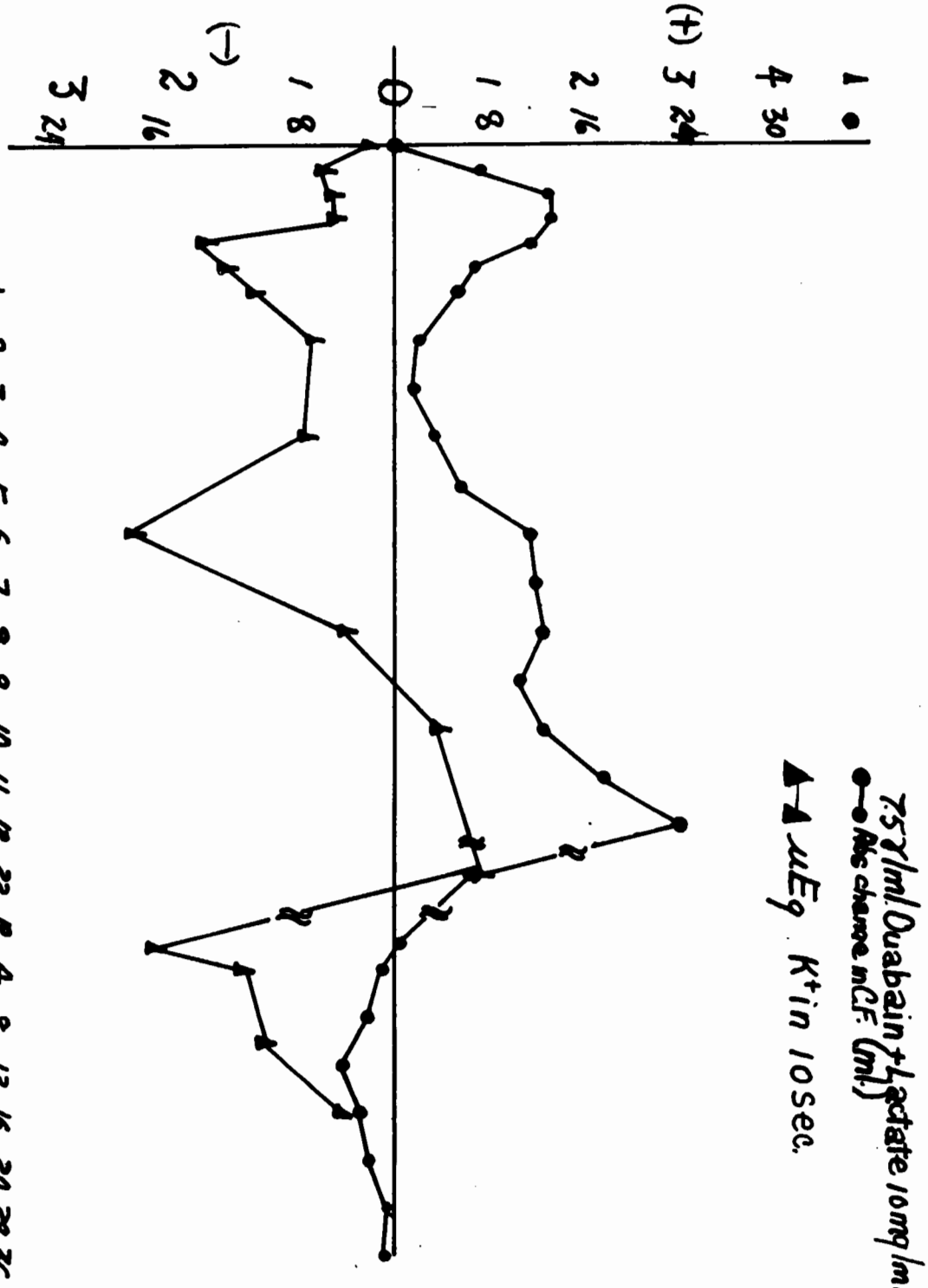


Figure 28. See legend of Figure 6 and 7. Ouabain (7.5 γ/ml.) added to Locke containing added lactate (10 mg./ml.).

Finally, the associated electrocardiograms show an exaggeration of ST-T changes (B to G) followed by ventricular slowing with an alternating idioventricular rhythm (J), and terminal ventricular fibrillation (K to L). During the recovery phase, the electrograms show sustained complete arrest of the heart for as long as 76 minutes of perfusion with normal Locke.

The coronary flow and associated potassium movement in these preliminary experiments are summarized in Table XIII and XIV and correlated in Figure 38.

These data indicate that during the first minute of perfusion there was an increased coronary flow associated with a net loss of potassium from the heart (see initial section of Figure 38). Subsequently, there was a short period of reduced coronary flow concomitant with reduction in net loss of potassium, and, followed by a progressive coronary dilatation, associated with a continued loss, followed by a terminal gain of potassium—more or less the normal type of responses previously demonstrated during perfusion with ouabain in normal Locke solution. During the post-ouabain perfusion period, both the coronary flow and the potassium uptake of the heart decreased, when the perfusing fluid was changed to normal Locke.

(d) Summary and Conclusions

In the isolated rabbit or cat heart perfused with a fixed concentration (7.5 γ/ml.) of ouabain, it was observed that:

1. In the presence of added magnesium (0.36 mg./ml.), the average duration to cardiac arrest was 16.0 ±3.61 minutes, while in similar experiments without ouabain, the heart continued to beat in a relatively normal manner for approximately 60 minutes.

2. With added magnesium, there was a definite decrease in the occurrence of ventricular ectopic beats, and decrease in the intensity of the contractions, even when there was no significant increase in time required for cardiac arrest.

3. With added magnesium, there was an immediate intense coronary dilatation which became less marked although well sustained. These changes were not

accompanied by any marked alterations in the cardiac potassium equilibrium.

4. After magnesium-ouabain cardiac arrest, perfusion with normal Locke led to no consistent pattern in the recovery of hearts—some initiated strong ventricular contractions within minutes, while other showed only a weak activity after prolonged perfusion with normal Locke, although three out of six returned to normal sinus rhythm.

It is therefore concluded from the foregoing (Nos. 1 to 4), that,

(a) Magnesium added to the perfusing fluid antagonizes the cardiac irregularities induced by ouabain, but do not delay the onset of cardiac standstill.

(b) Magnesium antagonizes the usual observed changes in cardiac potassium balance, following ouabain.

(c) Magnesium *per se* exerts a marked coronary dilatation, which is independent of potassium movement and which is not effected by ouabain.

(d) Since no significant changes in potassium balance occur in these experiments, magnesium *per se* abolishes the usually observed ouabain potassium changes, and this effect might be responsible for the absence of the cardiac arrhythmias.

(e) These findings would also emphasize that there is no relationship between the onset of ouabain cardiac arrest and potassium equilibrium in the heart.

5. During the perfusion of the isolated heart with Locke solution containing no *sodium*, the onset of cardiac arrest developed equally rapidly, whether ouabain was added or not.

6. Perfusion with the sodium-free Locke leads to coronary constriction and rapid cardiac stimulation, followed by arrhythmias and ventricular fibrillation, and there were no marked alterations in the potassium equilibrium either in the presence or absence of ouabain.

7. Following cardiac arrest in all sodium-free Locke experiments, perfusion

with normal Locke, induced prompt recovery of the heart associated with an intense coronary dilatation.

8. The overall changes in coronary flow and potassium balance, both during the development of arrest and during the recovery period, in the sodium-free perfusion experiments, resemble rather closely those observed earlier with ouabain during perfusion with increased potassium.

9. During perfusion with dextrose-free Locke, the onset of ouabain cardiac arrest is not altered, and the coronary flow, heart contractions and observed changes in potassium balance, were similar to those generally seen with ouabain in normal Locke.

10. Following ouabain cardiac arrest, during perfusion with dextrose-free Locke, perfusion with normal Locke led to no recovery.

The foregoing preliminary observations (Nos. 5 to 10) suggest that, the cardiac alterations induced by sodium-free Locke perfusion may be due to the relative increase of potassium. It would appear then (i) that, the sodium-potassium ratio is important in maintaining both normal heart function and coronary circulation, and (ii) that, an alteration in this ratio in the heart might be a fundamental factor in the mechanism of the action of ouabain. The problem however, requires further study. These data also show that, (a) dextrose is not essential for either the occurrence of the effects of ouabain on the coronary circulation or potassium balance, or for the development of cardiac arrest; and, (b) following digitalization of the heart in the absence of dextrose, the toxicity is irreversible, rather like that observed following digitalization in the absence of calcium.

11. During perfusion of isolated hearts (rabbit or cat) with ouabain (7.5 γ/ml.) and Locke solution, containing either one or ten mg./ml. lactate, the average time interval to cardiac arrest, was shortened (12.5 and 10.0

±1.64 minutes, respectively), in comparison with the ouabain response in normal Locke.

12. Definite decrease in the number of ventricular ectopic beats and the intensity of the contractions were observed with 10 mg./ml. lactate although the toxicity of the ouabain was increased.

13. During perfusion with the low concentration of lactate (1 mg./ml.) and added ouabain, typical initial coronary constriction followed by dilatation ensued. The usual associated potassium changes were also present. With the high lactate concentration (10 mg./ml.) there was an immediate coronary dilatation (associated with myocardial depression), followed by a slight reduction in coronary flow, and subsequent increased coronary dilatation. These changes were associated with a loss of potassium throughout the duration of the lactate-ouabain perfusion.

It is therefore concluded from these preliminary observations (Nos. 11 to 13), that,

(a) Added lactate increased ouabain toxicity, although the arrhythmias were completely prevented, indicating again no relationship between ouabain arrhythmias and ouabain toxicity (cardiac arrest).

(b) Both the coronary flow and potassium alterations with ouabain are somewhat distorted by the associated myocardial depression due to the lactate.

(c) In the presence of lactate, digitalization of the heart led to an irreversible toxicity, rather like that previously shown with calcium-free or dextrose-free Locke.

DISCUSSION

CORONARY FLOW AND POTASSIUM MOVEMENT.

The data presented above show clearly that under the influence of ouabain characteristic concomitant changes in coronary flow and potassium movement occur in the isolated perfused rabbit and cat heart. It has been observed that, under the conditions employed, with normal Locke as the perfusing fluid, during the initial period, there was a reduction in coronary flow and an associated "net gain" of potassium by the heart. These effects were followed by subsequent coronary dilatation associated with a "net loss" of potassium. The change from "net gain" to "net loss" of potassium preceeded the change from coronary constriction to dilatation.

As is well known, changes in the heart action can induce important secondary changes in coronary flow. Thus, in general, increasing the systolic contractions tends to retard coronary flow mechanically by increasing extravascular compression; and conversely, with better diastolic relaxation coronary flow may be mechanically increased. Recent studies by Wiggers (1954) however, suggest that increasing the force of ventricular contraction "does not throttle coronary flow but actually promotes it". Moreover, it is now abundantly clear that unknown cardiac metabolites, associated with myocardial stimulation and released into the coronary circulation, can influence the vasomotor 'tone' of the vessels, as reported in various types of experiments by Melville and Lu (1950), Lu and Melville (1951), Melville and Mazurkiewicz (1955), Hackel and Clowes (1956). It has long been known that during conditions of anoxia, there is an increased coronary flow (Drill, 1954). Alella, Williams, Bolene-Williams and Katz (1955) have recently shown that coronary flow is predominantly determined by the existing level of oxygen consumption, and with increasing consumption of oxygen, coronary dilatation occurs to meet the needs of the myocardium for oxygen. How the oxygen needs

of the heart lead to increased coronary flow, is still unknown.

The findings presented indicate that the observed initial "net gain" of potassium during ouabain perfusion might be the cause of the initial decreased coronary flow; and conversely, the subsequent coronary dilatation, the result of the "net loss". It has been noted under these conditions, that in the majority of experiments the "net loss" of potassium from the heart begins prior to the initiation of the coronary dilatation, and therefore appears to be the cause.

It has been previously observed (Katz and Linder, 1938, and Melville and Mazurkiewicz, 1955) that after injections (small doses) of potassium, there is coronary dilatation, but since the myocardium is also simultaneously depressed, it is difficult to assess the significance of these observations. However, excessive doses of potassium have been shown by the same workers, to lead predominantly to coronary constriction, despite myocardial depression.

When the effects of ouabain were studied during perfusion of the heart with either excess or reduced potassium, it was further observed that either in the presence or absence of ouabain, increasing the potassium concentration in the perfusing fluid, leads to coronary constriction associated with a "net gain" of potassium, All of these findings favor the hypothesis that potassium may be an important "metabolite" in the physiological control of the coronary circulation.

In the presence of ouabain, during perfusion with decreased potassium, a short initial coronary constriction was still observed, but there was no "net gain" of potassium. In fact, in these experiments the "net loss" of potassium again clearly precedes the initiation of coronary dilatation. Presumably, coronary dilatation cannot ensue until after the potassium level in the heart has been decreased below a "critical" level.

In these experiments, when the potassium concentration was increased only

slightly (ten per cent above normal), the coronary flow changes were rather similar to those observed with normal Locke, except that contrarily, the dilatation began while there was still a positive potassium balance. Why this was so, is not clear.

It had been previously reported (Korol, 1956) that during cardiac stimulation by various drugs (noradrenaline, adrenaline, isoprenaline and aminophylline), there was, in each case, a "net loss" of potassium associated with coronary dilatation. However, it was also noted that various cardiac depressants (acetylcholine, methoxamine and nitroglycerine) led to a "net uptake" of potassium and coronary dilatation. In these latter experiments again, the associated myocardial depression might conceivably complicate the overall coronary flow-potassium balance relationship.

The possibility that change in either the calcium or the calcium-potassium ratio might influence the coronary vascular bed, has been suggested (Melville and Mazurkiewicz, 1955). However, under the influence of ouabain, when the normal potassium-calcium ratio was altered in various proportions, only the effects of the corresponding potassium alterations could be observed. This would indicate that calcium is not involved in the actions of ouabain upon coronary flow and cardiac potassium balance.

It is well known that magnesium can exert a potent coronary dilator effect. Relative to the problem of ionic movement, Stanbury (1948) has reported that magnesium prevents the stimulating effect of potassium ions on the superior cervical ganglion. Similarly, Bryant, Lehmann, and Knoefel (1939) have demonstrated that calcium antagonizes, both the central and peripheral nervous system actions of magnesium. Katz (1936) has also concluded that at the myoneural junction, magnesium can exert a curare-like blockade, and this blockade can be antagonized by potassium; and finally, Brosnan and Boyd (1936, 1937) have reported that calcium chloride, potassium

chloride and adrenaline can all counteract magnesium "curarization". The latter authors have also suggested that, because the antagonism with adrenaline develops more slowly, this effect is probably an indirect one and possibly due to a mobilization of potassium. In our experiments, the presence of magnesium completely abolished the initial ouabain coronary constriction, but enhanced the subsequent dilatation. Since the ouabain potassium changes were prevented, it appeared that magnesium both exerts a "direct" coronary dilator action, and antagonizes potassium movements. The possibility arises that these two actions might be related, and perhaps then the coronary dilator effect of magnesium might also involve potassium movement. This problem again, requires further investigation.

It has also been previously suggested (de Burgh Daly and Clark 1920) that the cardiac effects resulting from a lack of sodium resemble those induced by digitalis in the isolated frog heart. Our data indicate that the lack of sodium, either in the presence or absence of ouabain, leads to coronary constriction, and that this develops without significant potassium imbalance. Although the number of experiments performed was small, it might be again that the resultant relative increased potassium-sodium ratio is responsible for this coronary constriction.

In the case of the lactate ion, low concentrations without added ouabain, on prolonged perfusion (90 minutes) produced only a slight coronary constriction and no detectable associated change in potassium balance. The usual responses to ouabain were however still observed in the presence of low lactate perfusion. With high lactate concentrations myocardial depression ensued and therefore the results are equivocal.

One could conclude from the above findings, that the observed changes in the potassium balance in the heart may be the primary mechanism through which ouabain exerts its effect upon the coronary circulation. However, some of

the data suggest that secondary alterations in the sodium-potassium ratio might also be involved in this action. Finally, the results presented might explain in part, some of the opposing views in the literature concerning the effects of ouabain on coronary circulation and myocardial potassium (see Historical Review).

OUABAIN-INDUCED CARDIAC ARRHYTHMIAS AND ELECTROLYTES.

The data presented show that the characteristic cardiac arrhythmias (sinus bradycardia, ectopic beats, ventricular tachycardia) induced by ouabain can be significantly influenced by changes in the electrolytes in the perfusing fluid. Thus, these arrhythmias can be clearly diminished or prevented by perfusion with Locke containing, either excess of potassium, or added magnesium, or a high concentration of lactate, or reduced calcium content. Conversely, the arrhythmias are enhanced or potentiated by either decreased potassium, or increased calcium, or omission of sodium from the perfusing fluid.

In the case of the initial sinus bradycardia, it would appear that this is due to the initial "net gain" of potassium by the heart, since excess potassium in the perfusing fluid augments the bradycardia, while reduced potassium decreases it. The later arrhythmias (ectopic beats, ventricular tachycardia) also appear to be due to the "net loss" of potassium by the heart, since they are lessened by excess potassium and enhanced by reduced potassium. In addition, magnesium which antagonizes potassium movements as shown above, also reduces these ouabain arrhythmias.

In addition, it is clear that either excess of calcium or an omission of sodium from the perfusing fluid, can enhance the arrhythmias. On the other hand, either decreasing calcium, or adding lactate abolished these arrhythmias. Whether or not these effects involve relative or secondary changes in potassium cannot be stated from our data. However, curiously enough, under none of these conditions was the time required for cardiac standstill prolonged when

the heart was perfused with a fixed (7.5 γ/ml.) ouabain concentration.

Since the cardiac actions of the digitalis glycosides involve "peculiar characteristics connected with their penetration and fixation in heart muscle" (Sollmann, 1948), this latter effect might be due to the fact that the ultimate toxic action of ouabain on the heart depends only on a progressive "cumulation" or"fixation" of the glycoside, which occurs irrespective of any of the electrolyte changes which have been investigated. These findings would agree in some respects with those recently described by Hazard, Hazard and Thouvenot (1956), concerning the influence of various potassium salts upon the toxicity of digitoxin on the guinea pig heart in vivo. These authors found that with high potassium concentrations, the average lethal dose of digitoxin was not significantly increased. It would then appear from our observations that the basic mechanism of the arrhythmias is not identical with the basic mechanism leading to ultimate cardiac arrest by ouabain. This curious phenomenon might indeed, be related to the cumulation or physical fixation of the glycoside in the heart. It is conceivable that these arrhythmias might be simply "triggered" perhaps by the potassium imbalance induced by ouabain, but even when these effects are blocked or antagonized by one means or another, the progressive cumulation of the glycoside still continues until a toxic maximum leading to cardiac arrest is reached.

POST-OUABAIN RESTITUTION OF THE HEART AND ELECTROLYTES.

Although only a limited number of experiments were performed in connection with this problem, it was observed that after a fixed concentration of ouabain was perfused, there were considerable variations in initiating recovery, depending on the ionic composition of the ouabain perfusing fluid employed.

Using decreasing concentrations of ouabain in normal Locke, while it required longer to stop the heart, the recovery also developed more slowly, and at times was impossible. This might indicate that the duration of

exposure to ouabain was the primary factor involved in the degree of irreversible "fixation" of the glycoside.

It would appear that resuscitation of the ouabain arrested heart depends upon the presence of potassium in the perfusing fluid employed during the recovery period. Thus, as the potassium concentration in this fluid decreased, the initiation of recovery was delayed and with potassium-free Locke, no recovery ensued. Recovery was also accelerated, following perfusion with excess potassium, or after the heart had been ouabainized in sodium-free Locke. In the latter case also, there was a relative increased potassium-ouabain concentration. In both of these conditions, therefore the heart might have contained a high potassium concentration at the time of arrest. The rapid recovery could then be due to an accelerated outward movement of potassium. Scarinci (1953) has also reported that in the isolated frog heart after systolic arrest, induced by perfusion with ouabain in Clark solution, restoration of contractions could be readily obtained when the perfusing fluid was changed to potassium-free Clark. There must however, be other factors involved, since recovery could not be achieved when the heart was ouabainized either in a calcium-free or in a dextrose-free or in a high lactate medium. In addition, after perfusion with magnesium-ouabain, recovery was rather variable. Indeed, under none of these conditions was there any detectable evidence of potassium retention.

Finally, it would appear that in the absence of calcium during ouabain perfusion, although ouabain toxicity developed in the usual manner, recovery was not possible in five out of six experiments. This might indicate that in the absence of this ion the ouabain becomes more firmly "fixed" in the heart. No definite explanations for the lack of recovery with these latter types of perfusions can be given at present, but this aspect of the problem appears to warrant further investigation.

THE EFFECT OF DIGITALIS ON SOME METABOLIC PROCESSES.

The available information concerning the biochemical reactions affected by digitalis is limited. Indeed, the biochemical bases for the normal heart contractions or the changes involved in cardiac failure are still not known. This problem has been reviewed by Wollenberger (1949).

That the action of digitalis does not involve oxidative metabolism, the Krebs tricarboxylic acid cycle or the Embden-Meyerhof reaction, has been demonstrated by Ellis (1953). He showed that the responses of the isolated frog heart to strophanthin-k were not dependent on metabolic activity limited by the application of various enzyme inhibitors—nitrogen, sodium fluro-acetate, sodium iodoacetate, iodoacetamide or 2.4 dinitrophenol. Ellis concluded that the site of action of the glycosides is in the area of metabolism involved in the utilization of chemical energy for muscular work.

Recently Bellet, Wasserman and Brody (1955) have revived interest in the possible use of sodium lactate to restore the failing heart. As early as 1935, Lovatt-Evans showed that the heart can use lactic acid as a source of energy.

Clark, Eggleton, Eggleton, Gaddie and Stewart (1938) have also reported that the frog heart can oxidize lactic acid both when it functions normally and after it had been poisoned by iodoacetic acid, and in the latter lactate could revive the heart.

From in vitro studies on the influence of lactate on the dog's heart (left ventricle) by Wollenberger (1953), it was concluded that following addition of C^{14} labelled lactate to ouabain (5×10^{-7} M.), there was an acceleration of the uptake of lactate and the production of $C^{14}O_2$.

Although the mechanism of the action of lactate in diminishing the arrhythmias, as described above, is not clear, the data presented leave no doubt that sodium lactate does not prevent ouabain cardiac arrest. It would appear that a proper antidote to digitalis would have to alter not

only the cardiac arrhythmias, but also antagonize or prevent the normal physical "fixation" or "cumulation" of the glycosides in the heart. This latter is apparently also not affected by any of the other ions (potassium, calcium, sodium) studied. This aspect of the findings warrants further investigation.

GENERAL SUMMARY

In the isolated perfused rabbit and cat heart, it has been shown:

1. that, increasing the concentrations of ouabain (1.88 to 30 γ/ml.), in the perfusing fluid, leads to a corresponding decrease in the time required to reach cardiac standstill. The least variability from experiment to experiment was observed with a concentration of 7.5 γ/ml. under these conditions.

2. That, increasing the concentrations of ouabain in the perfusing fluid leads to transient initial sinus bradycardia, followed by a more sustained cardiac stimulation associated with increased amplitude of contractions and increased degrees of arrhythmias (shifting pacemaker, ectopic beats, paroxysmal and ventricular tachycardia and final ventricular fibrillation).

3. That, all concentrations of ouabain studied lead to an initial moderate transient decrease in coronary flow rate associated with a transient "net uptake" in potassium, and followed by a more sustained increased coronary flow, associated with a more sustained "net loss" of potassium.

4. That, following ouabain cardiac arrest, perfusions with normal Locke lead to slight irregular coronary dilatation, followed by complete recovery. Similar perfusion with Locke containing 50 per cent of normal potassium lead to prompt and sustained coronary dilatation, and eventual restoration to normal sinus rhythm, but prolonged perfusion with potassium-free Locke led to no recovery. Perfusion with added sodium lactate or simple injections of potassium chloride, calcium chloride, or adrenaline did not enhance the recovery as observed with normal Locke.

5. That, using a fixed ouabain perfusing concentration (7.5 γ/ml.) during perfusion with <u>excessive potassium</u> (16.8 mEq./l.) in Locke, there is no significant difference between the average times required to lead to cardiac arrest, as compared with similar "control" experiments <u>without ouabain</u>.

In both groups of experiments there was a rapid and sustained coronary constriction associated with a net uptake of potassium, but no coronary dilatation. Rapid ventricular fibrillation ensued in both groups.

6. That, with the fixed ouabain concentration, and twice normal potassium (11.2 mEq./l.), the average period for cardiac arrest was 15.17 ±3.05 minutes, while in similar experiments without ouabain, the heart continued to beat relatively well, for at least 60 minutes of observation. During perfusions with ouabain (7.5 γ/ml.) in normal Locke, cardiac standstill occurred in 17.56 ±0.63 minutes.

7. That, during similar perfusion with twice normal potassium (11.2 mEq./l.), there is a prompt and sustained coronary constriction associated with initial marked increase in "net uptake" of potassium, followed by a slight "net loss". In the controls (without ouabain) there was more gradual coronary constriction associated with little change in potassium equilibrium.

8. That, during perfusion with ten per cent excess potassium (6.16 mEq./l.) the toxicity of ouabain was enhanced, as judged by the average time required for cardiac arrest (13.0 ±1.53 minutes), and as compared with that required when normal Locke was the perfusion fluid (17.56 ±0.63 minutes). There was a brief coronary constriction followed by marked and sustained dilatation, and associated with an average "net uptake" of potassium, followed by a "net loss".

9. That, with all concentrations of potassium above that in normal Locke, there was a definite diminution in the tendency to the development of ventricular ectopic beats, although there was no significant increase in the perfusion times required for cardiac arrest.

10. That, with decreased potassium in the perfusing fluid, ouabain induced relatively less initial coronary constriction followed by greater coronary dilatation. Concomitantly, there was no evidence for the usual initial "net uptake" of potassium, but rather a continuous and sustained "net loss".

11. That, following cardiac arrest during perfusion with high potassium (16.8 and 11.2 mEq./l.) normal Locke perfusion led to rapid recovery, that is, initiation of ventricular activity associated with striking coronary dilatation. Conversely, after previous cardiac arrest with decreased potassium, recovery with normal Locke was definitely more delayed in the rabbit and absent in the cat.

12, That, with the fixed ouabain concentration, and using either increased (16.8 mEq./l.) or decreased (zero) calcium, there was no significant difference between the average times required to lead to cardiac arrest.

13. That, perfusion with normal Locke led to recovery and resuscitation of the hearts, when previously arrested by ouabain (7.5 γ/ml.) in excess calcium; but, that no recovery ensued after similar arrest by ouabain in calcium-free or calcium-potassium-free Locke.

14. That, in the presence of excess calcium, accentuated cardiac stimulation and arrhythmias ensued, and conversely, in zero calcium, there was no sustained stimulation, but only a short period of preterminal arrhythmias.

15. That, during perfusion with either increased calcium or calcium-free solutions, there was no significant deviation from the usually observed ouabain effects on coronary flow or potassium balance.

16. That, in the presence of added magnesium (0.36 mg./ml.), the average duration to cardiac arrest was 16.0 ±3.61 minutes, while in similar experiments without ouabain, the heart continued to beat in a relatively normal manner for approximately 60 minutes.

17. That, with added magnesium, there was an immediate intense coronary dilatation which became less marked although well sustained. These changes were not accompanied by any marked alterations in the cardiac potassium equilibrium.

18. That, with added magnesium, there was a definite decrease in the

occurrence of ventricular ectopic beats, and decrease in the intensity of the contractions, even when there was no significant increase in time required for cardiac arrest.

19. That, after magnesium-ouabain cardiac arrest, perfusion with normal Locke led to no consistent pattern in the recovery of hearts—some initiated strong ventricular contractions within minutes, while others showed only a weak activity after prolonged perfusion with normal Locke, although three out of six returned to normal sinus rhythm.

20. That, during perfusion of the isolated heart with Locke solution containing no sodium, the onset of cardiac arrest developed equally rapidly, whether ouabain was added or not.

21. That, perfusion with the sodium-free Locke leads to coronary constriction and rapid cardiac stimulation, followed by arrhythmias and ventricular fibrillation, and there were no marked alterations in the potassium equilibrium either in the presence or absence of ouabain.

22. That, following cardiac arrest, in all sodium-free Locke experiments, perfusion with normal Locke induced prompt recovery of the heart associated with an intense coronary dilatation.

23. That, the overall changes in coronary flow and potassium balance, both during the development of arrest and during the recovery period in the sodium-free perfusion experiments, resemble rather closely those observed earlier with ouabain during perfusion with increased potassium.

24. That, during perfusion with dextrose-free Locke, the onset of ouabain cardiac arrest is not altered, and the coronary flow, heart contractions and observed changes in potassium balance, were similar to those generally seen with ouabain in normal Locke.

25. That, following ouabain cardiac arrest, during perfusion with dextrose-free Locke, perfusion with normal Locke led to no recovery.

26. That, during perfusion of isolated hearts (rabbit or cat) with ouabain (7.5 γ/ml.) and Locke solution containing either one or ten mg./ml. lactate, the average time interval to cardiac arrest was shortened (12.5 and 10.0 ±1.64 minutes, respectively), in comparison with the ouabain response in normal Locke.

27. That, definite decrease in the number of ventricular ectopic beats and the intensity of the contractions were observed with 10 mg./ml. lactate although the toxicity of the ouabain was increased.

28. That, during perfusion with the low concentration of lactate (1 mg./ml.) and added ouabain, typical initial coronary constriction followed by dilatation ensued. The usual associated potassium changes were also present. With the high lactate concentration (10 mg./ml.) there was an immediate coronary dilatation (associated with myocardial depression), followed by a slight reduction in coronary flow, and subsequent increased coronary dilatation. These changes were associated with a loss of potassium throughout the duration of the lactate-ouabain perfusion.

29. That, following cardiac arrest and perfusion with normal Locke, the hearts arrested with low lactate concentration (1 mg./ml.) with added ouabain, recovered in 12 - 16 minutes. On the other hand, hearts arrested with high lactate (10 mg./ml.) plus ouabain, showed no tendency to recovery.

GENERAL CONCLUSIONS.

The findings presented above suggest the following conclusions:

1. There is an intimate relationship between changes in coronary flow, heart action and potassium balance in the beating heart which can be correlated with varying concentrations of ouabain.

2. Ouabain leads to an initial transient coronary constriction associated with sinus bradycardia and net gain of potassium, followed by sustained coronary dilatation, cardiac stimulation and net loss of potassium.

3. The initial coronary constriction is associated with an increased potassium uptake, while the subsequent dilatation is preceded by and appears to be due to the observed potassium loss.

4. Subsequent resuscitation of the ouabain-arrested heart appears to depend upon the presence of potassium in the perfusing fluid.

5. Excess potassium in the perfusing fluid can antagonize cardiac irregularities induced by ouabain, but no mutual antagonism appears to exist between the two agents in respect to the onset of cardiac standstill.

6. Decreased potassium, but not complete absence of potassium, in the perfusing fluid, augmented cardiac irregularities and enhanced cardiac standstill, in comparison with controls.

7. The onset of ouabain cardiac standstill is not influenced by either calcium concentration per se, or by the calcium-potassium ratio in the perfusing fluid, although the appearance of arrhythmias were clearly modified by the presence or absence of calcium.

8. With excess calcium, there was normal recovery of the heart, but in the absence of calcium, ouabain toxicity was irreversible, suggesting either an enhanced fixation or retention of the glycosides in the absence of calcium.

9. The effects of ouabain on the coronary flow and on the cardiac potassium

equilibrium are not dependent on calcium.

10. Magnesium added to the perfusing fluid antagonizes the cardiac irregularities induced by ouabain, but do not delay the onset of cardiac standstill.

11. Magnesium antagonizes the usual observed changes in cardiac potassium balance, following ouabain.

12. Magnesium per se exerts a marked coronary dilatation, which is independent of potassium movement and which is not effected by ouabain.

13. Magnesium per se abolishes the usually observed ouabain potassium changes, and this effect might be responsible for the absence of the cardiac arrhythmias. These experiments again emphasize that there is no relationship between the onset of ouabain cardiac arrest and potassium equilibrium in the heart.

14. Characteristic cardiac alterations induced by sodium-free Locke perfusion may be due to the relative increase of potassium and would suggest: (i) that, the sodium-potassium ratio is important in maintaining both normal heart function and coronary circulation and (ii) that, an alteration in this ratio in the heart might be a fundamental factor in the mechanism of the action of ouabain.

15. Dextrose is not essential for either the occurrence of the effects of ouabain on the coronary circulation or potassium balance, or for the development of cardiac arrest.

16. Following digitalization of the heart in the absence of dextrose, the toxicity is irreversible, rather like that observed following digitalization in the absence of calcium.

17. Added lactate increased ouabain toxicity, although the arrhythmias were completely prevented, indicating again, no relationship between ouabain arrhythmias and ouabain toxicity (cardiac arrest).

18. Both the coronary flow and potassium alterations with ouabain are somewhat distorted by the associated myocardial depression due to the lactate.

19. In the presence of lactate, digitalization of the heart led to an irreversible toxicity, rather like that previously shown with calcium-free or dextrose-free Locke.

CLAIMS OF ORIGINAL WORK

1. The application of a new technique of simultaneously recording coronary flow and heart action (mechanical and electrical) and associated potassium exchanges, to the study of the cardiac action of digitalis.

2. A systematic study on the effects of various concentrations of ions on the coronary circulation and heart action in the presence or absence of ouabain.

3. The use of ouabain as a means for elucidating some of the chemical factors that may effect coronary circulation.

4. The results of these investigations clarify several conflicting observations in the literature, relative to the problem of the influence of the electrolytes on the heart and establish the physiological importance of potassium movements in the control of the coronary circulation.

5. To the best knowledge of the author, the findings presented in this study are the first data in the literature; (a) to indicate a definite relationship between cardiac potassium disequilibrium and the calibre of the coronary vessels; (b) to show that digitalis induced arrhythmias are not necessarily related to digitalis induced cardiac arrest; (c) to show that the reversibility of digitalis "fixation" in the heart is dependent upon various electrolytes, or (d) to show that cardiac depressants, such as magnesium and lactate, can inhibit digitalis arrhythmias, without effecting ultimate digitalis cardiac arrest.

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