STUDIES ON THE EFFECT OF COLD-STORAGE ON RAT ATRIA

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EXTRAIT

Etudes de l'effet de la préservation par le froid sur les oreillettes de rats

L'effet de la préservation par le froid a été étudié sur les oreillettes de rats. La préservation par le froid ne change pas la fréquence de contraction spontanée mais produit une diminution de la contractilité. Les oreillettes préservées ont une concentration basse en potassium intracellulaire, un potential de repos reduit ainsi qu'une diminution de l'amplitude, de la durée, de l'aire et de la rapidité de dépolarisation du potentiel d'action. L'incubation du tissu dans un milieu pauvre en K^+ produit une augmentation de la contractilité des oreillettes preservées au froid mais non des oreillettes fraîches. Les oreillettes fraîches, incubées dans un milieu contenant 12 mM K^+ , ont des caractéristiques électrophysiologiques semblables à celles des oreillettes preservées au froid, mais elles ont une force contractile supérieure. L'incubation des oreillettes dans un milieu contenant de l'adénosine augmente leur contenu en ATP sans augmenter leur contractilité. Les effets inotrope et chronotrope de la noradrénaline, de la ouabaïn ou du Ca^{++} sont comparables sur les oreillettes fraîches et preservées. Il en est conclu que cette diminution de la contractilité des oreillettes préservées par le froid est partiellement due à des changements électrophysiologiques ainsi qu'à une diminution du contenu en ATP.

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ABSTRACT

The effect of cold-storage was studied on rat atria. Storage did not change the spontaneous rate but caused a reduction in the contractility. Stored atria had a low intracellular K^+ concentration and had a reduced resting potential, action potential height, duration, area and depolarization rate. Incubation in low K^+ produced an increase in the contractility of the stored but not of fresh atria. Fresh atria incubated in 12 mM K^+ had electrophysiological characteristics similar to but had a contractile force greater than those of stored atria. ATP content of stored atria was reduced. Incubation of stored atria with adenosine increased the ATP content but not the contractility. The positive inotropic and chronotropic effects of noradrenaline, ouabain or Ca^{++} on fresh and stored atria were comparable. It was concluded that the decrease in contractility of stored atria was due partly to electrophysiological changes and partly to the reduced ATP content.

STUDIES ON THE EFFECT OF COLD-STORAGE ON RAT ATRIA

by

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PkEFACE

The effects of cold-storage on the response of various smooth muscles to drugs has been studied in this laboratory (Varma and McCullough, 1969). It was therefore of interest to study the effects of cold-storage on heart muscle. Preliminary experiments showed that rat atria could be stored in the cold for periods of up to 72 hours. During the period of storage, the contractile force steadily decreased but the spontaneous rate remained unaltered. An examination of the literature revealed that although cold-storage of heart is known to lead to a reduction in the contractile force without any change in the spontaneous rate (Athreya et al, 1968; Garzon et al.,1968; Ferrans et al., 1970; Kamiyama et al., 1970; Trunkey et al., 1970), no study had been undertaken to determine the causes for this reduction in the force of contraction or the reasons for the lack of change in the spontaneous rate. This thesis therefore is a study of the above problems. For the purpose of the investigation, rat atria were cold-stored for periods of up to 72 hours in a physiological solution.

INTRODUCTION

A. RATE AND CONTRACTILE FORCE IN COLD-STORED ATRIA

Storage in the cold has been used for many years as a method of preservation of tissues. However, physiological and biochemical changes occur during the period of storage and these changes alter the functional state of the stored tissue or organ. The nature and magnitude of alteration in the functional state depends, among other things, on the tissue involved. In the case of the heart, prolonged storage in the cold has been reported to cause a reduction in the contractile force. Trunkey et al. (1970) found that storage of dog heart for 24 hours at 8-10^{o} C resulted in mitochondrial swelling and a loss of glycogen. Garzon et al. (1968) found that the maximal rate of rise of the ventricular pressure of dog hearts stored for 24 hours at 4^{o} C was approximately one third of the control. Ferrans et al. (1970) studied cold-stored dog hearts and found that the function and ultrastructure remained normal for up to 14 hours of storage; these authors observed that storage for 18-24 hours caused a decrease in the rate of rise of ventricular pressure and structural changes. Kamiyama et al. (1970) reported that the storage of rat hearts in the cold for 48 hours resulted in approximately 50% reduction in contractile force with no change in the spontaneous rate. Athreya et al. (1968) found a reduced contractile force in rabbit hearts stored for 24 hours at 4^{o} C; they reported that the storage of the hearts for 24 hours in 11-17 mM K^{+} prevented the decrease in force of contraction. However, after storage in this medium for 48 hours mechanical recovery was poor but electrical activity was normal. In all of the studies mentioned above, the whole heart was stored and was perfused during the storage period. It was thus possible that some changes were due to the perfusion and not the storage per se as difficulty with perfusion has been reported during the

long storage period (Webb, 1968; Childs and Lower, 1969).

Although the contractile force is decreased by cold storage, no change in spontaneous rate has been reported. Kamiyama et al. (1970) reported no change in spontaneous rate of whole rat hearts stored for 48 hours in the cold, despite a 50% reduction in force. It has been reported, however, that severely damaged stored hearts cannot be defibrillated indicating a change in excitability (Ferrans et al., 1970).

B. EFFECT OF DRUGS ON COLD-STORED TISSUES

No studies dealing with the effect of drugs on cold-stored heart muscle could be found. However, several studies performed on smooth muscle have been reported. Varma and McCullough (1969) found that 7 days after storage at 6° C, responses of rabbit aortic strips to noradrenaline reduced while those of rabbit splenic strips and rat vasa deferentia were increased. There was no change in the response of the cat nictitating membrane. The maximum response to noradrenaline was reduced in the splenic strips and vasa deferentia but not in the other preparations. Shibata (1969) found an increase in the sensitivity to noradrenaline and adrenaline in rabbit aortic strips stored in cold for up to 7 days, but a decreased sensitivity after longer periods of storage. Lum et al. (1966) found that after 24-72 hours cold-storage there was a decrease in the response of rabbit jejunum to phenylephrine and methoxamine but not to noradrenaline or adrenaline; they attributed this phenomenon to a selective loss of α receptors produced by cold-storage. Shibata (1969) reported reduced responses to nicotine and tyramine in cold-stored rabbit aortic strips; he attributed this change to the degeneration of the sympathetic nerves and a loss of catecholamine

stores, since these agents act by releasing noradrenaline. McDougal and Borowitz (1972) reported reduced responses to serotonin and tetramethylammonium but no change in responses to barium in cold-stored guinea pig ileum. Cold-storage is also known to cause a decrease in responses to high K^+ in various preparations (Shibata, 1969; Varma and McCullough, 1969; McDougal and Borowitz, 1972). This was probably due to low intracellular concentrations of K^+ which resulted in a partial depolarization of the muscle fibers (Shibata, 1969).

C. ATTRIBUTED MECHANISMS FOR THE DECREASED CONTRACTILE FORCE IN COLD-STORED HEARTS

1) Electrophysiological changes

Incubation of red blood cells (Harris, 1941; Danowski, 1941), smooth muscle (Daniel, 1965; Friedman and Friedman, 1967) and heart (Page and Storm, 1965) in the cold is known to cause a loss of intracellular K^+ and an increase in intracellular Na^+. The response of smooth muscle to K^+ has been shown to be less after cold-storage (Shibata, 1969; Varma and McCullough, 1969; McDougal and Borowitz, 1972), which would indicate that the cells were partially depolarized after the prolonged storage period. No experiments have been reported investigating the contractile force and any electrophysiological changes in the heart after cold-storage. Matsuda et al. (1956) reported normal resting and action potentials in dog ventricle after 72 hours of cold-storage. Matsuda et al. (1967) also reported normal action potentials in dog papillary muscle stored in the cold for 24 hours. Matsuda (1960) found that action potentials and resting potentials were normal in dog terminal Purkinje Fibers after 24 hours of storage but indicated that

"recovery became less satisfactory" after 48 hours of cold-storage. On the other hand, Tamai and Kagiyama (1968) found a marked hyperpolarization in kitten atria and ventricle after storage for 15-72 hours at 4° C. However, no indication of changes in the contractile force after cold-storage was reported in any of these experiments. The studies on the effect of cold-storage on the electrophysiological properties of cold-stored heart muscle are thus conflicting. However, it is not unlikely that electrophysiological changes do occur after extended periods of storage in some tissues. However, it is not known if these changes are responsible for the decrease in the contractile force produced by cold-storage. Experiments were therefore designed to determine if electrophysiological changes occurred after cold-storage and if these changes could be responsible for the low contractility of cold-stored atria.

2) Structural changes

Changes in the ultrastructure have been found after cold-storage and these are considered to be a possible cause for the decreased contractility of cold-stored hearts. Trunkey et al. (1970) found mitochondrial swelling and a loss of glycogen granules in whole dog hearts stored for 24 hours at 8-10° C. Ferrans et al. (1970) reported that the storage of dog hearts in the cold for 3-14 hours had little effect on the ultrastructure and contractile force but storage for 18-24 hours caused mitochondrial swelling, damage to the mitochondrial cristae, interstitial edema and swelling of the sarcoplasmic reticulum. These findings, they reported, could be correlated with the decrease in the contractile force of the hearts; when the contractility was poor, the structural damage was prominent and when the contractility was good, damage was slight. However, as these hearts were perfused during storage,

it is not known whether the changes were due to the lack of adequate perfusion as discussed above or to storage in the cold itself. Electron microscopy and measurements of extracellular space were therefore undertaken in the present work.

3) Metabolic changes

Changes in the metabolic processes might be expected to result in a decreased contractile force. However, little work has been done on the metabolic effects of prolonged cold-storage. Lee and Webb (1959) showed that dog hearts were able to metabolize glucose and lactate normally after 6-8 hours of cold-storage. Calman et al. (1970) reported that adenosine triphosphate (ATP) levels in rat hearts stored at 4^{o} C correlated with the "percentage survival" of the hearts. Loss of glycogen has also been reported in dog hearts stored at low temperatures for 24 hours (Ferrans et al., 1970; Trunkey et al., 1970). The reported increase in survival time of hearts stored in the cold with hyperbaric oxygen or with metabolic inhibitors such as magnesium sulphate indicate that metabolic changes occur during cold-storage and may be responsible for decreases in the contractile force of cold-stored hearts (Webb, 1968; Childs and Lower, 1969). In the present work an attempt was made to determine if changes in the ATP content could account for the decrease in contractile force produced by cold-storage.

D. ELECTROPHYSIOLOGY OF HEART MUSCLE

The action potential is thought to be the process which initiates the contraction of the heart under physiological conditions. The action potential recorded in atrial muscle is characterised by a sharp spike, as in other

tissues, followed by a smooth repolarization phase. The repolarization phase of the action potential in atrial muscle does not exhibit the "plateau" seen in ventricular muscle and is of shorter duration.

1) Ionic basis of electrophysiological activity

Electrical events in various tissues have for a long time been explained by ionic partitions and movements. Hodgkin (1951) postulated that the partition of K^+ was responsible for the resting potential. This has also been shown to be the case in the heart. Burgen and Terroux (1953a, 1953b) found that the resting potential in cat atrium varied in a linear relation with the log of the extracellular K^+ concentration. Draper and Weidmann (1951) found that the resting potential was largely unaffected by changes in the extracellular Na^+ concentration. In their classical work on the giant squid axon, Hodgkin and Huxley (1952a, 1952b, 1952c, 1952d) and Hodgkin et al. (1952) formulated equations to describe the movements of Na^+ and K^+ during the action potential. They demonstrated that the action potential could be explained by an abrupt increase in the Na^+ permeability which allowed Na^+ to flow down its electrochemical gradient and cause the action potential spike. There is much evidence to suggest that the cause of the spike of the action potential in heart muscle occurs through the same process (Cranefield et al., 1951; Draper and Weidmann, 1951; Brady and Woodbury, 1960; Noble, 1962). Hodgkin and Huxley (1952d) found that the initial increase in Na^+ conductance associated with the spike of the action potential fell off abruptly and was followed by an increase in K^+ permeability. These processes are believed to be responsible for the repolarization process. In heart muscle, a delayed

decrease in the Na^+ conductance after the action potential spike has been reported by several authors (Brady and Woodbury, 1960; Johnson and Tille, 1960; Noble, 1962; Deck and Trautwein, 1964). In addition, there is much evidence to indicate that there is, in heart muscle, a delayed increase in K^+ conductance (Weidmann, 1951, 1952) or a decrease in K^+ conductance (Brady and Woodbury, 1960; Johnson and Tille, 1960; Johnson and Wilson, 1962; Noble, 1962). These observations are thought to explain the plateau phase of the action potential in heart muscle. Although Na^+ and K^+ are the main ions responsible for the resting and action potentials, Ca^{++} has also been implicated in the action potential of some species (Niedergerke and Orkand, 1966; Coraboeuf and Vassort, 1968; Johnson and Lieberman, 1971).

The characteristic feature of the transmembrane potential of pacemaker fibers is a slow depolarization during the period of diastole of phase 4 of the action potential. Weidmann (1956) suggested 3 possibilities for this phenomenon: 1) a decrease in K^+ conductance, 2) a slow increase in Na^+ permeability, and 3) a reduction in the Na^+ pumping rate. The last possibility has the least evidence for it (Langer, 1968). Studies on membrane impedance on Purkinje fibers (Weidmann, 1951; Vassalle, 1966) and rabbit sinus fibers (Dudel and Trautwein, 1958) indicated that the resistance of the membrane increases throughout phase 4. Dudel and Trautwein (1958) found that a reduction in the extracellular concentration of Na^+ did not markedly reduce the slope of phase 4 in the sinoatrial node and Vassalle (1966) found an inward ionic current which was present in the absence of extracellular Na^+. Both authors concluded that the changes in membrane impedance are caused by the decrease in K^+ conductance, which results in a slow diastolic depolarization. Trautwein and Kassebaum (1961) found a

significant hyperpolarization in the absence of external Na^+ in Purkinje fiber and rabbit sinoatrial node and suggested that there is an appreciable resting Na^+ conductance during phase 4. They also found a voltage and time dependent decrease in K^+ conductance and suggested that the high resting Na^+ current acts as the generator of the impulse and that the decrease in the K^+ conductance re-establishes the condition of threshold. Trautwein and Dudel (1958) and Dudel and Trautwein (1958) have proposed that the maximum diastolic membrane potential is an after-potential resulting from the high K^+ conductance and the slow depolarization is a return of the membrane potential to the resting potential.because of a decrease in the K^+ conductance. DeMello (1963) has also implicated Cl^- in the current flow during phase 4; he found a significant increase in the slope of phase 4 when Cl^- was replaced by larger anions such as $SO_4^=$ in rabbit sinoatrial node and Purkinje fibers.

The resting membrane potential is due to the high intracellular concentration of K^+ and the low intracellular concentration of Na^+. The differences in the concentrations of these ions between the extracellular and intracellular fluid is governed by the resting permeabilities of these ions as well as a Na^+-K^+ pump. It is well established that the energy source for this pump comes from the hydrolysis of the terminal phosphate group of ATP by a membrane ATPase (Langer, 1968). This results in the active extrusion of Na^+ and accumulation of K^+. It is thus obvious that a reduction in the Na^+ pumping rate or an increase in the Na^+ permeability would result in the decrease in the resting potential. It is possible that a reduction in the energy stores could result in a reduction in the pumping rate and thus a depolarization of the cell membrane. A reduction in the resting potential would be expected to cause reductions in the other parameters of the transmembrane potential

Hoffman and Cranefield, 1960). In the present work, this possibility was investigated.

2) Effect of electrophysiological changes on contractile force

Direct correlations between the action potential and contractile force have been attempted (Reichel and Bleichert, 1959; Brady, 1964), but such correlations are difficult to make (Cranefield and Hoffman, 1958; Brady, 1964). In some cases, reductions in the various parameters of the action potential are accompanied by reductions in the contractile force. Incubation of heart muscle in high K^+ leads to reductions in action potential duration, height, depolarization rate, area and resting potential and these changes are associated with a reduction in the contractile force (Brooks et al., 1955; Cranefield and Hoffman, 1958; Hoffman and Cranefield, 1960). This reduction in the electrophysiological properties are probably due to the depolarizing actions of K^+ as Weidmann (1955a) found that the electrical repolarization of Purkinje fibers depolarized by high K^+ restored the action potential height and depolarization rate to normal. Acetylcholine has been shown to decrease the duration of the action potential in various preparations as well as decrease the contractile force (Webb and Hollander, 1956; Furchgott et al., 1960). Adrenaline has been shown to exert the opposite effects on the contractile force to acetylcholine and also to increase the duration of the action potential (Furchgott et al., 1960; Webb and Hollander, 1956). However, Furchgott et al. (1960) found that the addition of adrenaline after acetylcholine completely antagonized the effects of acetylcholine on the contractile force but only partially antagonized the effects on the transmembrane potential. Hollander and Webb (1955) found in

rat atria that the increase in stimulating frequency resulted in the decrease in the contractile force as well as in the duration and area of the action potential. However, examples are also numerous in the literature where the effect on the transmembrane potential is opposite to that on the contractile force. For example, Lu et al. (1968) and Spear and Moore (1971), in studying alternations in mammalian cardiac muscle, found that the action potential with the shorter duration was associated with the contraction with the smaller force. In some species, ouabain is known to decrease the duration of the action potential but increase the force of contraction (Reiter et al., 1966; Edmands et al., 1967; Prasad and Callaghan, 1970). However, in these cases, it is possible that the increase in force has occurred through a process unrelated to the transmembrane potential and the effects on the action potential are secondary. This is almost certainly the case with the cardiac glycosides. It is obvious that not all changes in the contractile force are produced by changes in the electrophysiological properties of the heart, but one would expect from the results of experiments performed by many workers, that changes in the transmembrane potential would result in changes in the contractile force as long as the agent producing these changes did not act on other processes as well.

3) Effect of electrophysiological changes on rate

There is a change in the rate at which the heart beats when the interval between the impulses generated by the sinoatrial node is altered. The interval between the impulses depends on either the rate of depolarization during phase 4 of the action potential, or on the amount of depolarization required to produce an action potential. The latter process depends on the

maximum diastolic membrane potential and the threshold potential (Hoffman and Cranefield, 1960). Correlations have been made between the effect on the rate and the change in the electrical activity of the sinoatrial node using various agents. Castillo and Katz (1955) and Hutter and Trautwein (1955, 1956), studying the effects of vagal stimulation on the frog sinus venosus, reported that the slope of phase 4 was reduced upon stimulation. They found that with stronger vagal stimulation, hyperpolarization and arrest occurred. West et al. (1956) found similar effects after the addition of acetylcholine to rabbit sinoatrial node. Hutter and Trautwein (1956) found that stimulation of the sympathetic fibers to the frog atrium resulted in an increase in the slope of phase 4 of the action potential, but no change in the threshold potential. West et al. (1956) found that the addition of adrenaline to rabbit atria resulted in similar findings. Hoffman and Cranefield (1960) also reported a lowering of the threshold potential in cat and rabbit sinoatrial nodes after adrenaline administration. Marshall (1957) studied the effects of temperature on the rabbit sinoatrial node and reported that a decrease in the temperature resulted in a decrease in the slope of phase 4 of the action potential which corresponded with the reduction in rate of the preparation. She also found a depolarization of the pacemaker cells, but no change in the threshold potential. The slight depolarization was offset by the decrease in slope of the generator potential. Weidmann (1955b), in studying the effects of Ca^{++} on pacemaker activity in calf and sheep Purkinje fibers, found that the reduction in the extracellular Ca^{++} concentration resulted in a lowering of the threshold potential. All of the above changes in electrical activity

corresponded with the changes in the rate produced by the various agents.

E. HIGH ENERGY COMPOUNDS IN HEART MUSCLE

The energy necessary for the contraction of the heart is thought to come from the hydrolysis of the terminal phosphate group from ATP. Most of the high energy phosphate bonds occur either in ATP or in creatine phosphate (CP). It is reasonably certain that the direct source of energy for the contraction is ATP and not CP. CP replenishes the supplies of ATP through the Lohmann reaction: CP + ADP = ATP + C (Needham, 1960; Ruch and Patton, 1965).

1) ATP, CP and contractile force in heart

Although changes can occur in the contractile force without significant alterations in the high energy metabolism, reductions in the ATP or CP content are accompanied by reductions in the contractile force of heart muscle. However, under conditions which inhibit metabolism, the reduction in contractile force seems to be more closely related to reductions in CP content rather than in ATP content (Furchgott and Lee, 1961). Reductions in the contractile force and in either the ATP or CP content have been found during anoxia in rat heart (Fleckenstein et al., 1959; Scheuer and Stezoski, 1968; Dhalla et al., 1972), cat papillary muscle (Lee et al., 1961) and guinea pig ventricle (Furchgott and Lee, 1961), in dog ventricle poisoned with 2,4-dinitrophenol (DNP) (Fawaz and Tutunji, 1957) and fluoroacetate (Fawaz, 1956) and in guinea pig ventricle treated with phenylbutazone (Hochrein and Döring, 1958). However, in many cases of pathological and experimentally induced heart failure, ATP levels have not been found to be

reduced. In addition, ouabain, which effectively increases the contractile force of failed hearts, has no effect on high energy compound levels (Bing, 1965; Lee and Klaus, 1971). The reports in the literature seem to indicate that any condition which produces a reduction in either CP or ATP stores would also cause a reduction in the contractile force of cardiac muscle.

2) ATP, CP and spontaneous rate in heart

It has been reported that reductions in ATP content are accompanied by reductions in the spontaneous rate of the heart. Both anoxia and fluoroacetate reduce the ATP and CP content of heart muscle (Furchgott and Lee, 1961). Gardner and Farah (1954) found that these compounds reduced the rate of spontaneous contractions in rabbit atria. However, DNP, which causes a large reduction in the CP content but not in the ATP content (Gardner and Farah, 1954; Furchgott and Lee, 1961), has no effect on spontaneous rate. Harry et al. (1967) studied changes in the ATP content and spontaneous rate of cultured rat heart cells. They found that 2-deoxyglucose plus oligomycin inhibited spontaneous contractions and caused a reduction in the ATP content. They reported similar results for iodoacetate plus oligomycin as well as DNP. They attributed the reduction in or loss of spontaneous contractions to the decrease in ATP levels. However, cold-storage of rat heart has been shown to cause a decrease in the ATP content (Calman et al., 1970) without changing the spontaneous rate (Kamiyama et al., 1970). It thus appears that while in many cases reductions in ATP content are accompanied by reductions in rate, there are some conditions in which the ATP content is decreased without affecting the spontaneous rate.

METHODS

A. GENERAL PROCEDURE

Male rats (Sprague-Dawley) weighing between 150 and 300 g were killed by a blow on the head and the heart rapidly excised. In some experiments, the hearts were left in the dead animal for 2 minutes before they were excised. The whole atria (right and left attached together) were cut free from the ventricular muscle and cleaned in a procelain dish containing oxygenated Krebs-bicarbonate solution at room temperature and set up in 50 ml tissue baths at 30° C. Unless specified otherwise, atria refers to the whole atria (right and left). The physiological solution used in all experiments was Krebs-bicarbonate (Krebs) of the following composition (mM): Na^+, 143.5; K^+, 5.9; Mg^{++}, 1.18; Ca^{++}, 2.5; Cl^-, 128.2; HCO_3^-, 25; SO_4^{--}, 1.18; $H_2PO_4^{--}$, 1.2; dextrose, 11. The Krebs solution was continuously bubbled with 95% O_2 and 5% CO_2. Spontaneous contractions were measured isometrically with Grass FT.03 force transducers and recorded on a Grass polygraph. The resting tension was adjusted to give maximum contractions (approximately 500 mg). Both the rate and contractile force were recorded in all experiments. The temperature of the baths could be maintained at any temperature between 2° C and 30° C by means of a refrigerator-heating unit and the temperature raised or lowered at a rate of 1 C°/min. All atria were allowed to stabilize for 1 hour in the bath.

B. COLD-STORAGE

Atria to be stored were set up in tissue baths as described above and allowed to stabilize for 1 hour, the rate and contractile force being recorded for post-storage comparisons. The tissues were then cooled slowly to below 10° C, transferred to flasks containing cold Krebs solution and

placed in the refrigerator at 6° C for 8-72 hours. Atria stored at temperatures lower than 6° C did not exhibit spontaneous contractions when they were set up in the organ bath. In some experiments, atria were stored in Krebs solution containing 1.2, 12 or 18 mM K^+ instead of the usual 5.9 mM K^+. After the required period of storage, the atria were placed directly into the tissue baths at the desired temperature (see Results). The rate and contractile force of the cold-stored atria were observed over a 1 hour period after the atria were placed in the tissue baths.

C. RELATION OF TEMPERATURE TO SPONTANEOUS CONTRACTIONS

Cold stored atria were incubated in the tissue baths at 2° C in oxygenated Krebs solution for 1 hour and warmed up at the rate of 1 C°/min. The temperature at which spontaneous contractions started was recorded. The warming was continued until the temperature reached 30° C and the tissues were allowed to stabilize for 1 hour at this temperature. The atria were then cooled at the same rate and the temperature at which spontaneous contractions ceased was again recorded. The same procedure was followed in the case of the fresh atria except that the tissues were cooled slowly first, kept at 2° C for 1 or 4 hours and then rewarmed. The temperature at which spontaneous contractions stopped and restarted was recorded.

D. DETERMINATION OF Na^+ AND K^+

Fresh and cold-stored atria were incubated in the tissue baths for 1, 2 and 4 hours at the required temperatures. In one series of experiments, fresh atria were cooled to 2° C for 4 hours as described above, rewarmed and taken out of the tissue baths immediately upon the resumption of spontaneous contractions. The atria were removed at the end of the required period of time, gently blotted on filter paper to remove the excess water, weighed on an analytical balance and transferred to test tubes containing 2 ml of distilled water. The Na^+ and K^+ were extracted overnight at room temperature and measured after the appropriate dilutions with a Beckman flame photometer using lithium as an internal standard. Water used in the dilutions and preparations was first distilled and then passed through a deionizing column and had an ionic concentration of less than 0.1 ppm. Total atrial Na^+ and K^+ contents were expressed as mEq/kg of wet tissue. Total tissue water was determined by drying the atria overnight at 100° C; the difference between the wet and dry weight was taken as the amount of water. Extracellular space was determined with ^{14}C inulin (Page and Solomon, 1960). The atria were incubated in Krebs solution containing the inulin in a concentration of 100 μg/ml for 2 hours. The tissues were then weighed as above and the inulin extracted for 2 hours in 2 ml of distilled water. The ^{14}C was counted by liquid scintillation (see Counting Procedure). Extracellular space was expressed as ml/100 g of wet tissue. Separate tissues were used for measuring the ionic content, water content and inulin space.

Intracellular K^+ concentrations were calculated from the mean values of the total atrial K^+, water and extracellular space using the

following formula (Page and Solomon, 1960) and expressed as mEq/l:

$$[K^+_i] = ([K^+_t] - V_{in}[K^+_o])/(V - 0.987V_{in})$$

where $[K^+_i]$ = the intracellular K^+ concentration

$[K^+_o]$ = the K^+ concentration in the medium

$[K^+_t]$ = the total K^+ content of the atria

V_{in} = the volume of the inulin space

V = the volume of the total atrial water assuming a specific gravity of 1.00

0.987 = the weight fraction of the inulin space which is water

K^+ equilibrium potentials were calculated with the Nernst equation and expressed in mV:

$$E_K = (RT/F)\ln[K^+_i]/[K^+_o]$$

where E_K = the K^+ equilibrium potential

R = the gas constant

T = the absolute temperature

F = the Faraday

K^+_i = the intracellular K^+ concentration

K^+_o = the extracellular K^+ concentration

E. ELECTROPHYSIOLOGICAL MEASUREMENTS

Atria were mounted in a special 10 ml bath designed for the purpose of the electrophysiological experiments. The temperature of the bath could

be maintained at any temperature between 10° C and 30° C by means of a refrigerator-heating unit. Left atria were used in these experiments and were allowed to stabilize for 1 hour before intracellular recordings were made. Krebs solution was continuously circulated through the bath and bubbled with 95% O_2 and 5% CO_2. Electrophysiological measurements were made with glass intracellular microelectrodes filled with 3 M KCl (filtered with a Millipore filter using 0.3 μ Millipore filter paper) and having a resistance of between 10 and 50 megohms and a tip diameter of less than 1 μ. Resting membrane potentials were recorded using the standard fixed microelectrode technique and the action potentials were recorded using the floating microelectrode technique, the electrodes being suspended on fine tungsten wire. Chlorided silver wire was used as the indifferent electrode. Signals were amplified with a Bioelectric NF1 amplifier and displayed on one channel of a Tektronix 502A oscilloscope. Contractions were recorded simultaneously with a RCA 5734 force displacement transducer and displayed on the second channel of the oscilloscope. The resting tension was adjusted to give the maximum force of contraction. Photographic records were taken with a Grass Kymograph camera using Kodak Kind 1732 paper. Records shown in the thesis were tracings taken from the prints of individual potentials and contractions. Atria were stimulated through platinum electrodes with rectangular pulses of a supramaximal voltage and a 5 msec duration at a rate of 120/min. Atria at 10° C were stimulated with 5 and 50 volts at a rate of 15/min and a pulse duration of 30 msec. A Bioelectric stimulus isolation unit was used in all experiments.

Action potential durations were recorded using a sweep speed of 10 msec/cm and measured at the 95% repolarization level. Action potential

depolarization rates were recorded using a sweep speed of 2 msec/cm and measured from the beginning of the action potential to the maximum level of depolarization and expressed in V/sec. Resting potentials and action potential heights were expressed in mV. "Conduction velocity" was calculated by dividing the distance between the stimulating and recording electrodes, accurately measured with a micrometer, by the time interval between the stimulus artifact and the action potential and was expressed in cm/sec (Hollander and Webb, 1955). The contraction rise time, expressed in msec, was taken as the time for the contraction to reach its maximum force. The contraction rise velocity in g/sec was calculated by dividing the force of contraction by the contraction rise time. The stimulus contraction time in msec was taken as the time between the stimulus artifact and the beginning of the contraction. Areas of the action potentials and forces of contraction were measured with a polar planimeter and expressed in mV.sec and mg.sec, respectively. All measurements were made from enlarged images of the photographic records.

F. EFFECT OF DRUGS

These experiments were performed on whole (right and left together) spontaneously beating atria. Cumulative dose-response curves (rate and contractile force) to noradrenaline, ouabain, Ca^{++} and K^{+} were determined. The response to the initial concentration of the agent was allowed to reach its maximum before the next higher dose was added, and the procedure was continued until the maximum response was reached. In one series of experiments, the atria were incubated with 40 μM adenosine for 45 minutes followed by a 5 minute washout before the dose-response curve to ouabain was determined. In some experiments, DNP was added in a concentration of

0.03 mM and its effect on the force of contraction was measured until the spontaneous contractions ceased. All drugs were dissolved in 0.9% saline with the exception of DNP which was dissolved in ethanol. The volumes added to the bath never exceeded 2.5% of the total volume of the bath. Tetrodotoxin (TTX) was used in the electrophysiological experiments and added in volumes of 10, 30 and 50 µl/10 ml bath resulting in concentrations of 0.1, 0.3 and 0.5 µg/ml.

G. ELECTRON MICROSCOPY

Fresh atria and atria stored in the cold for 24 and 48 hours were fixed in 3% phosphate buffered glutaraldehyde at pH 7.3 for 3 hours and washed overnight in the same buffer at 6° C. The tissues were post-fixed in 2% osmic acid for 1 hour, alcohol dehydrated and embedded in Epon. Sections were stained with lead citrate and uranyl acetate and examined under a Philips electron microscope. These experiments were kindly performed by Dr. J.B. Richardson.

H. ATP DETERMINATIONS

ATP content of fresh and cold-stored atria was determined with the luciferase method of Strehler (1963). Whole spontaneously contracting atria were incubated for 1 hour at 6 or 30° C; the contractions were recorded during this period. At the end of the incubation period, the atria were removed quickly and frozen in a mixture of dry ice and ether. The tissues were weighed on an analytical balance, ground up with a mortar and pestle in 8% frozen perchloric acid, allowed to thaw and the ATP extracted for 15 minutes at 0° C. The extract was then filtered, neutralized

with NaOH to pH 7.2-7.4 and diluted to the appropriate concentrations with deionized water. One ml of tissue extract or ATP standard solution was mixed with 0.6 ml of 25 mM glycylglycine-0.1 M Mg^{++} buffer at pH 7.5 in a cuvette. One ml of firefly extract containing 50 mM arsenate and 20 mM Mg^{++} was rapidly injected into the cuvette and the light intensity measured with the photomultiplier section of an Aminco-Bowman Spectrophotofluorometer. The ATP content of the atria was calculated from a standard curve made from known concentrations of ATP. ATP content was expressed as μM/g of frozen tissue. ATP content was also determined in tissues previously incubated with adenosine in a concentration of 40 μM for 45 minutes with a 5 minute washout in adenosine-free Krebs solution.

I. COUNTING PROCEDURE

^{14}C was counted by liquid scintillation with a Nuclear Chicago liquid scintillation counter. The scintillation medium contained 1 litre toluene, 1 litre ethanol, 1 litre 2,4-dioxane, 240 g naphthalene, 15 g PPO (2,5-diphenyloxazole) and 187.5 mg POPOP (p-bis(2-(5-phenyloxazolyl))-benzene). Fifteen ml of scintillation medium was used and was sufficient for the volume of water in the samples. Samples were counted for 10 minutes each and the background was less than 10% of the total counts. A quench curve using ^{14}C quenched with nitric acid was made by the external standard method and the counts of all the samples were corrected for quenching.

J. STATISTICAL ANALYSIS

Student's "t" test was used in the statistical analysis, the results being judged significant with a p value of less than 0.05. Two-tailed tests

of significance were used throughout. The results are presented as mean values with their standard errors (S.E.). These were obtained from 15-20 atria in experiments determining rate and contractile force, from 6-10 atria in experiments determining Na^+ content, K^+ content, inulin space and ATP content, from 4-6 atria in experiments determining the dose-response curves of Ca^{++}, K^+, ouabain, noradrenaline and DNP, and from 30-50 penetrations (3-4 atria) in the electrophysiological measurements.

K. CHEMICALS AND DRUGS USED

Noradrenaline bitartrate (K & K Laboratories)

Ouabain (K & K Laboratories)

2,4-dinitrophenol (K & K Laboratories)

Tetrodotoxin (Calbiochem)

^{14}C-Inulin (New England Nuclear)

Adenosine triphosphate disodium (Aldrich)

Adenosine (Aldrich)

Glycylglycine (Sigma)

Arsenate buffer 0.1 M pH 7.4 (Sigma)

Firefly extract (Sigma)

RESULTS

A. CONTRACTILITY AND COLD-STORAGE

1) Effect of cold-storage on the contractile force

The effect of prolonged cold-storage on the contractile force of rat atria is shown in Fig. 1. In these experiments, atria were removed from the cold (6° C) and placed directly into the tissue baths containing oxygenated Krebs solution at 30° C. In general, they exhibited spontaneous contractions almost immediately (within 1 minute after being placed in the baths). The decrease in force of contraction within the first 24 hours of storage was steeper than in the subsequent 48 hours. This decrease in force of contraction was unaccompanied by any change in the spontaneous rate over the full 72 hour period of storage. Atria stored for 96 hours or more, in general, did not exhibit spontaneous contractions, nor could contractions be produced by electrical stimulation. The contractile force and rate of the rewarmed stored atria did not change during the 1 hour stabilization period indicating that atria attained their maximal contractility and rate very rapidly.

2) Relation of temperature to cessation of spontaneous contractions

Fresh atria were cooled slowly and the temperature at which spontaneous contractions ceased was noted. After the required period of time, the temperature was raised at the same rate and the point at which contractions restarted was noted. The same procedure was followed for the stored atria except that the cold-stored atria, after being rewarmed to 30° C were then recooled. The results are shown in Fig. 2. Upon cooling, cold-stored atria stopped beating at significantly higher temperatures compared to fresh atria. However, no significant difference in the

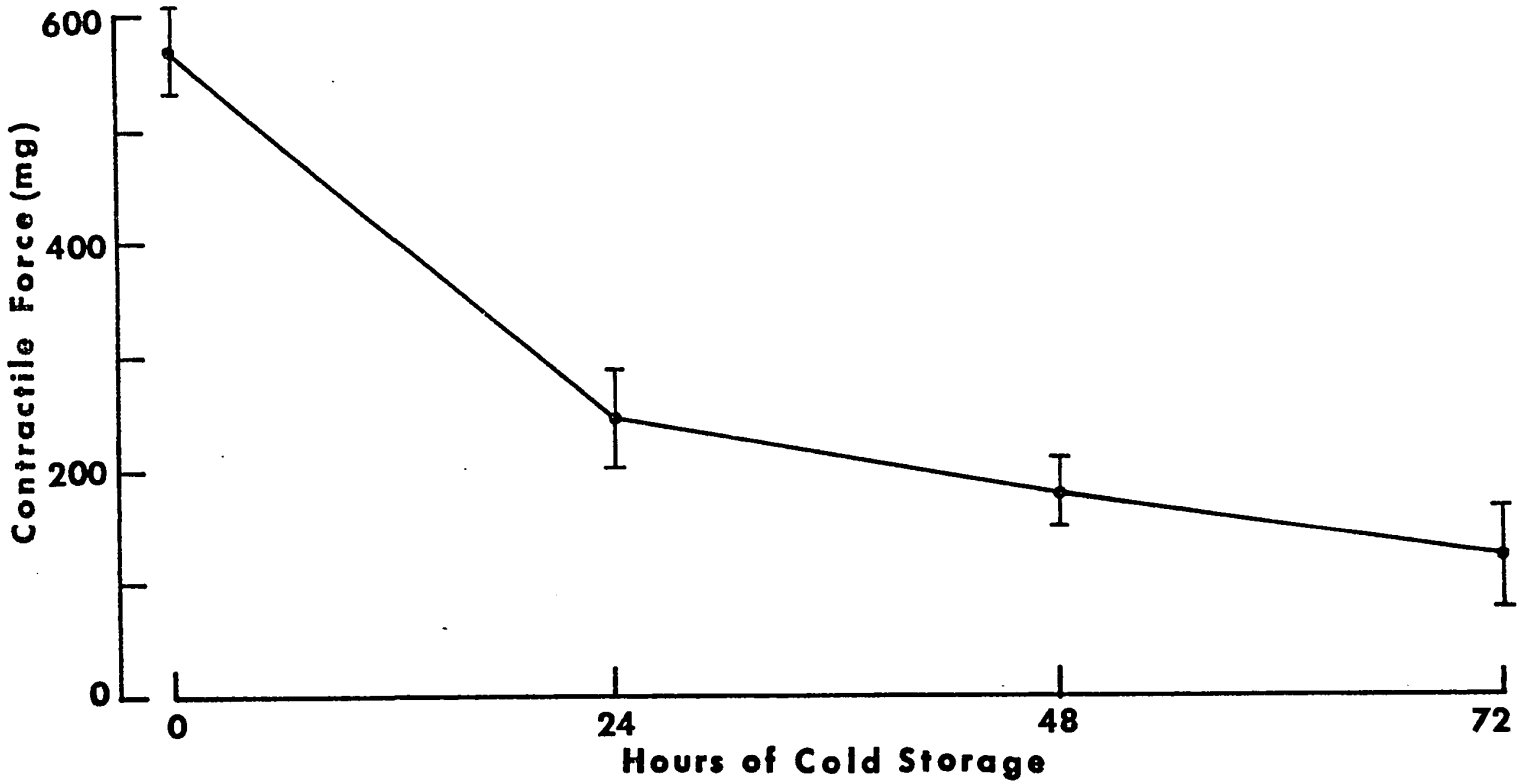

Fig. 1. Decrease in contractile force of spontaneously beating rat atria produced by storage at 6^{o} C for 24-72 hrs. Each point represents the mean $\pm$ S.E.

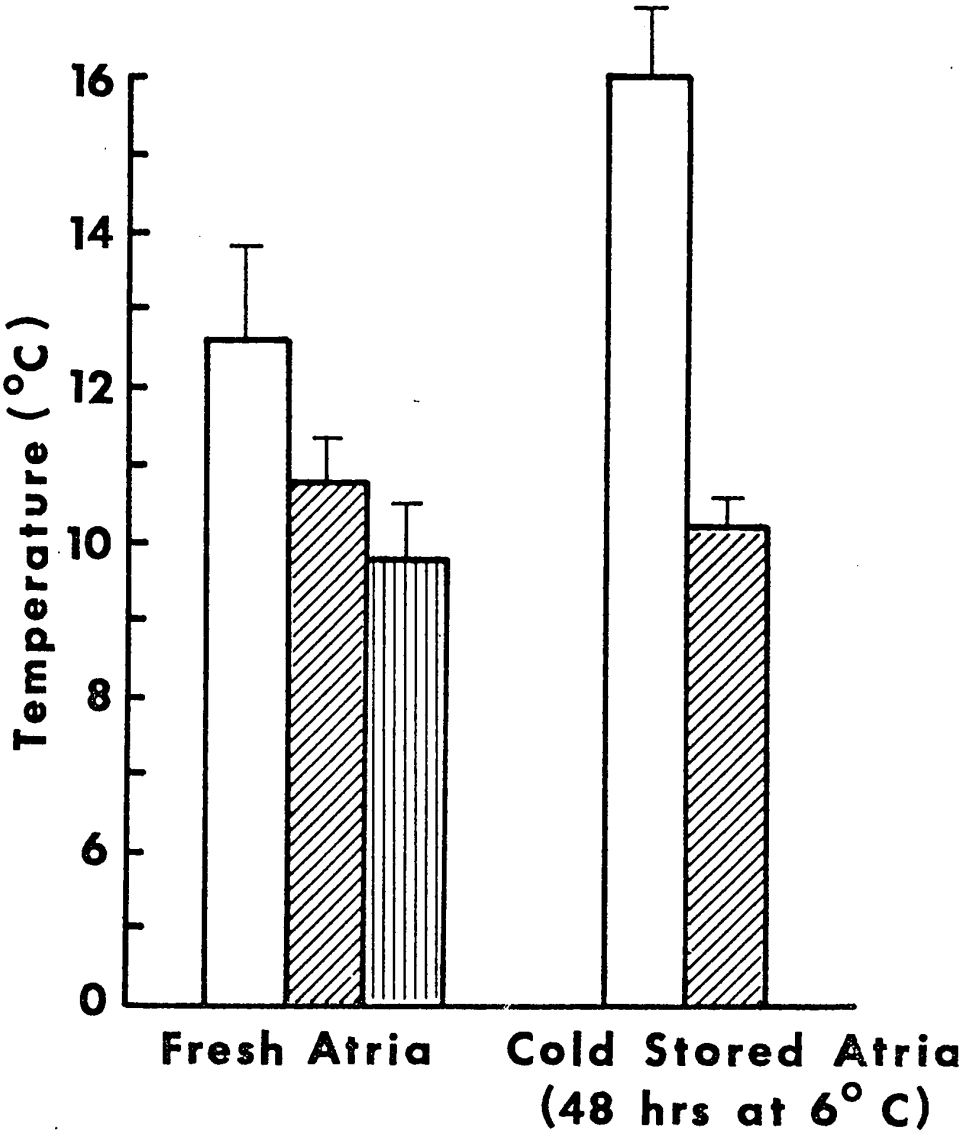

Fig. 2. Effect of cold-storage on the temperature at which spontaneous contractions stopped and started when rat atria were slowly cooled or warmed (1 C^{o}/min). Open columns represent the temperature at which contractions stopped. Cross hatched columns represent the temperature at which contractions restarted after incubation for 1 hr at 2^{o} C. The vertical hatched column is the temperature at which contractions restarted after incubation for 4 hrs at 2^{o} C. Each column represents the mean $\pm$ S.E.

temperature at which spontaneous contractions started between the fresh and cold-stored atria was observed. From these results, it would seem that the presence or absence of spontaneous contractions is determined by the temperature at which the tissue is incubated rather than any changes that may occur due to storage.

B. ELECTROPHYSIOLOGICAL CHANGES AND CONTRACTILITY IN COLD-STORED RAT ATRIA

1) Effect of cold-storage on the ionic content of rat atria

The importance of ionic metabolism in cellular processes and, in particular, in the function of the heart is well known. It was therefore decided to examine the Na^+ and K^+ content of the atria in order to determine if the reduction in contractile force of cold-stored atria could be related to any changes in their ionic content. The Na^+ and K^+ content of fresh and cold-stored atria is shown in Table 1. The total ionic content of these tissues is shown along with the intracellular K^+ concentration and the calculated K^+ equilibrium potential. The data shows that cold-stored atria contained significantly less K^+ and more Na^+ than did fresh atria at the same incubation temperature. Also, the intracellular K^+ concentration as well as the K^+ equilibrium potential of the stored atria were reduced when compared with the fresh. There was no significant difference in the Na^+ and K^+ content or in the intracellular K^+ concentration between the cold-stored atria which were incubated for 2 hours in oxygenated Krebs solution at 6° C and those which were incubated at 30° C. This indicated that very little if any recovery of the ionic gradients occurred when cold-stored atria were rewarmed.

TABLE 1. Effect of cold-storage on the ionic content of rat atria.

Preparation	Na^+ (total) mEq/kg ± S.E.	K^+ (total) mEq/kg ± S.E.	K^+ (intracellular) mEq/L	K^+ Equilibrium potential mV
Fresh atria 30° C (2 hrs)	70.4 ± 1.2	71.5 ± 1.1	127.4	79.8
Cold-stored atria (48 hrs at 6° C)				
6° C (2 hrs)	88.3 ± 4.9*	43.6 ± 2.6*	78.6	61.9
30° C (2 hrs)	86.2 ± 2.5*	43.4 ± 1.5*	78.6	67.2

*Significantly different from control (fresh) values, $p<0.05$.

All atria were incubated in fresh Krebs bubbled with 95% O_2 + 5% CO_2 for 2 hours before they were used for determinations of Na^+ and K^+.

2) Effect of cold-storage on the transmembrane potential of rat atria

The changes found in the ionic content and the calculated K^+ equilibrium potential of the cold-stored atria indicated a possible change in the transmembrane potentials of these atria. For this reason, it was decided to conduct an investigation into the electrophysiological properties of the cold-stored atria. Fig. 3 and 4 and Table 2 show the effects of 48 hours of cold-storage on the electrophysiological properties of rat atria. The resting membrane potentials of the stored atria were significantly lower than those of the fresh tissues (Table 2). This finding is consistent with the reduction seen in the calculated K^+ equilibrium potential. Tracings of typical action potentials of fresh (A) and cold-stored (B) atria are shown in Figs. 3 and 4; the tracings in Fig. 4 were taken from the same potentials shown in Fig. 3 but at a sweep speed 5 times greater to show the difference in the action potential depolarization rates. These tracings indicate that the action potential height and depolarization rate were reduced in the cold-stored atria. In addition, the peak of the action potential was more rounded in the stored tissues. The tracings of the contractile forces are shown beneath the action potentials in Fig. 3. Table 2 shows the mean data obtained from the fresh and cold-stored tissues. It should be noted that the resting potential, action potential height, duration, depolarization rate and area of the stored atria were all significantly reduced when compared with the respective values obtained in the fresh atria. There was no significant change in the conduction velocity. Significant reductions were also seen in the contractile force, contraction rise velocity and contractile force area; no change was detected in the contraction rise time or the stimulus contraction time. It should be

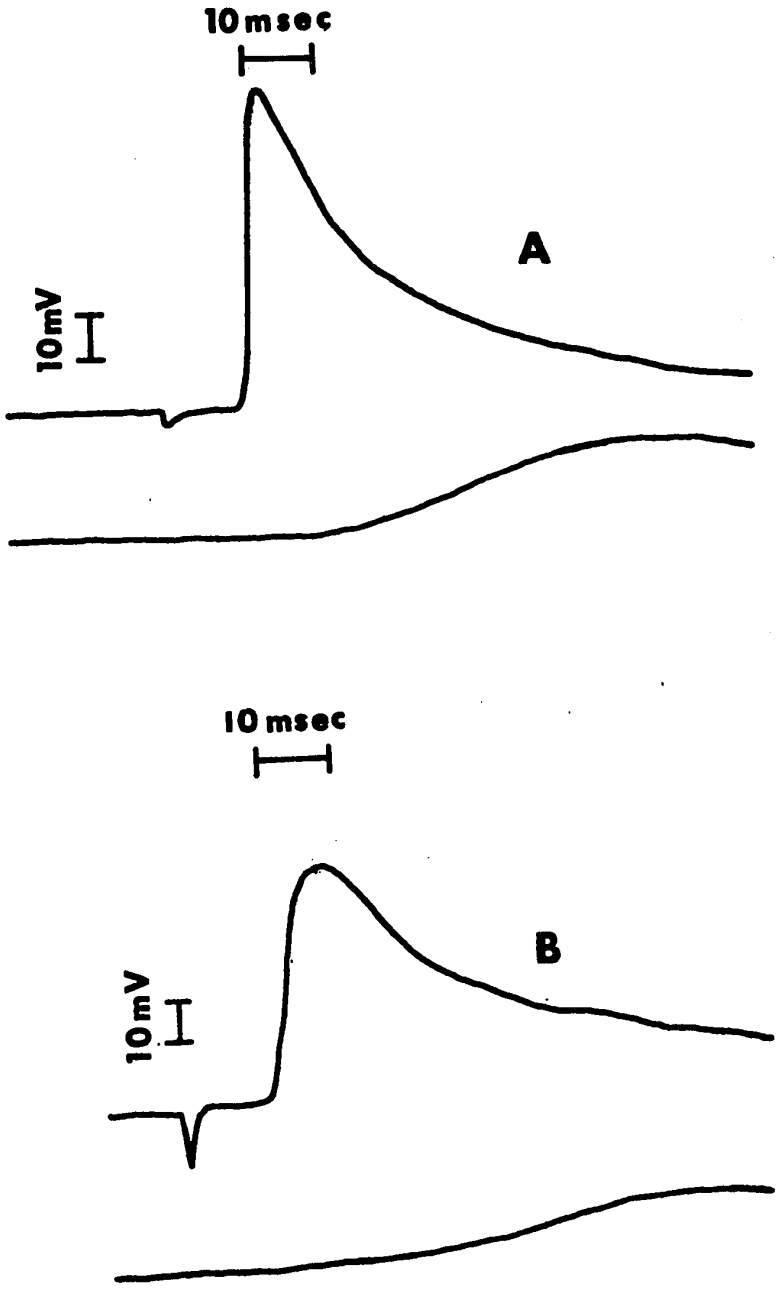

Fig. 3. Tracings of action potentials (above) and simultaneous contractions (below) of fresh left atrium (A) and cold-stored (48 hrs at 6° C) atrium (B) of rat.

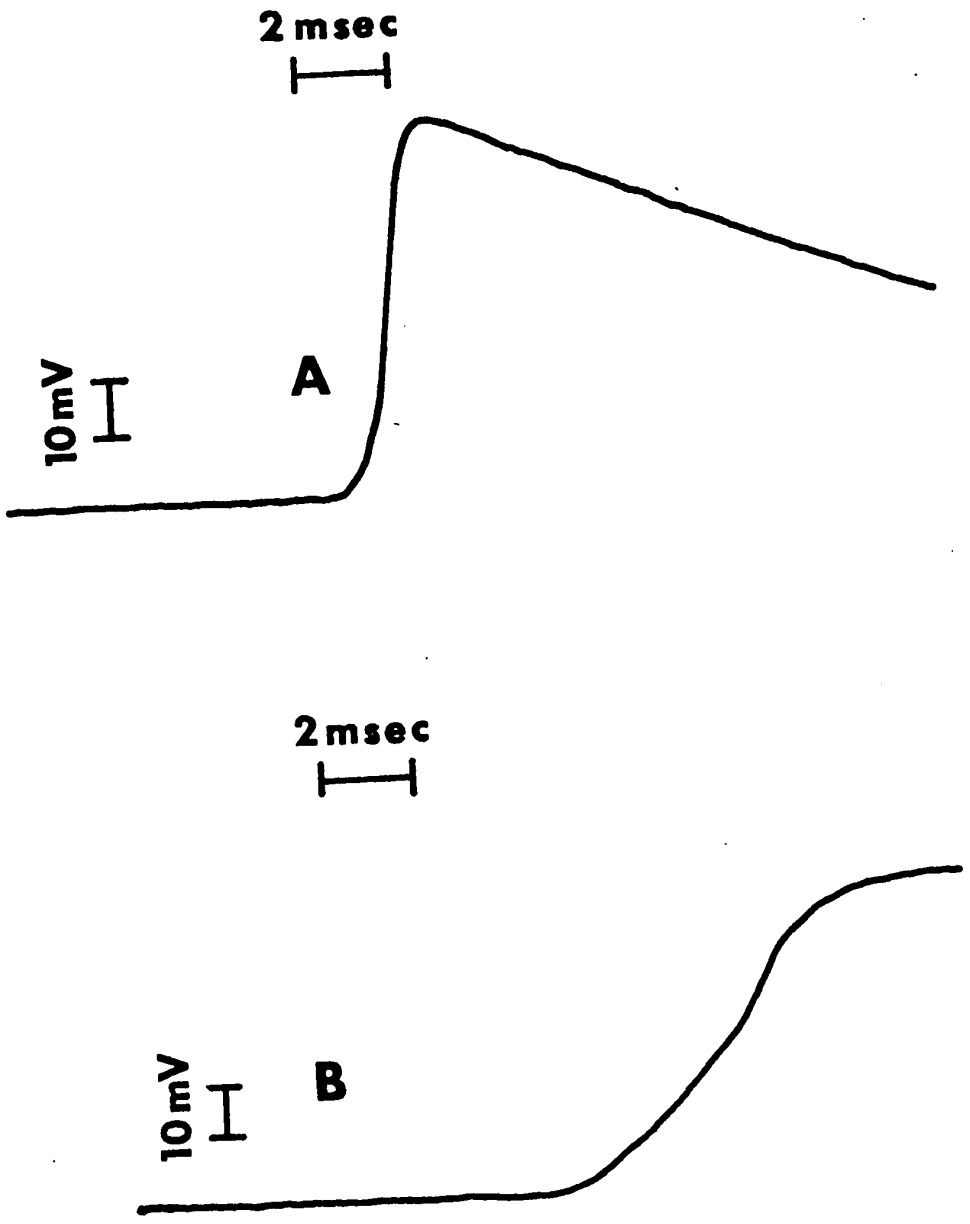

Fig. 4. Tracings of action potentials shown in Fig. 3 at high sweep speed (X5) to show the difference in the depolarization rates between fresh (A) and cold-stored (B) atria.

TABLE 2. Effect of cold-storage on transmembrane potentials of rat atria determined at 30° C.

Parameter	Fresh atria	Cold-stored atria (48 hrs at 6° C)
Resting potential (mV ± S.E.)	74 ± 1.1	61 ± 1.4*
Action potential height (mV ± S.E.)	80.7 ± 1.2	70.4 ± 2.1*
Action potential duration (msec ± S.E.)	58.2 ± 1.5	49.2 ± 2.7*
Action potential depolarization rate (V/sec ± S.E.)	52.8 ± 6.1	32.4 ± 3.1*
Action potential area (mV·sec ± S.E.)	2.15 ± 0.1	1.52 ± 0.1*
"Conduction velocity"(cm/sec ± S.E.)	53.3 ± 2.1	46.9 ± 2.5
Force of contraction (mg ± S.E.)	150 ± 19	50 ± 3*
Contraction rise time (msec ± S.E.)	41.5 ± 2.4	38.0 ± 1.2
Contraction rise velocity (g/sec ± S.E.)	3.6 ± 0.3	1.3 ± 0.1*
Contractile force area (mg·sec ± S.E.)	15.4 ± 2.4	4.8 ± 0.7*
Stimulus-contraction time (msec ± S.E.)	36.8 ± 1.5	37.3 ± 2.8

*Significantly different from control (fresh) values, $p<0.05$.

noted that the difference in the contractile force between the stored and fresh atria in these experiments was comparable to that in the original experiments seen in Fig. 1; the force of the stored atria was 33% of the fresh in the present series and 43% in the former.

3) Effect of K^+ on cold-stored atria

In an attempt to determine the role played by the electrophysiological changes in the reduction of the contractile force of cold-stored atria, responses to changes in the extracellular K^+ concentration were measured. The difference between the intracellular and extracellular K^+ concentrations is the main factor responsible for the resting membrane potential and the membrane potential is known to affect other electrophysiological properties of heart cells such as the action potential height, duration and depolarization rate (Hoffman and Cranefield, 1960). Experiments were therefore undertaken to determine if the lowering of the extracellular K^+ and the subsequent increase in the resting potential would increase the contractile force in the cold-stored atria to, or close to fresh values. The effect of varying concentrations of extracellular K^+ is shown in Fig. 5. The points on the graph were plotted as a percentage of the force of contraction of the control value of the same atrium in normal K^+ (6 mM). The contractile force of the fresh atria was unaffected by changes in the extracellular K^+ concentration between 1.2 and 12 mM. Contractility only began to decrease at higher concentrations of K^+. The cold-stored atria were much more sensitive to changes in the extracellular K^+. High K^+ (9.6 and 12 mM) caused a significant reduction in the force of contraction of the cold-stored atria, probably because the cells were already partially depolarized.

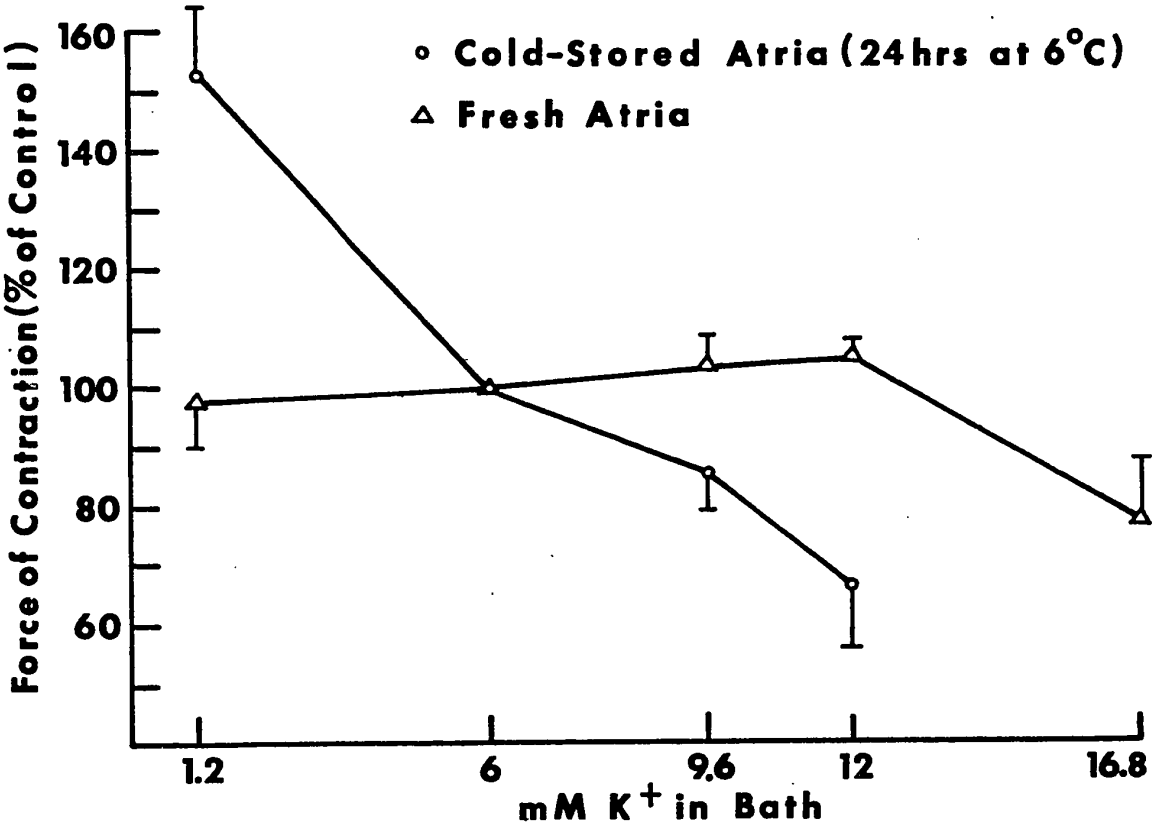

Fig. 5. Effect of K^+ on the contractile force of spontaneously beating rat fresh atria and atria stored at 6° C for 24 hrs. The force is expressed as a percent of the force recorded in 6 mM K^+ Krebs. Each point represents the mean $\pm$ S.E.

Low K^+ (1.2 mM) increased the force to some 150% of that observed in 6 mM K^+. These results suggest that the force of contraction, reduced after cold-storage, could be increased by increasing the resting potential and that electrophysiological changes were, in part, responsible for the decrease in contractile force. However, as Fig. 6 shows, the contractile force of the cold-stored atria in 1.2 mM K^+ was only some 60% of its control (i.e., the force before storage). These results indicate that although low K^+ increased the contractile force of cold-stored atria, it did not restore the contraction to normal values.

4) Effect of low temperatures on the ionic content of rat atria

In order to obtain more information on the relation between electrophysiological changes and the reduction of the contractile force, an attempt was made to produce by a different method electrophysiological changes similar to those produced by cold-storage and study the relationship between these changes and the contractile force. This was done by comparing fresh atria at 10° C with cold-stored atria at 30° C. Table 3 shows the effect of cold (2° C and 10° C) on the Na^+ and K^+ contents of fresh atria. Incubating the tissues at 2° C, as seen in lines 2 and 3, resulted in a slow loss of K^+ and gain in Na^+, as the 4 hour values were significantly different from the 1 hour values. Rewarming the tissues which had been incubated for 4 hours at 2° C to 10° C at a rate of 1 C°/min resulted in a partial recovery of the Na^+ and K^+ contents and the K^+ equilibrium potential. Atria incubated at 10° C for 4 hours had Na^+ and K^+ contents similar to those of control. The K^+ equilibrium potential at this temperature (10° C), however, was lower than that at 30° C. Values

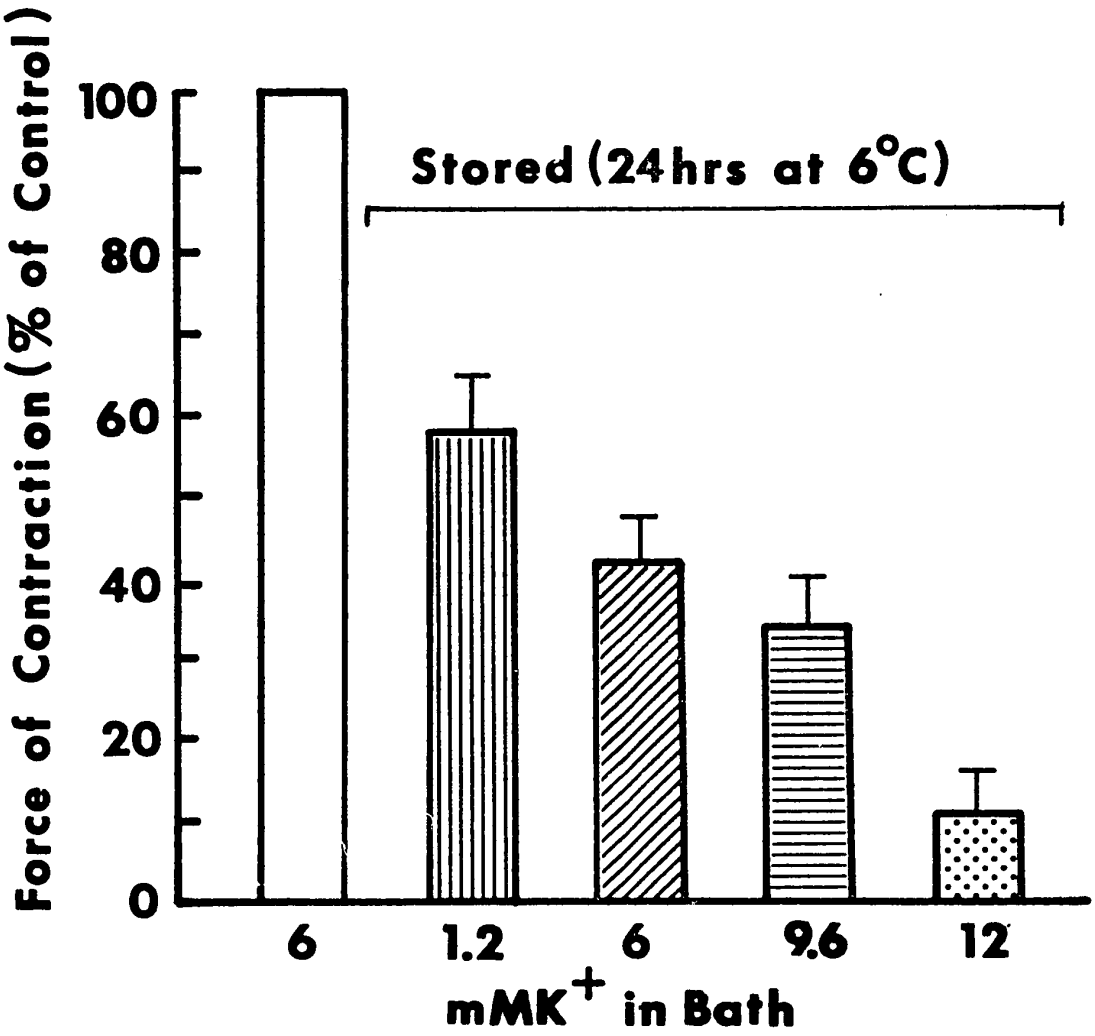

Fig. 6. Same results as shown in Fig. 5. The contractile force of the cold-stored atria is presented as a percent of that of the fresh atria incubated in 6 mM K^+ Krebs. Note that the force of contraction of the stored atria in 1.2 mM K^+ Krebs is only 55% of that of the fresh. Each column represents the mean $\pm$ S.E.

TABLE 3. Effect of cold on the ionic content of fresh rat atria.

Preparation	Na^+ (total) mEq/kg ± S.E.	K^+ (total) mEq/kg ± S.E.	K^+ (intracellular) mEq/L	K^+ equilibrium potential mV
30° C (2 hrs)	70.4 ± 1.2	71.5 ± 1.1	127.4	79.8
2° C (1 hr)	79.7 ± 2.9*	56.5 ± 3.2*	84.6	62.7
2° C (4 hrs)	83.5 ± 5.6*	36.5 ± 2.8*	54.6	52.3
2° C (4 hrs +10 min at 10° C**)	87.6 ± 2.9*	44.6 ± 3.9*	71.7	60.5
10° C (4 hrs)	72.8 ± 3.1	72.0 ± 2.0	123.4	73.7

*Significantly different from control values (30° C), $p<0.05$.

**Warmed to 10° C at 1°/min and removed when temperature reached 10° C.

of the resting potentials from atria incubated at 10° C were significantly lower than those of fresh and cold stored atria. The resting potentials were 55.8 ± 1.2 mV in fresh atria at 10° C, 74 ± 1.1 mV in fresh atria at 30° C and 61 ± 1.4 mV in the cold-stored tissues. In subsequent experiments, it was shown that the mean force of contraction of fresh atria at 10° C, stimulated electrically with sufficient voltage and pulse duration (50 V, 30 msec), was 70% of that of fresh atria at 30° C. This was significantly greater than the 43% in the cold stored tissues. It was therefore decided to study the action potentials of the fresh tissues at 10° C.

5) Effect of cold (10° C) on the action potential of rat atria

Tracings of typical action potentials of fresh atria recorded at 10° C and 30° C are shown in Fig. 7. The simultaneous contractions are shown beneath the action potentials. The configuration of potentials seen at 10° C were quite unlike those recorded at 30° C; rather than a fast rising phase with a much slower repolarization phase as seen at 30° C, the potentials at 10° C had depolarizing and repolarizing phases of approximately the same rate. In addition, the height of the potentials at 10° C was much smaller, the mean being 37.5 ± 2.4 mV as compared with 80.7 ± 1.2 mV at 30° C, a significant difference. The duration was significantly longer at 10° C, 219 ± 12 msec as compared with 58.2 ± 1.5 msec at 30° C.

6) Voltage dependency of the action potential at 10° C

Another interesting feature of the action potentials recorded at 10° C was the dependence of the height of the action potential on the

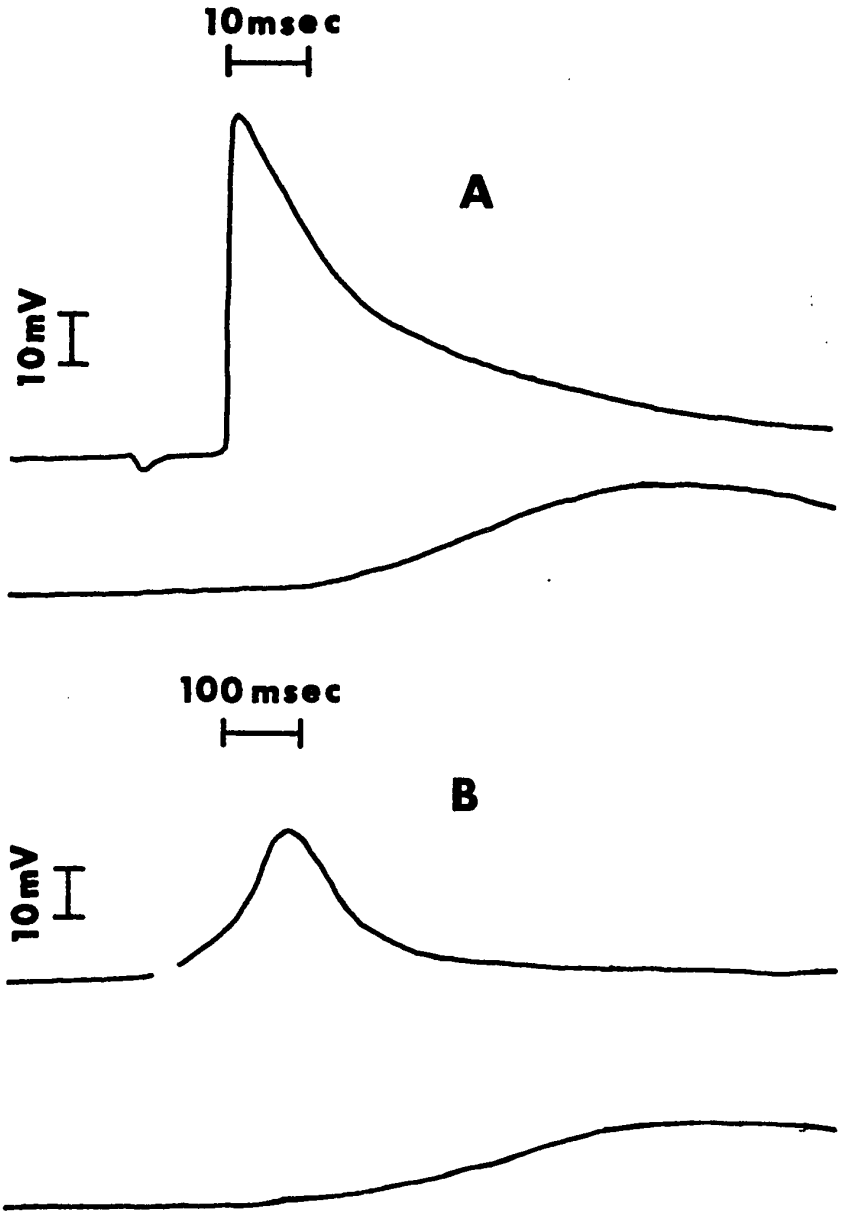

Fig. 7. Tracings of action potentials (above) and simultaneous contractions (below) from rat fresh left atrium at 30° C (A) and 10° C (B). Note that the time scale in B is 10X that in A.

stimulating voltage. A comparison of the tracings of the two potentials, one recorded with a stimulating voltage of 50 V (A) and the other at 5 V (B) is shown in Fig. 8. The mean data obtained at these two voltages is shown in Table 4. Both the height and the area of the action potentials of the atria stimulated with 5 V were significantly smaller than those of the atria stimulated with 50 V. However, the rate of depolarization and the action potential duration remained unaffected by the stimulating voltage. These results point out that the potentials seen at 10° C were not "all or none" phenomena like action potentials normally seen. The results with the contractile force show that this parameter also varied with the stimulating voltage at 10° C, a phenomenon that does not occur at higher temperatures. Significant differences were seen in both the magnitude and the area of the contractile force at 5 V when compared to 50 V. It is noteworthy that the contractile force at 30° C did not increase when the stimulating voltage was raised and there was no lag in the change in the force at 10° C if the voltage was changed suddenly. These results indicate that this phenomenon was not due to the release of noradrenaline due to the increased voltage.

7) Effect of TTX on the action potential at 10° C

Because of the peculiar nature of the potentials recorded at low temperatures, it was decided to determine if these potentials were due to the increase in Na^{+} conductance as is the case with normal action potentials. In these experiments, TTX, a known inhibitor of Na^{+} conductance was employed. Fig. 9 and Table 5 show the results obtained after the addition of TTX.

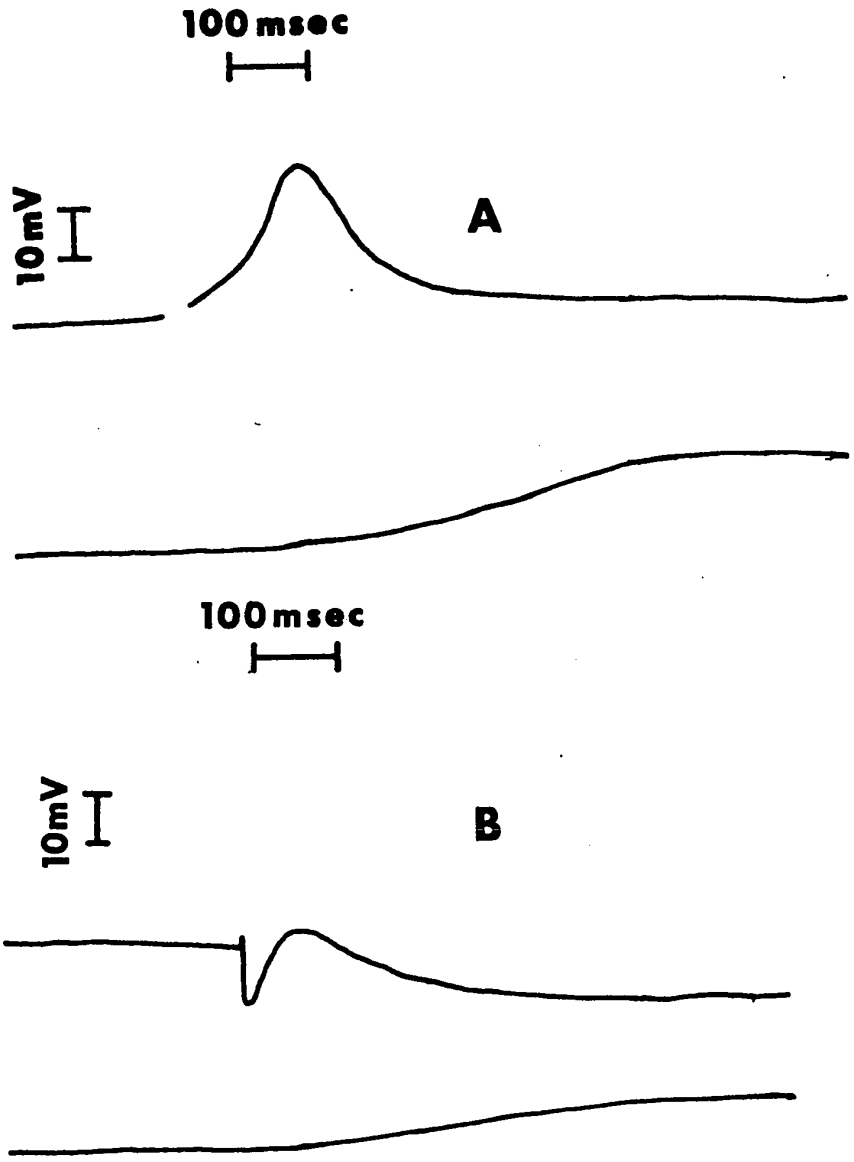

Fig. 8. Tracings of action potentials (above) and simultaneous contractions (below) from rat fresh left atrium at 10° C stimulated with 50 V (A) and 5 V (B). Note the voltage dependency of the height of the action potentials.

TABLE 4. Effect of cold (10° C) and varying stimulating voltage on transmembrane potentials of fresh rat atria.

Parameter	Stimulated with 50 volts	Stimulated with 5 volts
Resting potential (mV ± S.E.)	55.8 ± 1.2	55.8 ± 1.2
Action potential height (mV ± S.E.)	37.5 ± 2.4	13.5 ± 1.4*
Action potential duration (msec ± S.E.)	219 ± 12	261 ± 46
Action potential depolarization rate (V/sec ± S.E.)	0.5 ± 0.05	0.3 ± 0.07
Action potential area (mV·sec ± S.E.)	4.98 ± 0.6	3.47 ± 0.5*
Force of contraction (% of force at 30° C ± S.E.)	69 ± 9	36 ± 9*
Contractile force area (% of area at 30° C)	780 ± 71	360 ± 57*

*Significantly different from control (atria stimulated with 50 V) values, $p<0.05$.

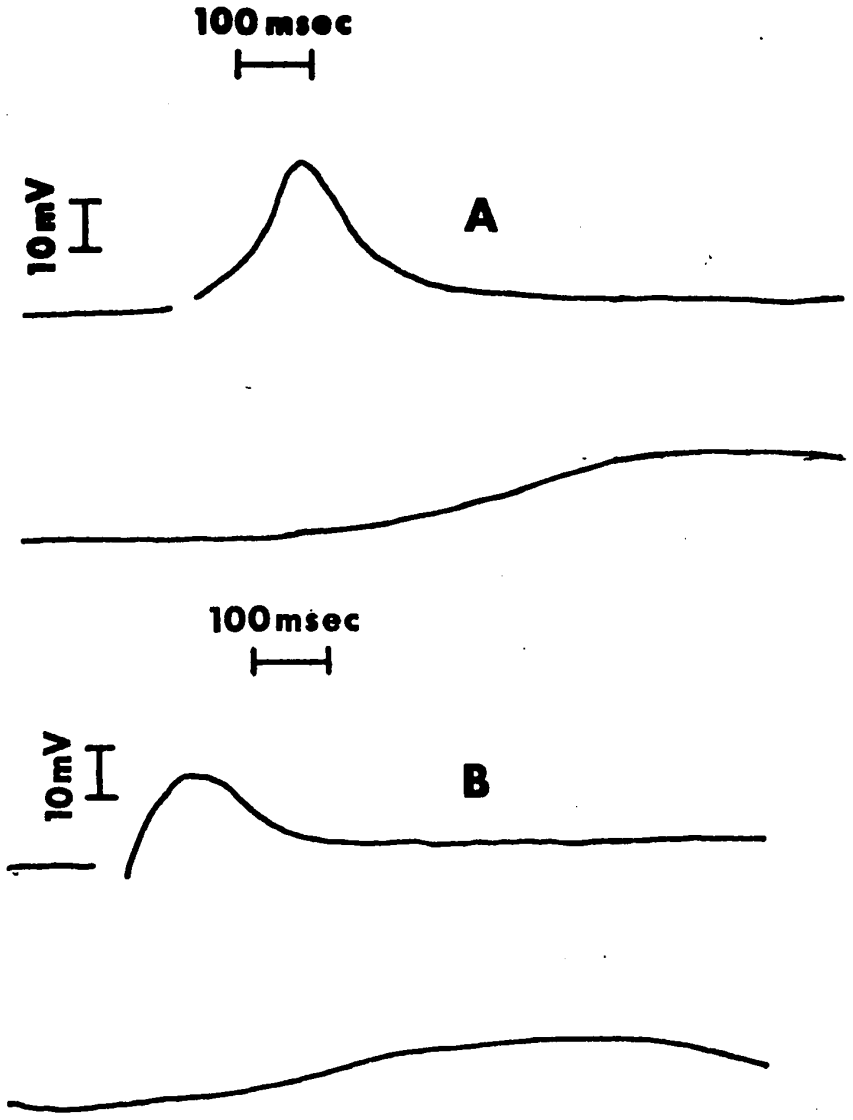

Fig. 9. Tracings of action potentials (above) and simultaneous contractions (below) from rat fresh left atrium at 10° C. Tracing A is from the control preparation and B after the addition of TTX 0.03 µg/ml. Note the reduction in height of the action potential produced by TTX.

TABLE 5. Effect of tetrodotoxin on transmembrane potentials of fresh rat atria at 10° C stimulated with 50 volts.

TTX (μg/ml)	Action potential height mV ± S.E.	Action potential duration msec ± S.E.	Action potential area mV·sec ± S.E.	Depolarization rate V/sec ± S.E.	Contractile force Percent of control ± S.E.
0.0	37.5 ± 2.4	219 ± 12	4.98 ± 0.6	0.5 ± 0.05	100
0.1	31.9 ± 5.6	240 ± 23	4.47 ± 1.0	0.5 ± 0.1	103 ± 10
0.3	15.5 ± 7.2*	221 ± 13	1.99 ± 1.0*	1.1 ± 0.5	31 ± 3*
0.5	6.2 ± 2.6*	230 ± 21	0.74 ± 0.32*	0.4 ± 0.02	19 ± 1*

*Significantly different from control (no TTX) values, $p<0.05$.

TTX, tetradotoxin

Fig. 9 shows tracings of potentials from atria stimulated with 50 V at 10° C. The top potential (A) is from the control situation and the bottom (B) after the addition of 0.3 µg/ml of TTX; a reduction in the height of the potential occurred after the addition of TTX. Table 5 shows the mean data obtained after the addition of TTX in concentrations of 0.1, 0.3 and 0.5 µg/ml. Successively higher concentrations of TTX resulted in the successive decrease of the action potential height and area as well as the contractile force. The action potential duration and depolarization rate remained unaffected even by the highest concentration of TTX (0.5 µg/ml). These results indicate that these potentials, like normal action potentials, were caused by the influx of Na^+ into the cells.

8) Effect of high K^+ (12 mM) on the ionic content of fresh rat atria

Experiments were also performed on fresh rat atria using K^+ as a depolarizing agent in an effort to produce electrophysiological changes similar to those seen in cold-stored atria. Table 6 shows the effect of 12 mM K^+ on the Na^+ and K^+ contents of fresh atria. The total K^+ content, as well as the intracellular K^+ concentration, increased as a result of the increase in extracellular K^+. The K^+ equilibrium potential was, however, lower in these tissues than in those incubated in 6 mM K^+, indicating that 12 mM K^+ had caused a depolarization of the atrial cells.

9) Effect of high K^+ (12 mM) on the transmembrane potential of fresh rat atria

Fig. 10 and Table 7 show the effect of 12 mM K^+ on the electro-

TABLE 6. Effect of high K^+ (12 mM) on the ionic content of fresh rat atria.

Preparation	Na^+ (total) mEq/kg ± S.E.	K^+ (total) mEq/kg ± S.E.	K^+ (intracellular) mEq/L	K^+ equilibrium potential mV
6 mM K^+	70.4 ± 1.2	71.5 ± 1.1	127.4	79.8
12 mM K^+	62.5 ± 1.2*	81.6 ± 1.5*	144.4	65.0

*Significantly different from control (6 mM K^+) values, $p<0.05$.

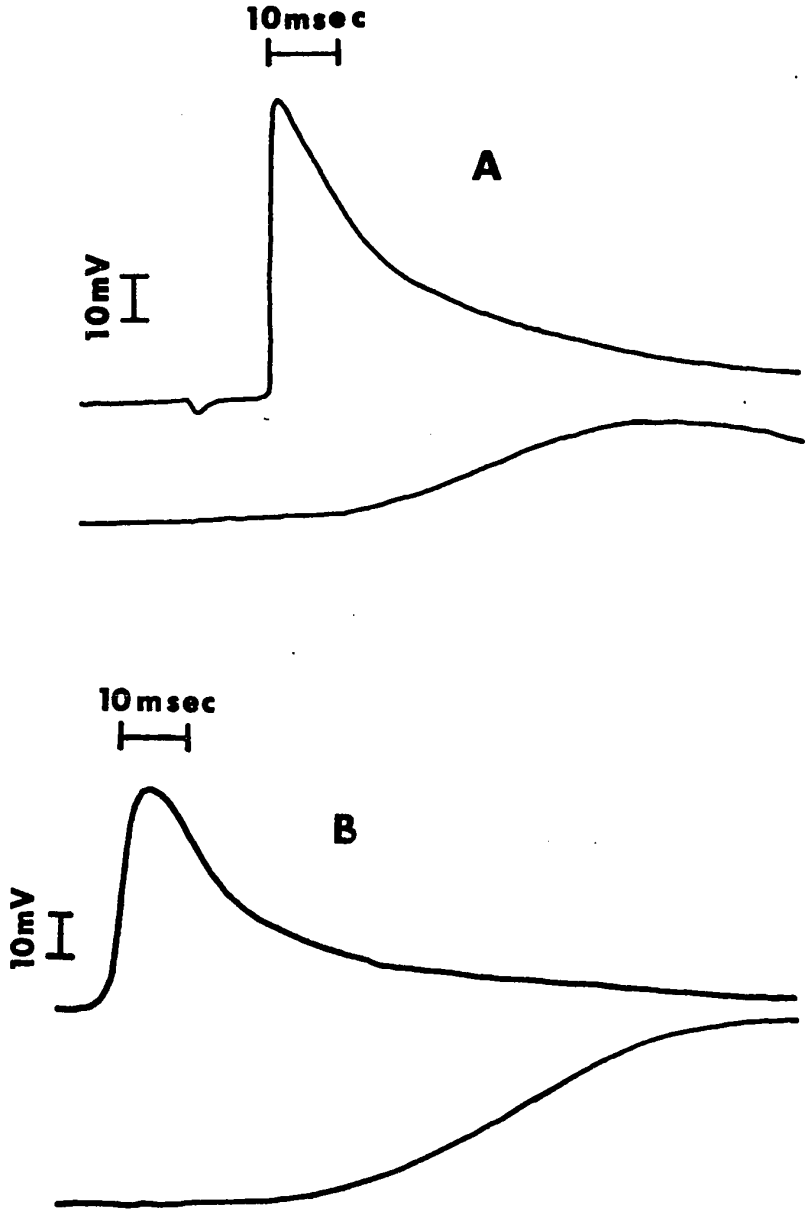

Fig. 10. Tracings of action potentials (above) and simultaneous contractions (below) from rat fresh left atria at 30° C in 6 mM K^+ Krebs (A) and 12 mM K^+ Krebs (B).

TABLE 7. Effect of high K^+ (12 mM) on transmembrane potentials of fresh rat atria.

Parameter	6 mM K^+	12 mM K^+
Resting potential (mv ± S.E.)	74 ± 1.1	58.6 ± 2.4*
Action potential height (mv ± S.E.)	80.7 ± 1.2	50.7 ± 1.9*
Action potential duration (msec ± S.E.)	58.2 ± 1.5	45.3 ± 1.9*
Action potential depolarization rate (V/sec ± S.E.)	52.8 ± 6.1	9.1 ± 0.9*
Action potential area (mV·sec ± S.E.)	2.15 ± 0.1	1.18 ± 0.07*
Force of contraction (% of force in 6 mM K^+ S.E.)	100	84 ± 7

*Significantly different from control (6 mM K^+) values, $p<0.05$.

physiological characteristics of fresh left atria. In Fig. 10, tracings of action potentials from an atrium incubated in 6 mM K^+ Krebs (A) and one incubated in 12 mM K^+ Krebs (B) are shown. The action potential height, duration and depolarization rate were reduced by the high K^+. Table 7 shows the mean data obtained from these experiments. Significant reductions were seen in the resting potential, action potential height, depolarization rate and area of those atria incubated in 12 mM K^+ when compared to 6 mM K^+. Despite the marked changes in the electrophysiological characteristics produced by 12 mM K^+, the contractile force was reduced by only 15%.

10) Comparison of electrophysiological data and contractile force in different preparations

An increase in the force of contraction of cold-stored atria to low K^+ (1.2 mM) suggested that the electrophysiological changes were, in part, responsible for the reduced contractile force of these atria. Since the electrophysiological changes produced by 12 mM K^+ and cold were similar to or greater than those produced by cold-storage, the effects of 12 mM K^+ and cold on the contractile force and the transmembrane potential were compared with the values obtained from the experiments on the stored atria. Table 8 shows this comparison. Only the mean data are shown; the standard errors were left out for the sake of clarity; they have been shown elsewhere. The contractile forces of the cold-stored atria and the fresh atria incubated at 10° C and stimulated with 5 V were not significantly different from each other. However, the electrophysiological properties were different in every case. The resting potential, action potential height and depolarization rate were all significantly lower in the atria at 10° C; however, the action potential

TABLE 8. Comparison of electrophysiological and mechanical activity in in different preparations.

Parameter	Cold-stored atria (48 hrs at 6° C)	Fresh atria 12 mM K^+	Fresh atria 10 (Stim 5 V)
Resting potential (mV)	61	59	56*
Action potential height (mV)	70	51*	14*
Action potential duration (msec)	49	45	261*
Action potential depolarization rate (V/sec)	32	9*	0.3*
Action potential area (mV·sec)	1.52	1.18*	3.47*
Force of contraction (% of cold stored atria)	100	263*	113

*Significantly different from control (cold-stored atria) values, $p<0.05$.

area and duration were significantly greater. The force of contraction of the fresh tissues in 12 mM K^+ was similar to that in 6 mM K^+ and over 2.5 times that of the cold-stored atria. The action potential height, depolarization rate and area in 12 mM K^+ were significantly lower than the respective values of the stored tissues, while the resting potential and action potential duration in the two preparations were not significantly different from each other. These results and a comparison of the results from the atria in 12 mM K^+ with those from the cold-stored atria, demonstrate that the electrophysiological changes cannot account for the observed large reduction in the contractile force of cold-stored atria.

C. ATP AND CONTRACTILITY IN COLD-STORED ATRIA

1) Effect of cold-storage on the ATP content of atria

Storage of red blood cells (Simon, 1967) and heart (Calman et al., 1970) in the cold is known to cause a decrease in the ATP content. Since the preceding experiments showed that electrophysiological changes could not account for the entire decrease in force of contraction of cold-stored atria, it was decided to study the ATP content of these tissues. Fig. 11 shows the effect of cold-storage on the ATP content of rat atria. The ATP content progressively decreased as the duration of cold-storage increased; it was 25% of fresh values after 72 hours of storage.

2) Effect of adenosine on the ATP content and contractile force of cold-stored atria

As the incubation of cold-stored red blood cells with adenosine is known to increase their viability and ATP content (Simon, 1967), it was

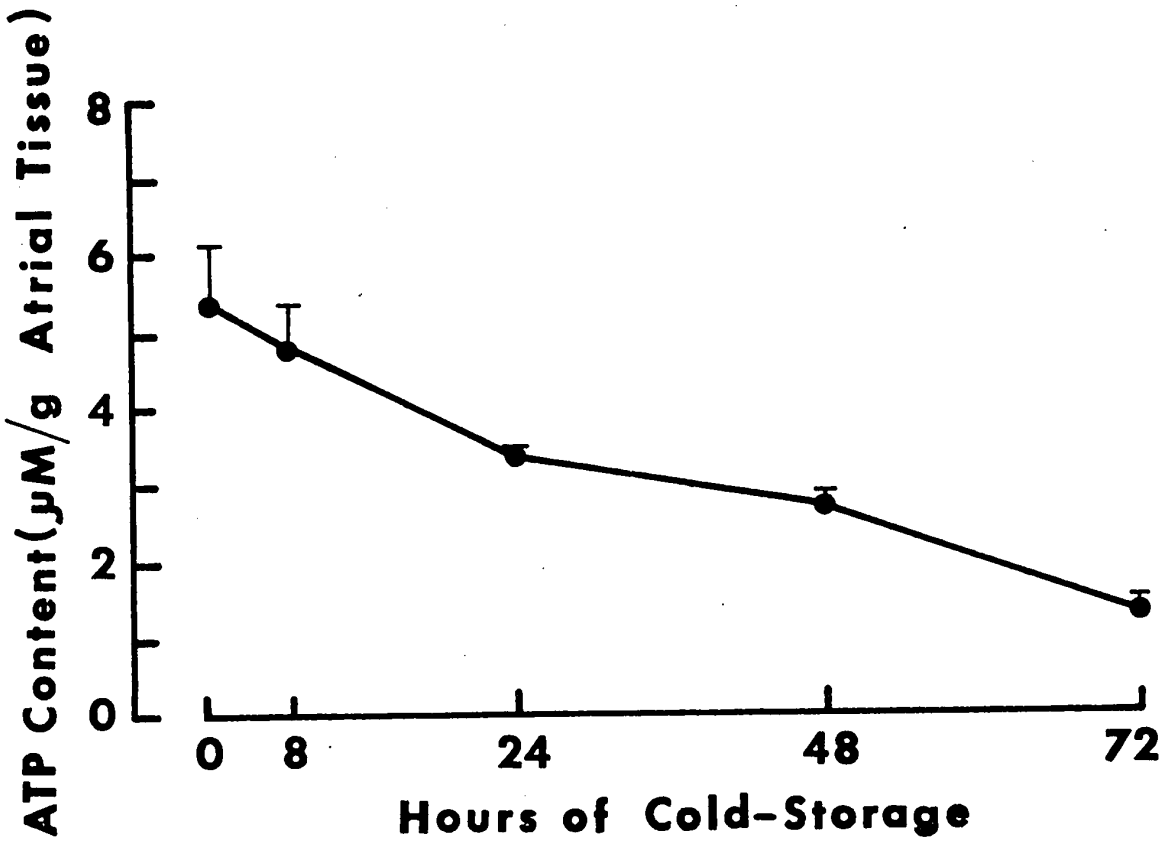

Fig. 11. Decrease in ATP content of spontaneously beating rat atria produced by storage at 6° C for 8-72 hrs. Each point represents the mean ± S.E.

decided to study the effect of adenosine on the ATP content and contractile force of cold-stored atria. The results of these experiments are shown in Fig. 12. Incubation of fresh atria resulted in a marked increase in ATP content, the post-incubation value being almost 4 times that of control. However, the incubation of the 24 hour cold-stored atria with adenosine caused a much smaller increase in ATP content; the increase was not highly significant ($0.1>p>0.05$). The ATP content of the cold-stored atria incubated with adenosine approached that of the control fresh atria. Adenosine itself had a depressant effect on the contractility of the atria. However, after adenosine was washed out, the force of contraction increased immediately. This slight increase, however, was not significant, although it was consistent. There was no significant difference between the effect of adenosine on the contractile force of fresh and cold-stored atria. It thus appears that there was no relation between the contractile force and the increase in ATP content resulting from incubation with adenosine in either the fresh or cold-stored atria.

3) Correlation between ATP content and the contractile force

The contractile forces and the respective ATP contents of various preparations are shown in Fig. 13 and 14. Fig. 13 shows the ATP content and contractility plotted as a percent of those values in the fresh atria. The atria shown in the figure were stored in cold for 8, 24, 48 and 72 hours; a group of atria was stored for 24 hours and was incubated with adenosine after storage and another group of atria was left in the animals for 2 minutes after death and then stored for 24 hours. In general, the decrease in contractile force of stored atria was associated with a decrease

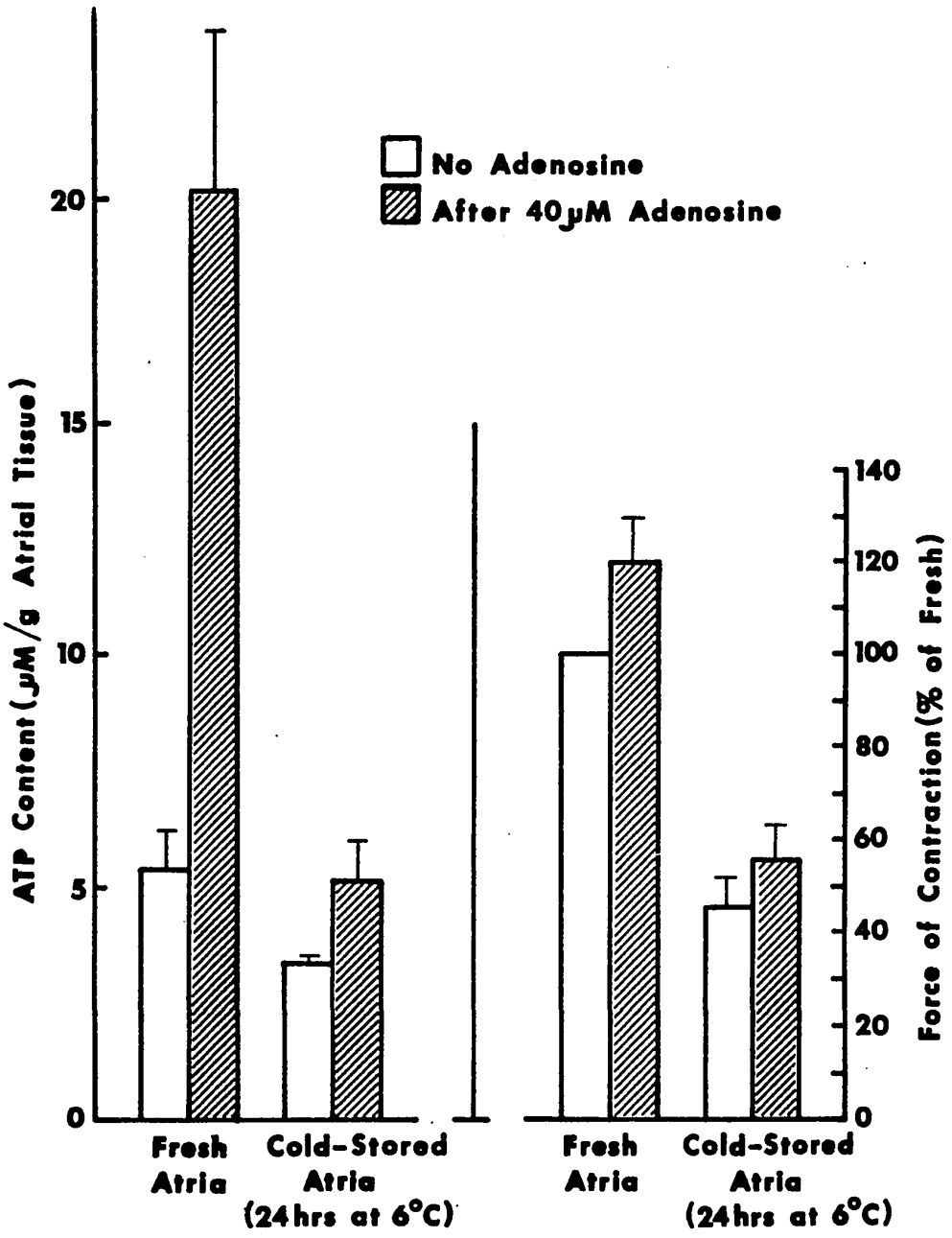

Fig. 12. Change in ATP content and contractile force of fresh and cold-stored rat atria after incubation in 40 μM adenosine for 45 min followed by a 5 min washout. Note the increase in ATP content without significant increases in the contractile forces. Also, note the large difference in the amount of ATP accumulation after adenosine incubation between the fresh and cold-stored atria. Each column represents the mean ± S.E.

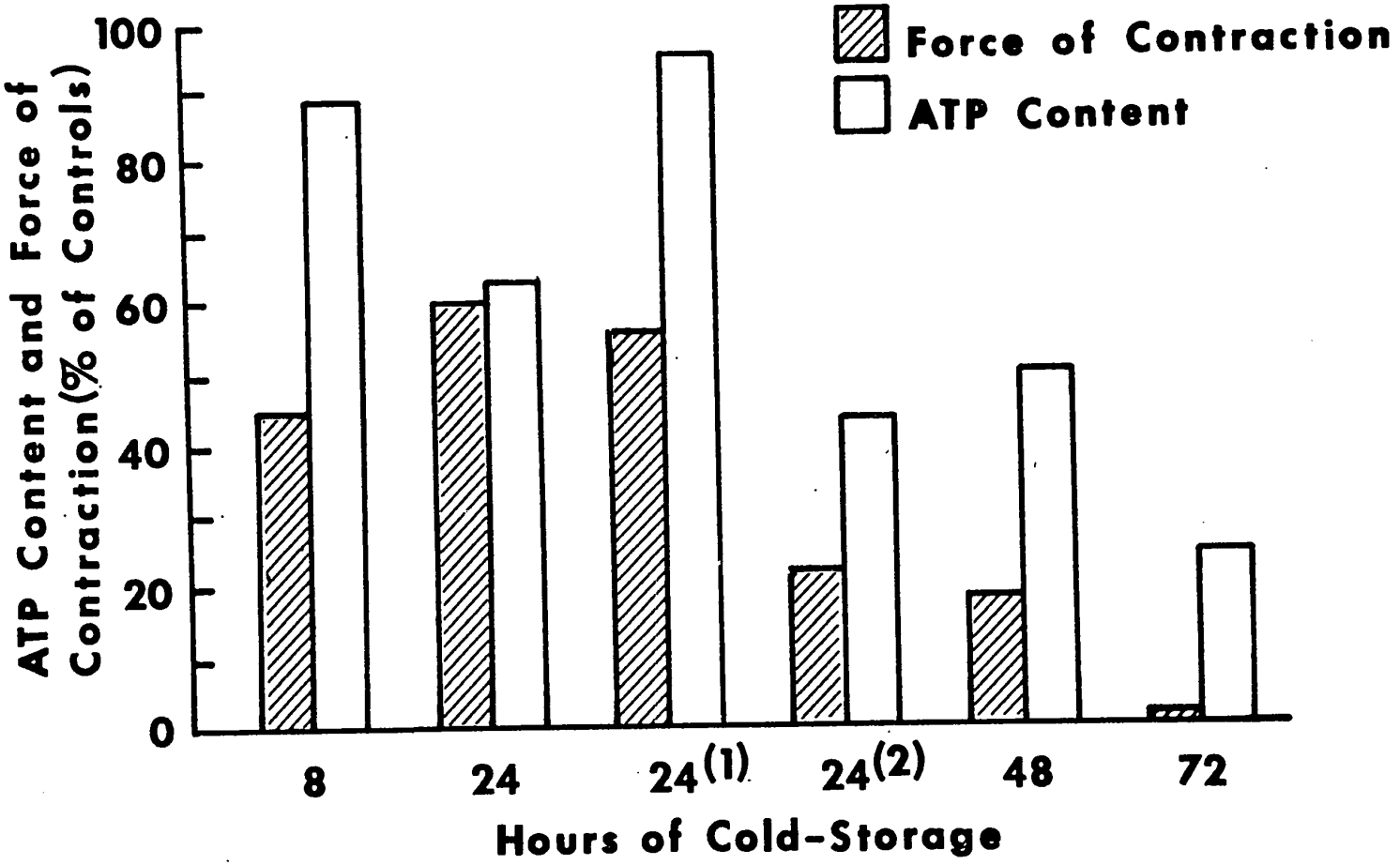

Fig. 13. ATP content and contractile force of spontaneously beating cold-stored atria of rat plotted as a percent of the values of fresh atria. (1) Atria stored for 24 hrs and incubated in 40 μM adenosine. (2) Atria left in the animal for 2 min after death and then stored for 24 hrs. Each column represents the mean value.

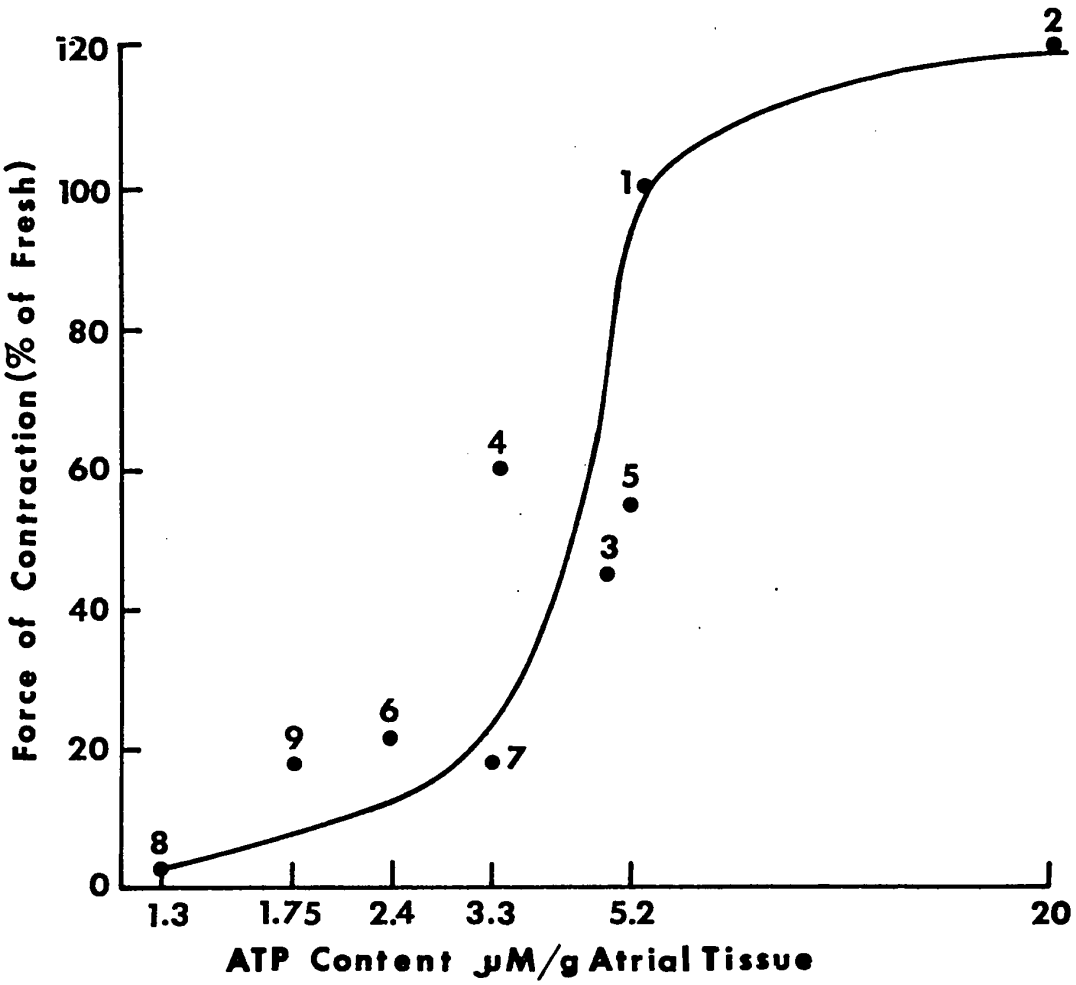

Fig. 14. ATP content and contractile force of spontaneously beating rat atria. 1- fresh atria; 2- fresh atria incubated with 40 µM adenosine; 3- atria stored for 8 hrs; 4- atria stored for 24 hrs; 5- atria stored for 24 hrs and incubated with 40 µM adenosine; 6- atria left in animal for 2 min after death and then stored for 24 hrs; 7- atria stored for 48 hrs; 8- atria stored for 72 hrs; 9- atria stored for 24 hrs in 1.2 mM K^+ Krebs. Each point represents the mean value.

in ATP content. Fig. 14 shows all preparations used in these experiments; the contractile force as percent of fresh values was plotted against the ATP content in μM/g on a semi-log scale. Here also, there is a correlation, as "S" curve, between the contractile force and the ATP content.

4) Effect of DNP on cold-stored atria

DNP, a metabolic inhibitor, is known to decrease the content of CP but not ATP in heart muscle (Furchgott and Lee, 1961). Fig. 15 shows the decrease in force of contraction of fresh and cold-stored atria as a function of time after the addition of DNP. DNP caused comparable reductions in the contractile force of the stored and fresh atria.

D. HISTOLOGICAL CHANGES IN COLD-STORED RAT ATRIA

1) Extracellular space

Storage of atria in the cold for 48 hours did not cause a significant change in the extracellular (inulin) space (Fig. 16).

2) Electron microscopy

Electron microscopy was performed on fresh atria and atria stored for 24 and 48 hours. Typical sections are shown in Fig. 17, 18 and 19. Fig. 17 shows a section from a fresh atrium and Fig. 18 and 19 from atria stored for 24 and 48 hours, respectively. In the stored atria, the myofibrils were somewhat less distinct and there was a decrease in the amount of glycogen. No other changes were detected in the stored atria; the plasma membranes, mitochondria and nuclei were all normal.

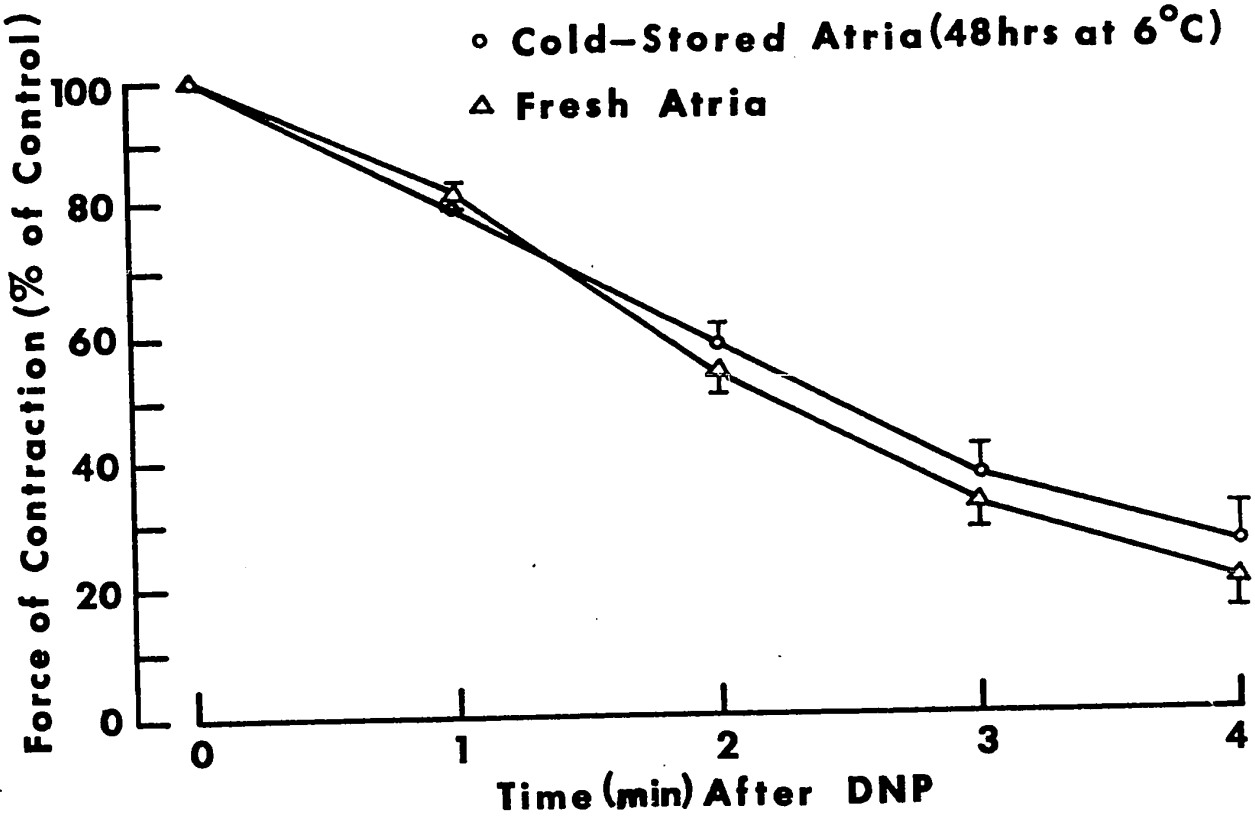

Fig. 15. Decrease in contractile force of spontaneously beating fresh and cold-stored atria of rats produced by incubation with 0.03 mM DNP. Each point represents the mean ± S.E.

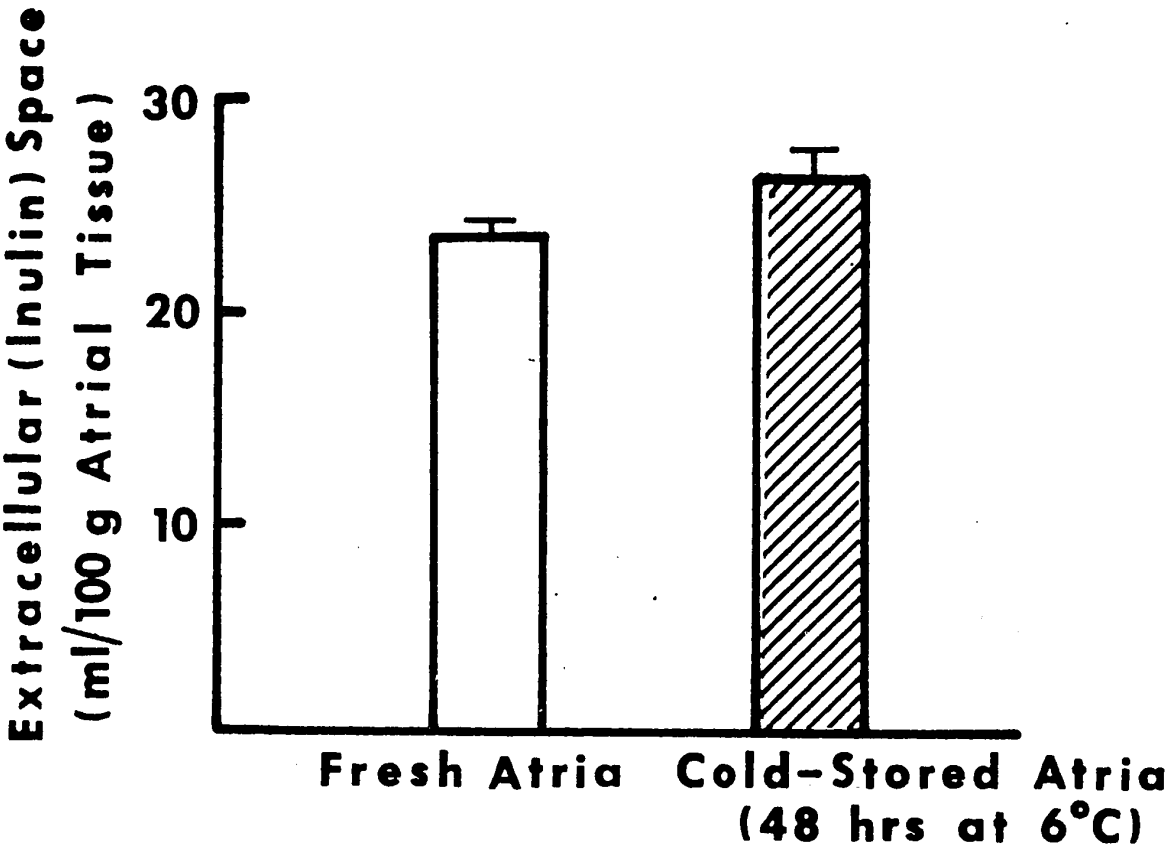

Fig. 16. Extracellular space of fresh and cold-stored spontaneously beating atria of rats measured with ^{14}C-inulin. Each column represents the mean ± S.E.

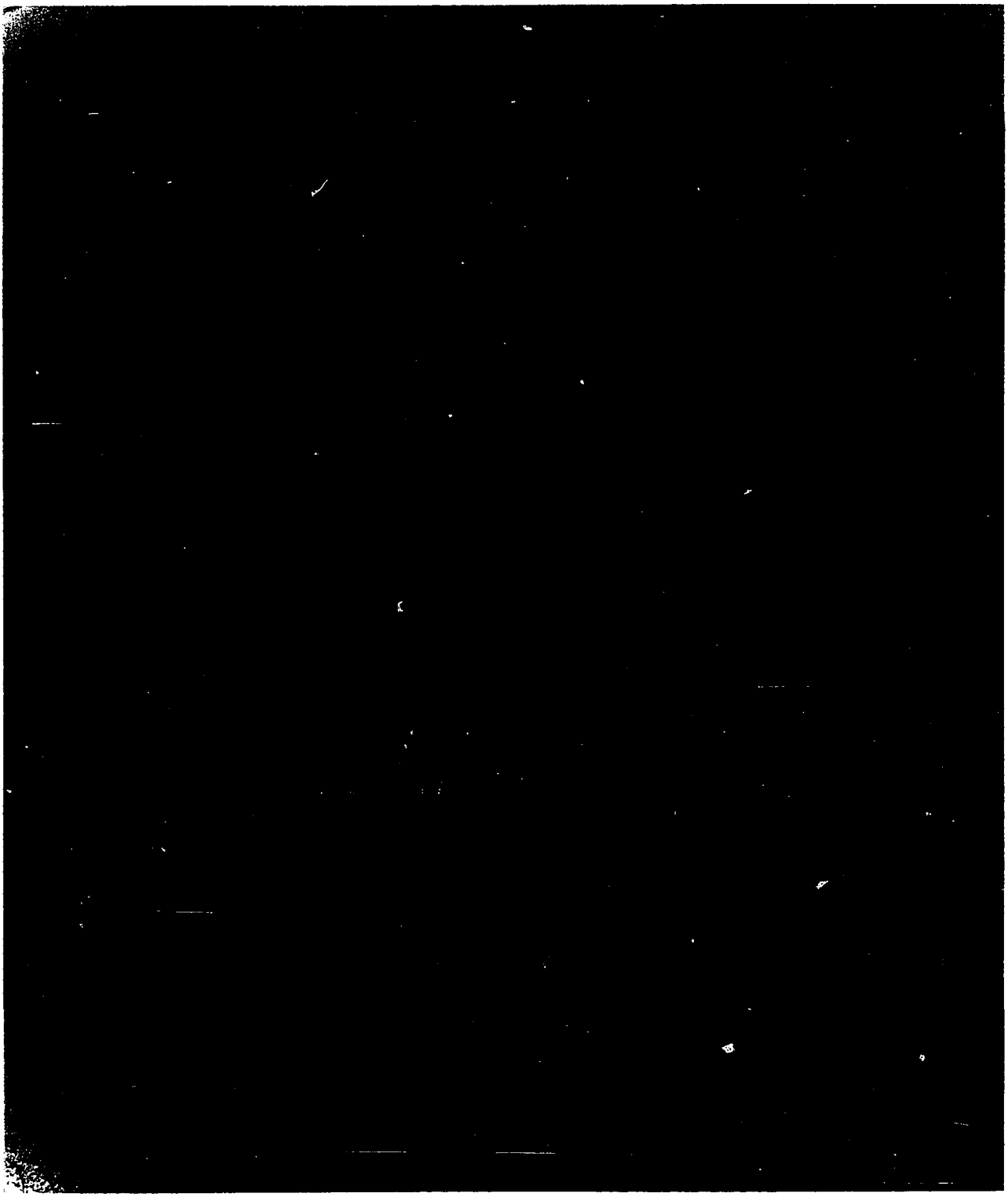

Fig. 17. Electron micrograph of a typical section from a fresh rat atrium. N-nucleus; M-mitochondrion; S-sarcomere; PM-plasma membrane. Small black particles are glycogen (G). Scale: 1μ.x14,600.

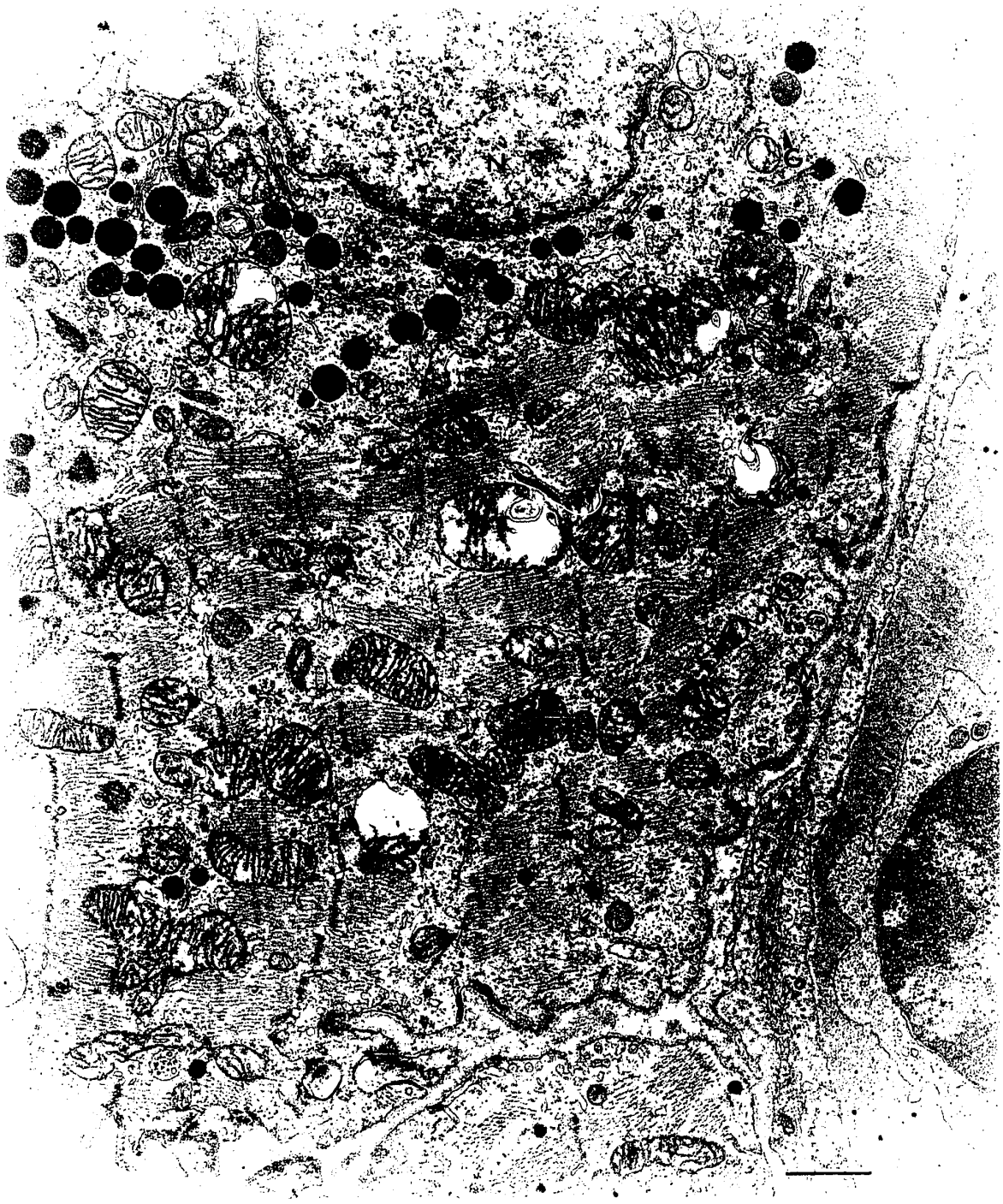

Fig. 17. Electron micrograph of a typical section from a fresh rat atrium. N-nucleus; M-mitochondrion; S-sarcomere; PM-plasma membrane. Small dense particles are glycogen (G). Scale: 1µ.x14,600.

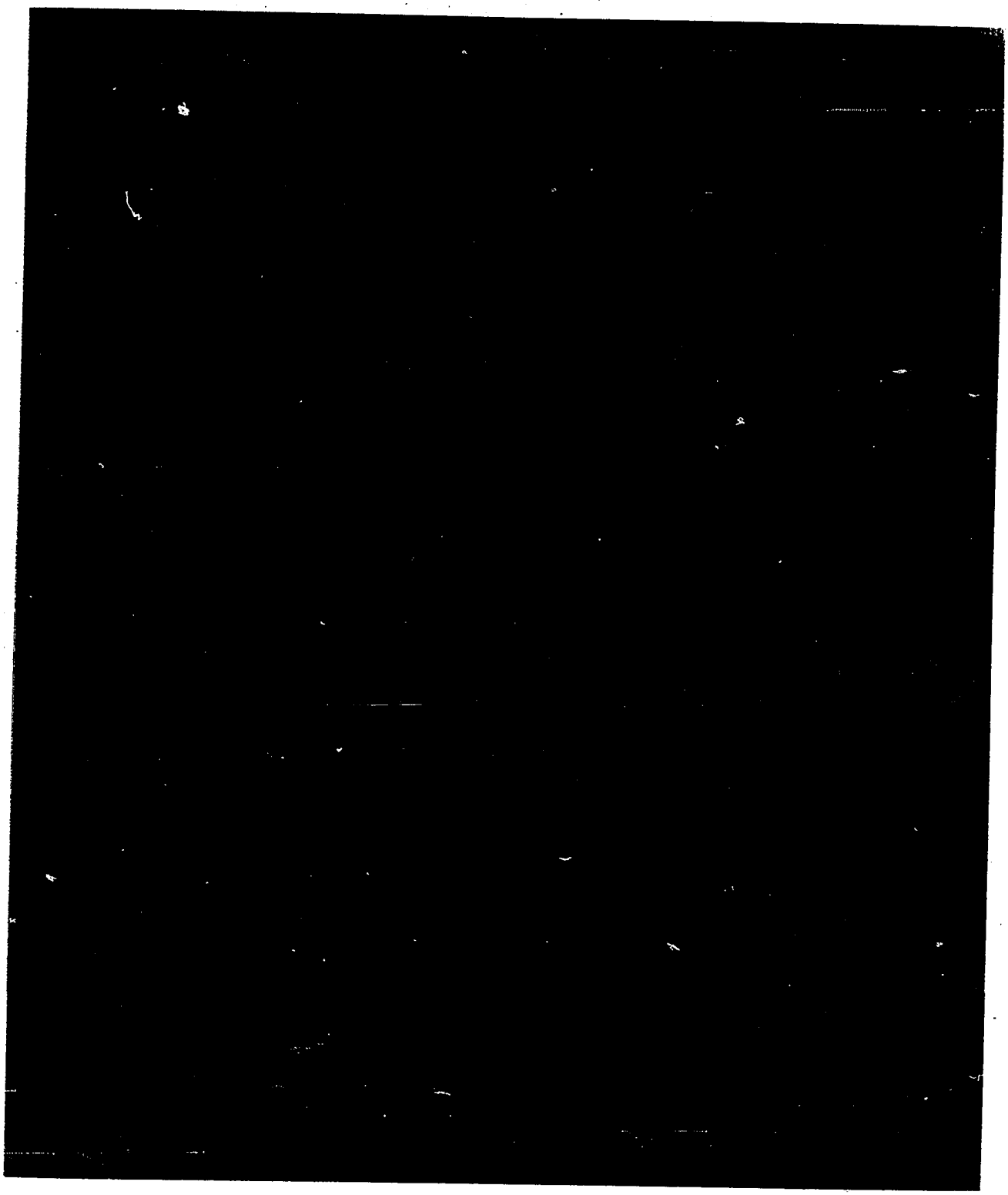

Fig. 18. Electron micrograph of a typical section from a rat atrium stored at 6° C for 24 hrs. M-mitochondrion; S-sarcomere; PM-plasma membrane. Scale: 1μ.x14,600.

Fig. 18. Electron micrograph of a typical section from a rat atrium stored at 6° C for 24 hrs. M=mitochondrion; S=sarcomere; PM=plasma membrane. Scale: 1µ.x14,600.

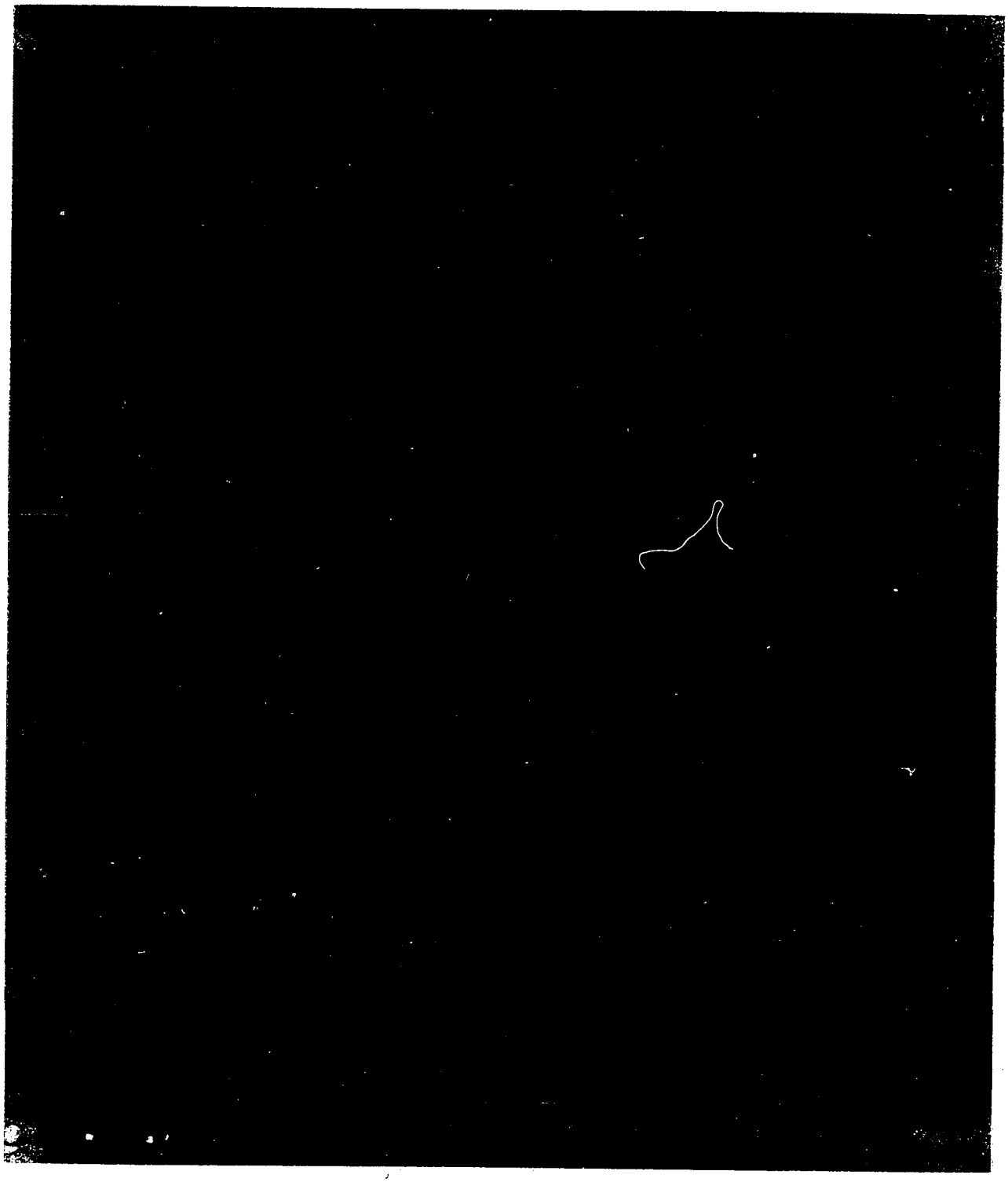

Fig. 19. Electron micrograph of a typical section from a rat atrium stored at 6° C for 48 hrs. N-nucleus; M-mitochondrion; S-sarcomere; PM-plasma membrane. Note the loss of glycogen. Scale: 1μ.x14,600.

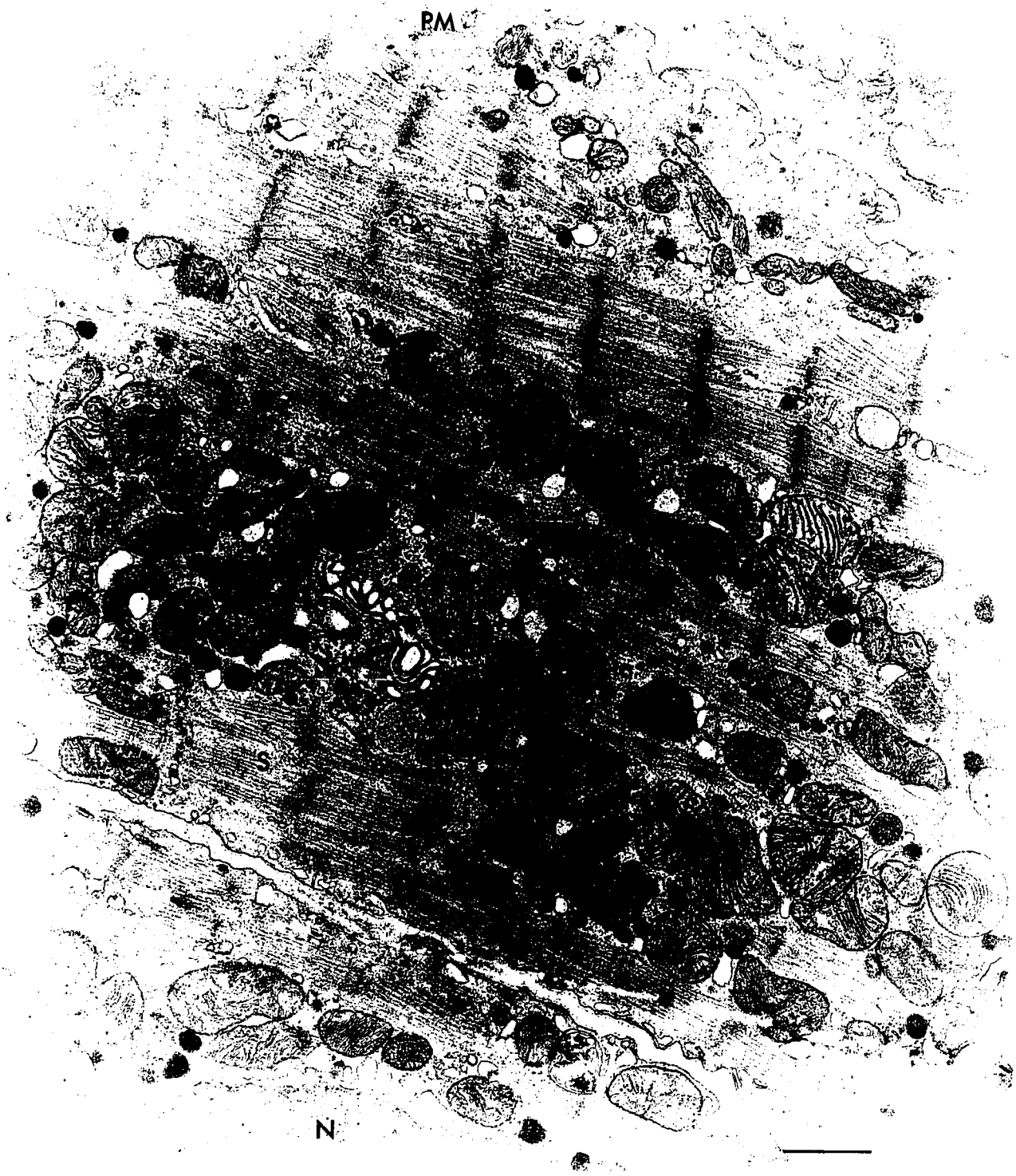

Fig. 19. Electron micrograph of a typical section from a rat atrium stored at 6° C for 48 hrs. N-nucleus; M-mitochondrion; S-sarcomere; PM-plasma membrane. Note the loss of glycogen. Scale: 1μ.x14,600.

E. EFFECT OF VARIOUS AGENTS ON THE CONTRACTILE FORCE OF SPONTANEOUSLY BEATING COLD-STORED ATRIA

1) Effect of noradrenaline

The dose response curves of fresh and cold-stored spontaneously contracting atria to noradrenaline are shown in Fig. 20. Noradrenaline caused comparable increases in the contractile force of the stored and fresh atria.

2) Effect of ouabain

As ouabain is frequently used in congestive heart failure and is known to cause a greater increase in the force of contraction of failed than of normal hearts, it was thought that ouabain might be able to restore the contractility of the cold-stored atria. The dose-response curves of fresh and cold-stored spontaneously beating atria to ouabain are shown in Fig. 21. There was no difference in the increase in contractile force of the fresh and cold-stored atria to this agent. Several experiments were performed in which fresh atria were incubated with high concentrations of noradrenaline (10 µg/ml). This resulted, in some cases, in the reduction in contractility after noradrenaline was washed out; when ouabain was added to these failed preparations, there was an increase of some 200-300% as compared to the 30-35% in the control fresh atria. On the other hand, ouabain did not cause a marked increase in the force of contraction when the contractile force was reduced by cold-storage, as it did when the contractile force was reduced due to high concentrations of noradrenaline.

It was thought desirable to test the effect of ouabain after the ATP content was increased by the prior incubation of the cold-stored atria

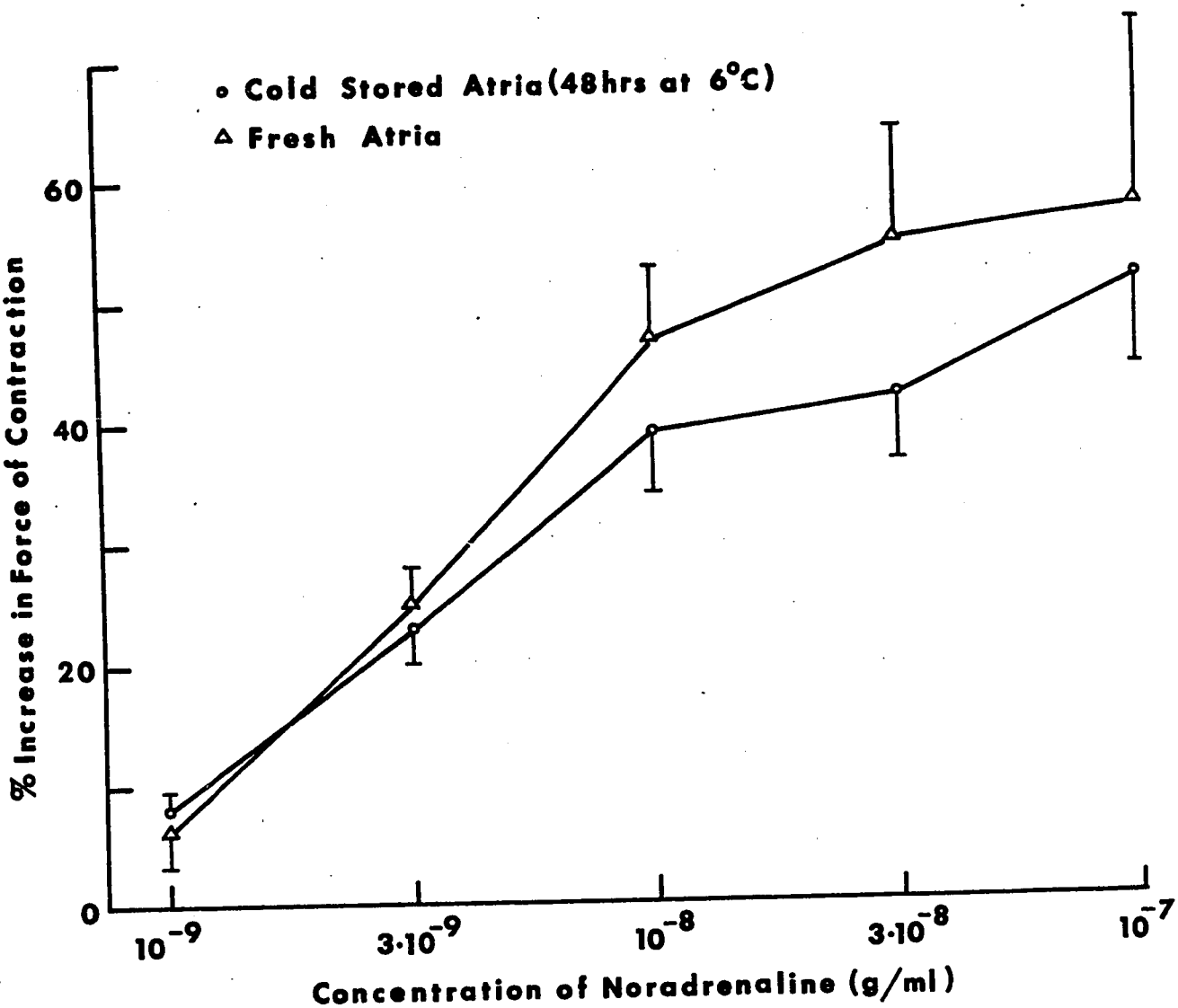

Fig. 20. Effect of noradrenaline on the force of contraction of spontaneously beating fresh and cold-stored atria of rats. Each point represents the mean ± S.E.

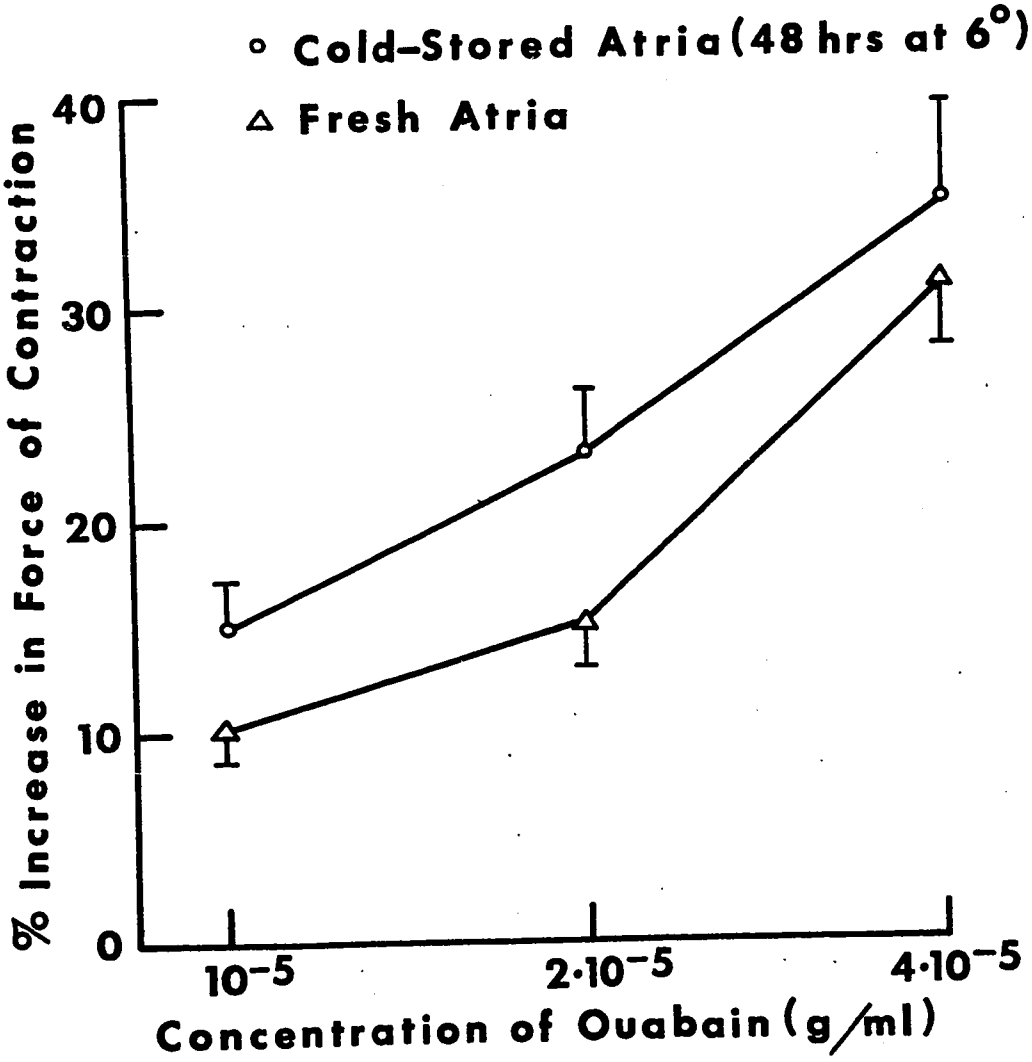

Fig. 21. Effect of ouabain on the force of contraction of spontaneously beating fresh and cold-stored atria of rats. Each point represents the mean ± S.E.

with adenosine. The results of these experiments are shown in Fig. 22. Even after the increase in ATP content, ouabain was no more effective in restoring the contractility of the stored atria than it was in the control stored atria.

3) Effect of Ca^{++}

The dose-response curves to Ca^{++} in fresh and cold-stored spontaneously beating atria are shown in Fig. 23. Ca^{++} increased the contractile force of the stored and fresh atria; the percent increase was not statistically different between the fresh and stored tissues.

4) Storage of atria in Krebs containing varying concentrations of K^{+}

Because of the loss of intracellular K^{+} during cold-storage and the inability of the stored atria to reaccumulate the lost K^{+}, it was decided to store the atria in Krebs solution containing high concentrations of K^{+} in an attempt to prevent the loss of cellular K^{+}. Atria were stored for 24 hours in 1.2, 6, 12 and 18 mM K^{+} and the results are shown in Fig. 24. The storage of the tissues in 1.2 mM K^{+} was shown to have adverse effects on the contractility of the atria, the contractile force being significantly lower in those atria when compared with those stored in 6 mM K^{+}. However, the storage of atria in higher concentrations of K^{+} did not prevent the reduction in the contractile force. It is also interesting to note that atria stored in 1.2 mM K^{+} had a mean ATP content of 1.75 μM/g; this was significantly lower than the 3.4 μM/g in the atria stored for the same length of time in 6 mM K^{+}. This indicates that it was not just a matter of the atria being unable to recover the K^{+} lost due to the low extracellular

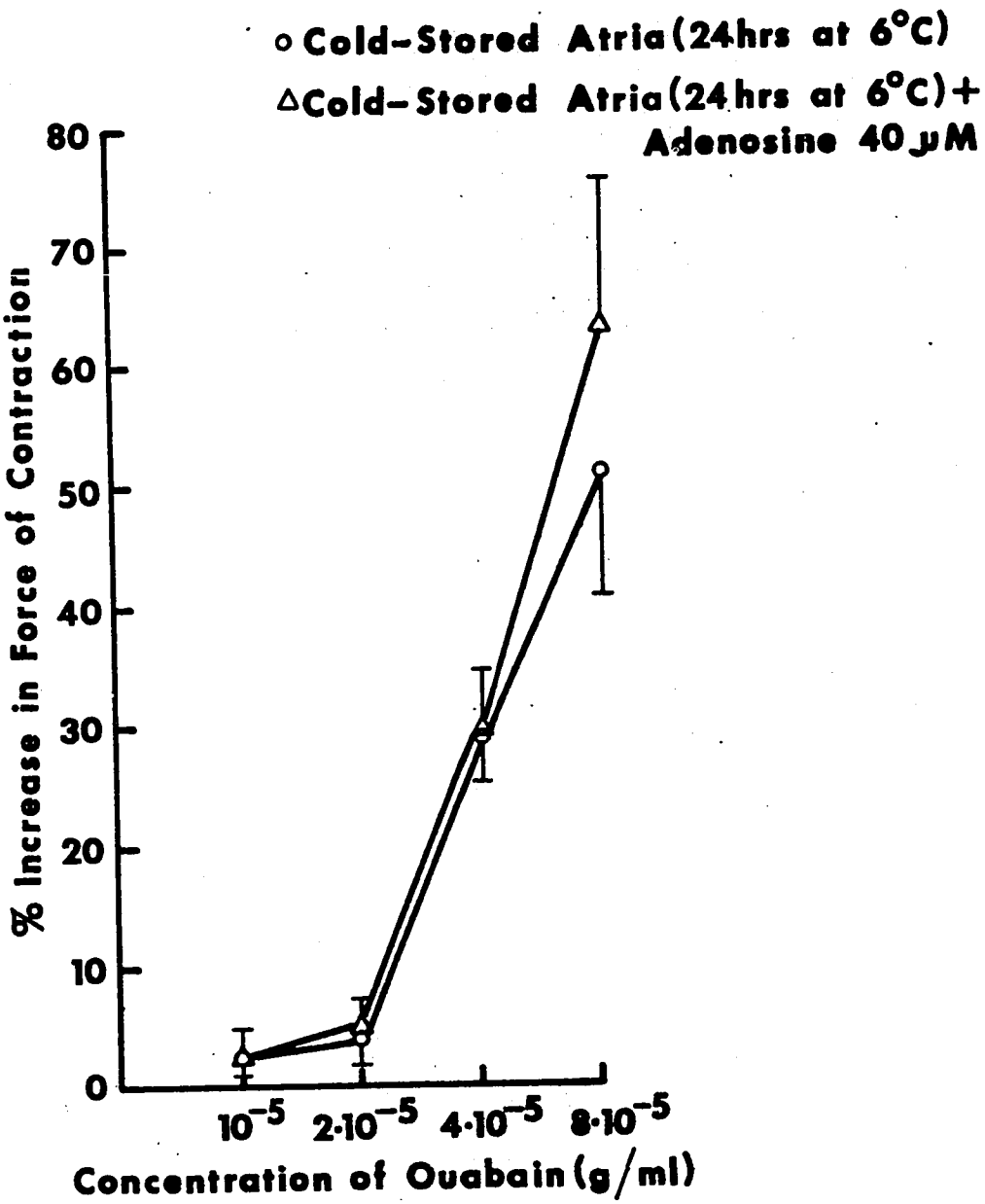

Fig. 22. Effect of incubation of atria with adenosine on the contractility of spontaneously beating cold-stored atria of rats to ouabain. Each point represents the mean ± S.E.

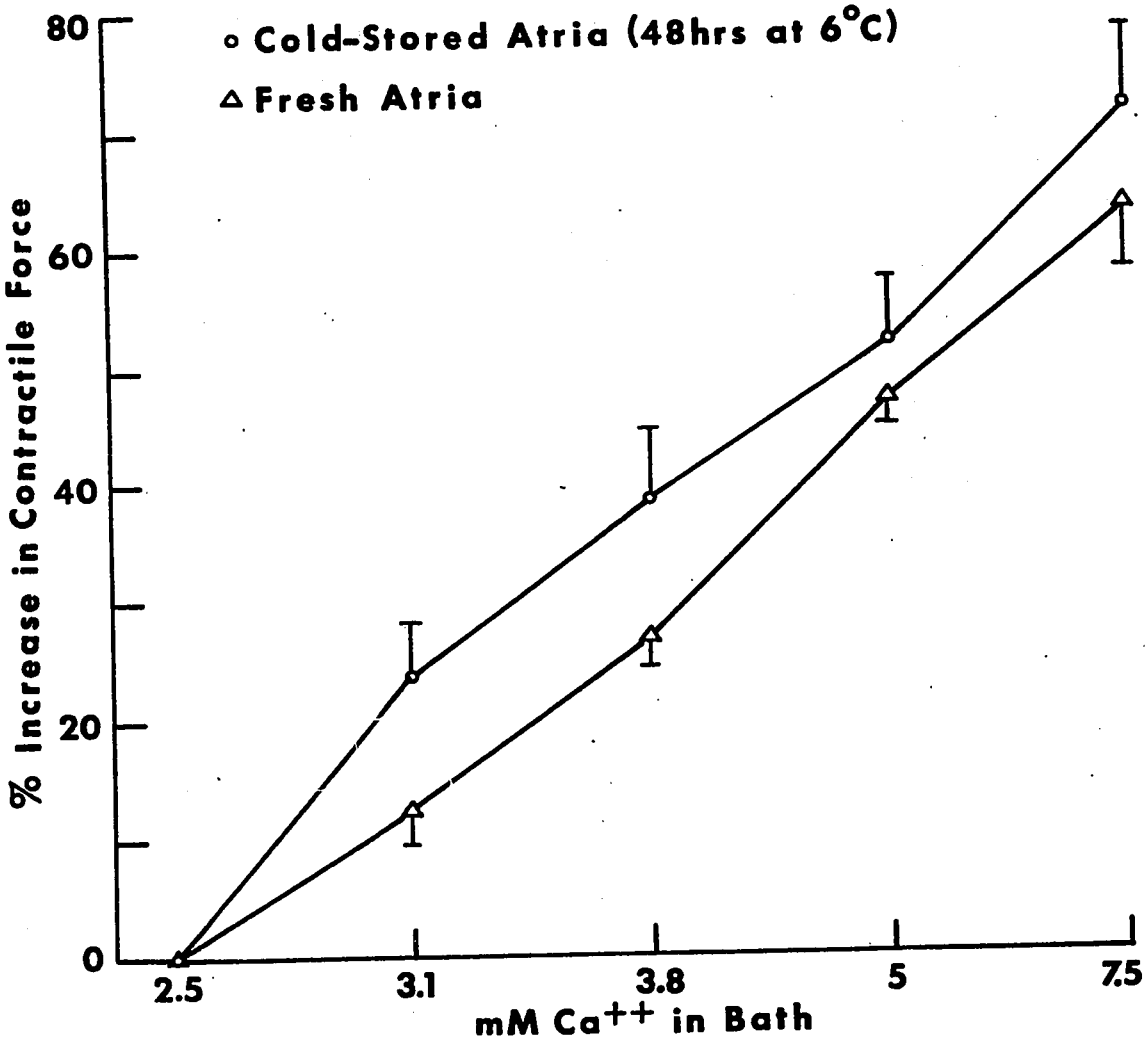

Fig. 23. Effect of Ca^{++} on the force of contraction of spontaneously beating fresh and cold-stored atria of rats. Each point represents the mean $\pm$ S.E.

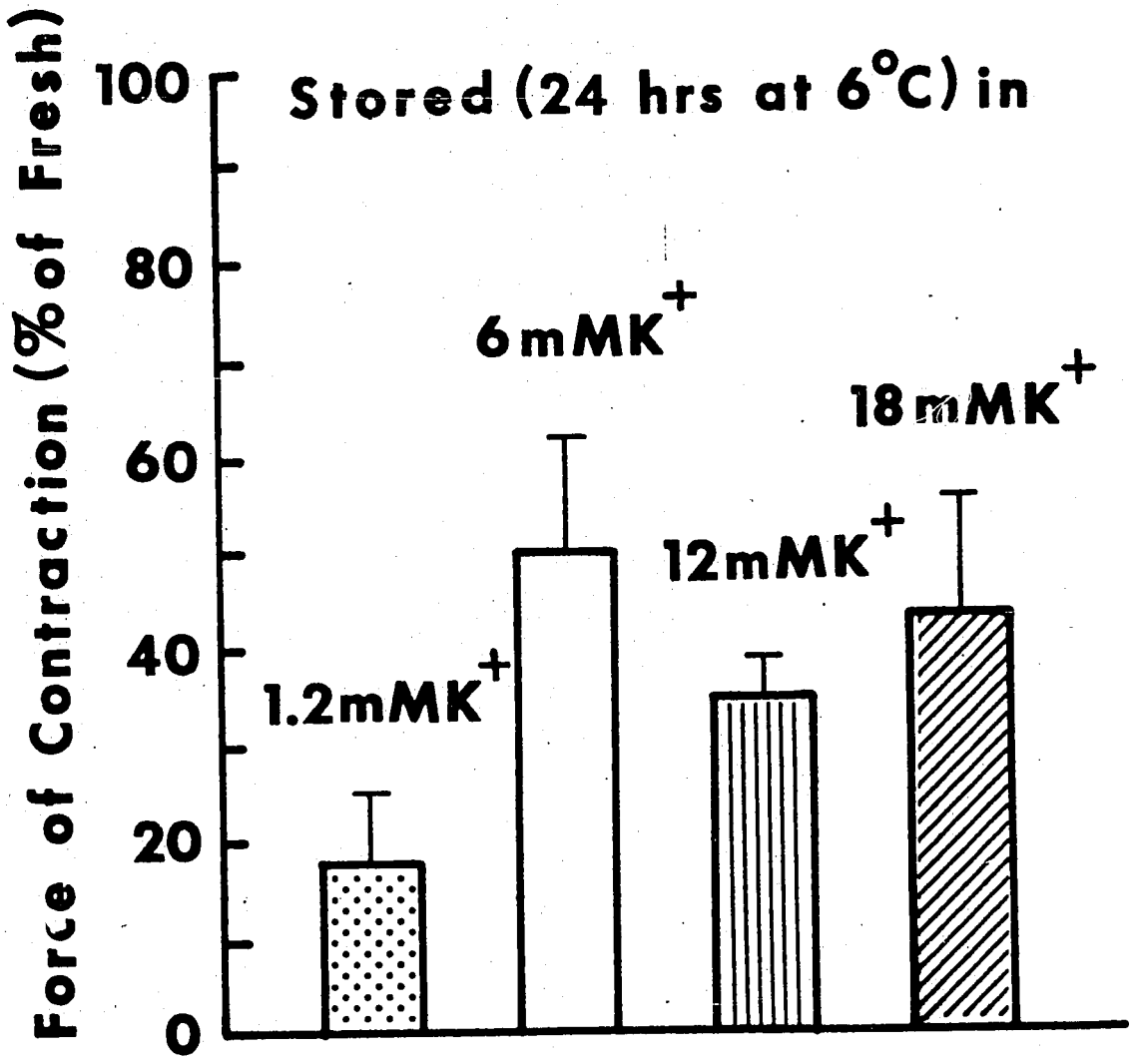

Fig. 24. Effect of storage in Krebs containing varying concentrations of K^+ on the contractile force of spontaneously beating cold-stored atria of rats. Each column represents the mean $\pm$ S.E.

K^+, but of damage to the myocardium caused by the lack of sufficient K^+ in the medium.

5) Summary of results

The maximum increases in the force of contraction produced by the various agents studied on fresh and cold-stored atria are shown in Table 9. Of all the experimental tests, the lowering of the extracellular K^+ to 1.2 mM was the only condition which caused a greater increase in contractile force of stored atria than in that of the fresh atria. However, the maximum contractile force of stored atria in 1.2 mM K^+ was still significantly lower than the force of the fresh atria (58% of the pre-storage force).

F. SPONTANEOUS RATE AND COLD-STORAGE

1) Effect of cold-storage on the spontaneous rate of rat atria

Fig. 25 shows the effect of cold-storage on the spontaneous rate of rat atria. There was no change in spontaneous rate for periods of storage of up to 72 hours, despite the fact that the contractile force after this period was only some 22% of that of the fresh atria. It should be noted that 48 hours after storage, the mean resting potential was 61 mV compared to a mean value of 74 mV of the fresh tissues; these values are significantly different from each other. However, these potentials were taken from the non-pacemaker cells of the left atrium and did not necessarily indicate a reduction in the resting potential of the pacemaker cells.

TABLE 9. Comparison of maximum increases in force of contraction produced by various agents in fresh and cold-stored rat atria.

Agent	Concentration	Fresh atria**	Cold-stored atria** (48 hrs at 6° C)
Noradrenaline	0.1 µg/ml	58 ± 18	52 ± 16
Ouabain	40 µg/ml	31 ± 6	35 ± 9
Ouabain + adenosine (40 uM preincubation)	40 µg/ml	---	64 ± 12[a] (52 ± 9)[b]
Ca^{++}	200 µg/ml	63 ± 6	72 ± 8
K^{+}	1.2 mM	-2 ± 8	53 ± 11*
Adenosine	40 µM	21 ± 9	25 ± 15

*Significantly different from control (fresh) values, $p<0.05$.

**% change in force of contraction, mean ± S.E.

[a]Atria stored for 24 hrs at 6° C.

[b]Force before incubation with adenosine.

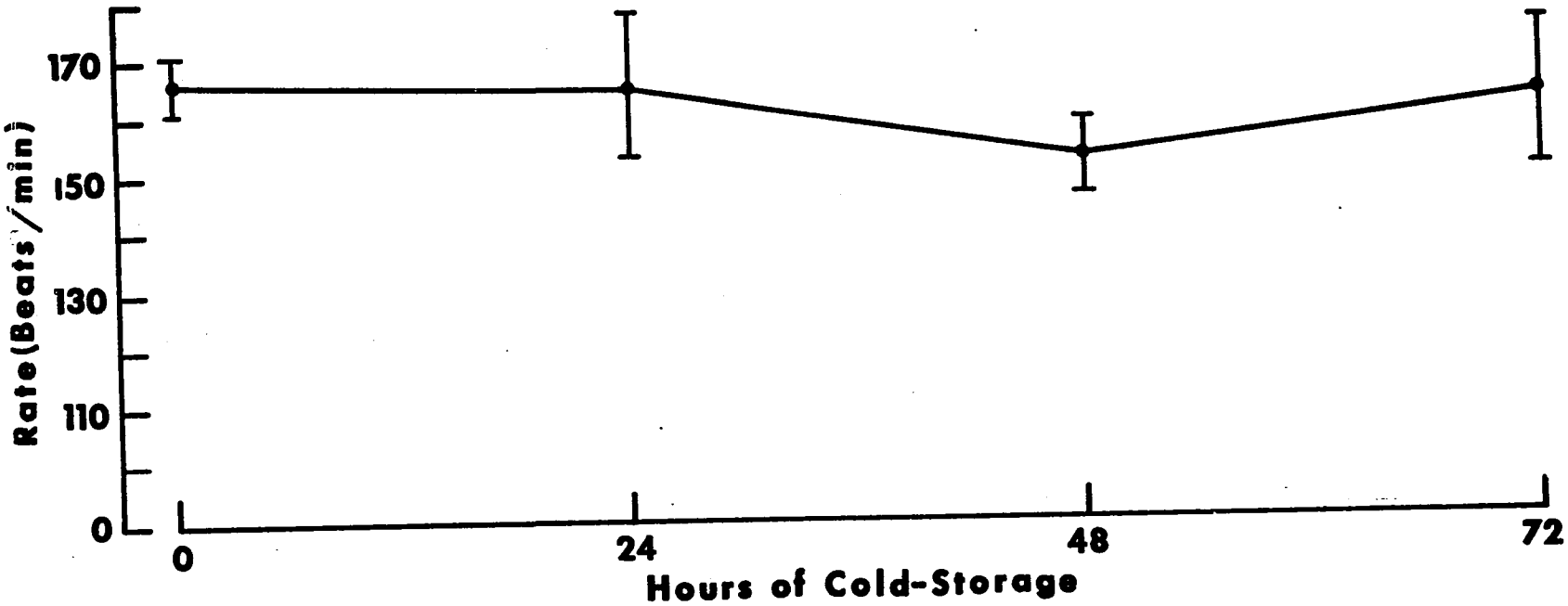

Fig. 25. Effect of storage at 6° C for 24-72 hrs on the spontaneous rate of rat atria. Each point represents the mean ± S.E.

2) Effect of 12 mM K^+ and cold-storage on rate and resting potentials of rat atria

Hoffman and Cranefield (1960) pointed out that the resting potentials in rabbit pacemaker cells remained unaffected by concentrations of extracellular K^+ which caused significant depolarization in other parts of the atrial muscle. For this reason, it was decided to test the effect of 12 mM K^+ on the rate and resting potential of fresh atria. Table 10 shows a comparison of the decrease in rate, intracellular K^+ concentrations, K^+ equilibrium potentials and resting potentials for fresh atria incubated in 12 mM K^+ and cold-stored atria. Although the resting potential of the non-pacemaker cells of the atria incubated in 12 mM K^+ were reduced to values corresponding to those seen after 48 hours of cold-storage, the rate was unchanged. These results are compatible with previous studies which showed that the cells of the muscle fibres can be depolarized without the resting potential of the pacemaker cells being altered. It is thus possible that the resting potential of the pacemaker cells was not lowered by cold-storage.

3) Effect of high K^+ on rate of cold-stored atria

Fig. 26 shows the dose-response curves of fresh and cold-stored spontaneously beating atria to K^+. The cold-stored atria were much more sensitive to high concentrations of K^+ than were the fresh tissues. This was probably due to the already partially depolarized state of the atrial cells caused by cold-storage.

TABLE 10. Effect of 12 mM K^+ and cold-storage on rate and resting potentials of rat atria.

Preparation	Decrease in rate % fresh	K^+ (intracellular) mEq/L	K^+ equilibrium potential mV	Resting potential mV
Fresh	0	127.4	79.8	74
Fresh 12 mM K^+	5	144.4	65.0	59*
Cold-stored (48 hrs at 6° C)	8	78.6	67.2	61*

*Significantly different from control (fresh) values, $p<0.05$.

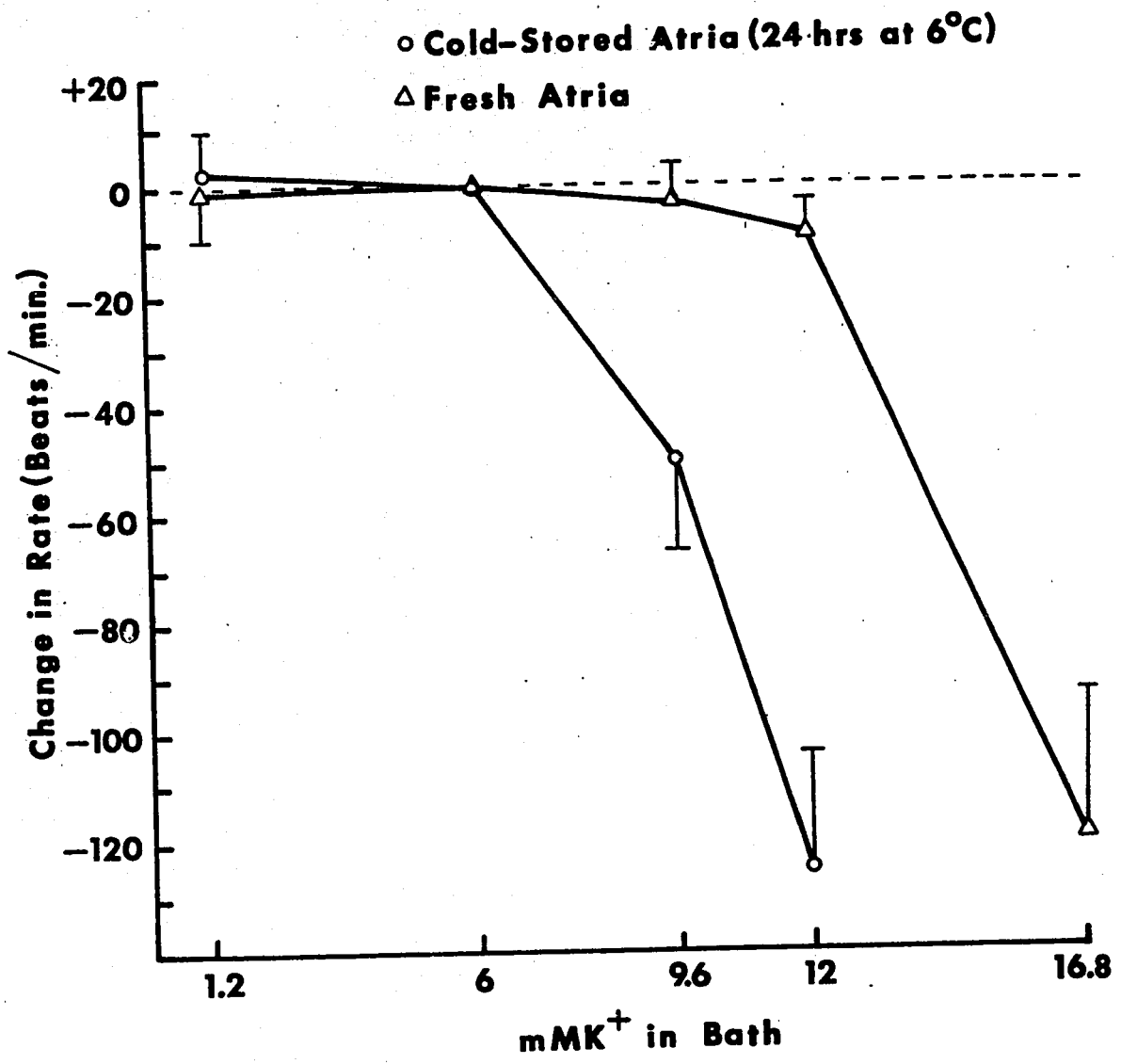

Fig. 26. Effect of K^+ on the spontaneous rate of fresh and cold-stored rat atria. Note the increased sensitivity of cold-stored atria to high K^+. Each point represents the mean $\pm$ S.E.

4) Rate and ATP content of fresh and cold-stored atria

The relation between the spontaneous rate and the ATP content of atria is shown in Table 11. The cold-stored atria, despite the large reduction in ATP content exhibited no change in the spontaneous rate. Also, there was no difference in the ATP content of atria incubated in fresh oxygenated Krebs at 6° C and at 30° C. The same phenomenon was seen in the fresh atria; there was no difference between the ATP content at 6° C and at 30° C. It should be emphasized that although there was a significant difference in the ATP content between the fresh and cold-stored atria, there was no difference in the spontaneous rate between the fresh and stored atria.

5) Effect of noradrenaline and Ca^{++} on the spontaneous rate of cold-stored atria

Fig. 27 and 28 show the dose-response curves to noradrenaline and Ca^{++} of fresh and cold-stored spontaneously beating atria. The two preparations responded in the same manner to the chronotropic effect of noradrenaline or Ca^{++}, no difference being seen either in the maximal response or in the sensitivity to these agents.

TABLE 11. Effect of cold on ATP content and spontaneous rate of fresh and cold-stored atria.

Duration of storage hrs	Incubation temperature °C	ATP content μm/g ± S.E.	rate % fresh at 30° C
0	30	5.4 ± 0.8	100
0	2	5.4 ± 0.9	0
48	30	2.7 ± 0.2*	92
48	6	2.9 ± 0.5*	0

*Significantly different from control (fresh)values, $p<0.05$.

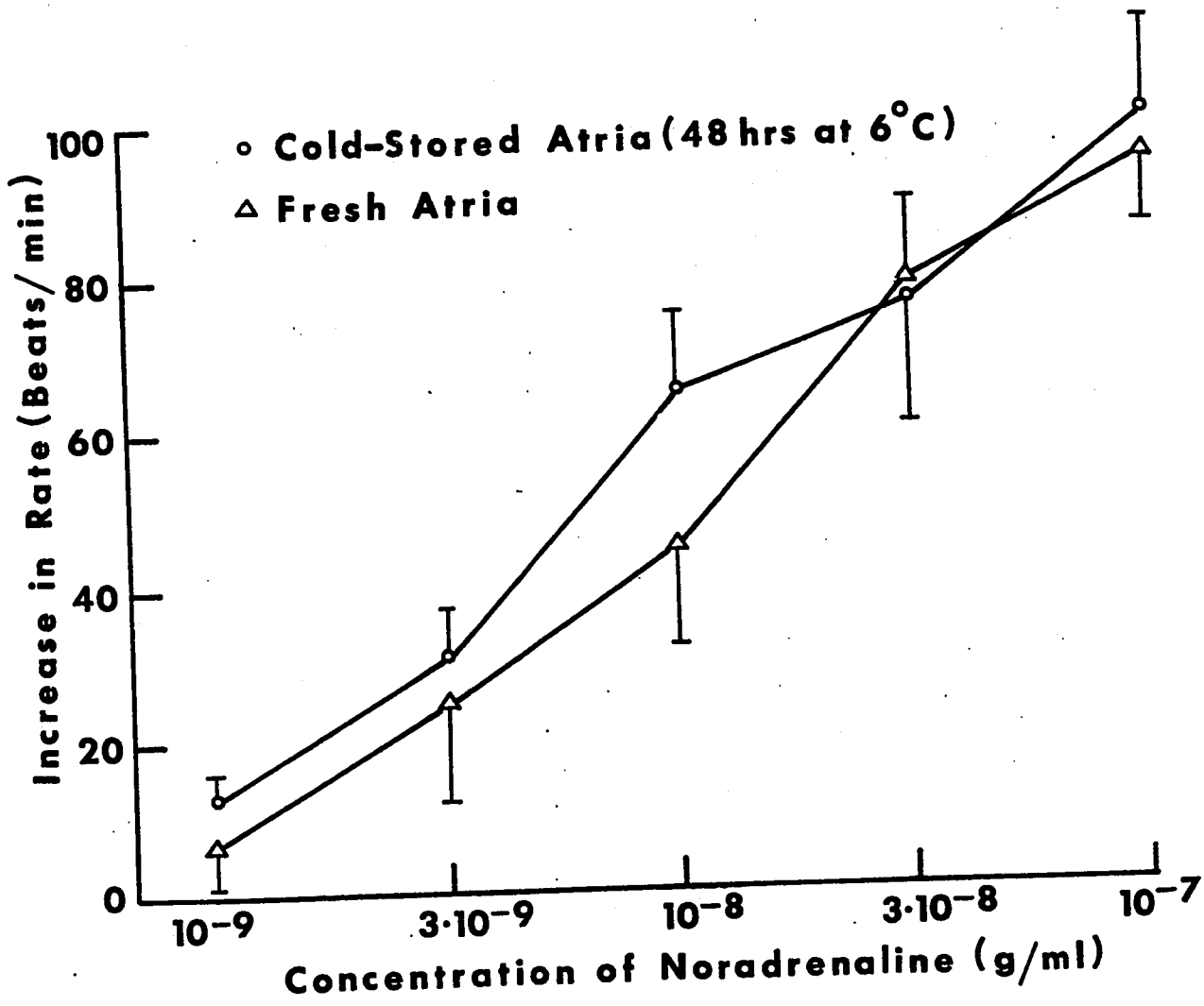

Fig. 27. Effect of noradrenaline on the spontaneous rate of fresh and cold-stored atria of rats. Each point represents the mean ± S.E.

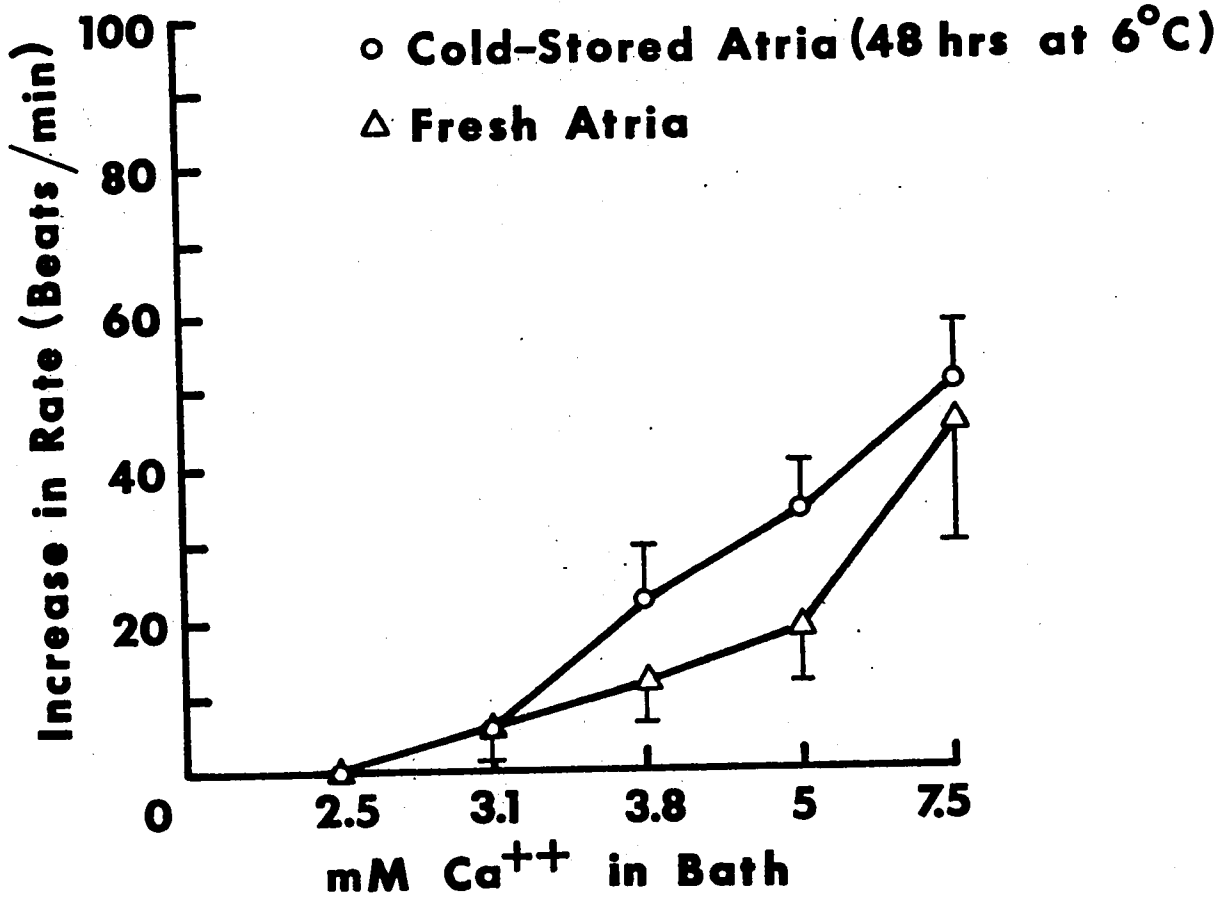

Fig. 28. Effect of Ca^{++} on the spontaneous rate of fresh and cold-stored atria of rats. Each point represents the mean $\pm$ S.E.

DISCUSSION

A. CONTRACTILITY AND COLD-STORAGE

The present experiments showed that the storage of atrial muscle in the cold for periods up to 72 hours resulted in a progressive decrease in the force of contraction; the rate of spontaneous contractions was unchanged in these tissues (Figs. 1 and 25). These results are consistent with the findings of other workers who reported similar results after storing whole perfused heart of rabbit (Athreya et al., 1968), rat (Kamiyama et al., 1970) and dog (Garzon et al., 1968; Ferrans et al., 1970). In the present study an attempt was made to find the principle changes responsible for this decrease in force of contraction. For this purpose, electrophysiological changes, changes in the ATP content and histological changes were studied.

It was found that the resting potential, action potential height, duration, area and depolarization rate of atria stored in cold for 48 hours were reduced (Table 2). Reports in the literature concerning electrophysiological changes produced by cold storage are conflicting. Matsuda et al. (1956, 1967) reported normal electrical activity in dog ventricle even after 72 hours of storage at 1-3° C. Matsuda (1960) found that action potentials and resting potentials were normal in terminal Purkinje fibers of the dog after 24 hours of storage at low temperature. However, he reported that "recovery became less satisfactory" after 48 hours of storage. He also reported that the false tendon "seemed less resistant to cold" than either the terminal Purkinje fibers or ventricular muscle. However, the author did not study the contractility of these tissues and gave no indication as to the nature of the changes seen in the above study. On the other hand, Tamai and Kagiyama (1968) found a marked hyperpolarization (the maximum was 184 mV in the atrium and 267 mV in the ventricle) of the cell membrane of kitten atria and ventricular muscle

after 15-72 hours of storage at 4° C. No such hyperpolarization was detected in the present work. This discrepancy in the results of these two studies is difficult to account for. However, it is possible that the species difference may account for the two different results. It is unlikely that the present results are incorrect as the measured intracellular resting potentials were in agreement with the calculated K^+ equilibrium potential which was determined from the measurements of the K^+ content of the stored atria (Table 1). The stored atria were also more sensitive to the depolarizing actions of high K^+ than the fresh tissues, a result that would not be expected if the resting potentials were normal or greater than normal. Although electrophysiological recordings from atria stored for varying periods of time were not done, it is not unreasonable to expect that storage for 24 and 72 hours would produce qualitatively similar changes.

The changes in the transmembrane potential of the cold-stored atria were probably due to the depolarized state of the cell membrane as a result of the low intracellular concentration of K^+ (Table 1). Reports in the literature show that the increase in extracellular K^+ results in the decrease of the resting potential, action potential height, duration and depolarization rate (Cranefield and Hoffman, 1958; Hoffman and Cranefield, 1960). Similar results were also found in the present study (Table 7). These electrophysiological changes produced by high K^+ are most probably due to the depolarizing actions of K^+ as Weidmann (1955a) found that the electrical repolarization of Purkinje fibers depolarized by high K^+ resulted in the restoration of the action potential height and depolarization rate. This suggests that the low resting potential produced by cold-storage caused the other electrophysiological changes. However, the situation is not so clear in the case of the decrease

in the duration of the action potential seen after cold-storage. High K^+ leads to a decrease in the duration of the action potential (Cranefield and Hoffman, 1958; Hoffman and Cranefield, 1960). However, Webb (1956) found that, under physiological conditions, rat atrial cells with lower resting potentials had longer action potential durations. It is thus unclear as to whether or not the depolarization of the cell membrane by cold-storage was responsible for the decrease in the duration of the action potential.

The reason for the depolarized state of the cold-stored atrial muscle seems to be the inability of the atrial cells to regain K^+ and extrude the excess Na^+ upon rewarming. This is indicated by the finding that the Na^+ and K^+ contents of the cold-stored atria incubated in fresh oxygenated Krebs for 2 hours at 6° C were the same as when stored atria were similarly incubated at 30° C (Table 1). This finding is seemingly in contradiction with the published reports in the literature. Various cold-stored tissues have been able, at least to some extent, to reaccumulate K^+ and extrude the excess Na^+ on incubation at normal temperatures. The recovery of the ionic gradients of red blood cells after cold-storage is well known (Danowski, 1941; Harris, 1941). Page and Storm (1965) found that ionic gradients in cat papillary muscle stored at 2° C for 2 hours were returned to normal upon rewarming. The same results have been observed in smooth muscle stored for 24 hours or less (Daniel, 1965; Friedman and Friedman, 1967). However, Shibata (1969) and Varma and McCullough (1969) found that smooth muscle stored for 4 days or longer exhibited a reduced response to high K^+ which was attributed to the incomplete recovery of the intracellular K^+ concentration. The apparent contradiction between the reports in the literature and the present findings could be explained by a lower resistance of heart muscle to the effects of

cold-storage than that of the other tissues studied. This is indicated by the observation that atrial muscle did not exhibit spontaneous contractions nor could they be stimulated electrically when stored for 96 hours or more, whereas smooth muscle exhibited contractions to noradrenaline even after 14 days of storage (Varma and McCullough, 1969). It was also found in the present work that atria which were stored for only 4 hours at 2^{o} C showed a partial recovery of the ionic gradients when rewarmed to 10^{o} C (Table 3).

The inability of stored atria to regain the K^{+}-Na^{+} balance on rewarming may be due to a decrease in the Na^{+} pumping rate resulting from the loss of ATP during cold-storage. The Na^{+}-K^{+}-ATPase system in the membrane is thought to be the main mechanism responsible for maintaining the ionic gradients (Langer, 1968). Atria stored in the cold for 48 hours were found to have a significantly lower ATP content than the fresh tissues (Fig. 11). Also the stored tissues were unable to replenish their ATP stores upon rewarming (Table 11). Thus the changes in the ATP content seem to correlate with the changes in the ionic gradients and may indicate a cause-effect relationship.

Reductions in the resting potential, action potential height, duration, area and depolarization rate were associated with a decrease in the contractile force 48 hours after cold-storage (Table 2). It would have been more appropriate to study the electrophysiological changes at each time period at which the contractile force was determined. However, for the sake of convenience, transmembrane potentials were only studied after 48 hours of storage.

Correlations between the contractile force and various parameters of the action potential are difficult to make (Cranefield and Hoffman, 1958; Brady, 1964). In some cases, a reduction in one or more of the parameters of the action potential is accompanied

by a reduction in force. Acetylcholine causes a reduction in the action potential duration and also in the force of contraction (Webb and Hollander, 1956; Furchgott et al., 1960). High K^+ causes a decrease in the resting potential and action potential height, duration and depolarization rate and the contractile force (Brooks et al., 1955; Cranefield and Hoffman, 1958; Hoffman and Cranefield, 1960). On the other hand, sometimes the opposite is the case. Ouabain causes a decrease in the action potential duration but an increase in force (Reiter et al., 1966; Edmands et al., 1967; Prasad and Callaghan, 1970). Lu et al. (1968) and Spear and Moore (1971) have demonstrated that the action potential with the shorter duration was associated with the contraction of the greater force during alternation in mammalian cardiac muscle. In the case of the cold-stored atria, however, as studied in the present work, it seems likely that the reductions in resting potential and action potential height, duration area and depolarization rate were responsible, at least in part, for the reduction in contractile force. This is indicated by the observation that the incubation of atria in 1.2 mM K^+ increased the force of contraction of cold-stored but not of fresh atria (Fig. 5). The low K^+ probably increased the already lowered resting potential as predicted by the Nernst equation. The improvement in the transmembrane potential probably increased the contractile force. However, in the case of fresh cardiac muscle, it has been reported that the incubation of the muscle in low K^+ failed to increase the resting potential (Burgen and Terroux, 1953a; Cranefield and Hoffman, 1958; Hoffman, 1959). However, these studies were performed in tissues under normal physiological conditions and the present studies were done on atria already partially depolarized, which may account for this discrepancy. It thus seems likely that the

electrophysiological changes were responsible for part of the reduction in the contractile force produced by cold-storage.

To further elucidate the contribution of the electrophysiological changes to the reduction in force produced by cold-storage, comparisons of the transmembrane potentials and contractile forces between the cold-stored atria and fresh atria incubated either at 10° C or in 12 mM K^{+} were made (Table 8).

High K^{+}, like cold-storage, caused significant reductions in the action potential height, duration, area and depolarization rate as well as the resting potential. In all cases, the reductions produced by high K^{+} in the fresh tissues were greater than (action potential height, area and depolarization rate) or the same as (resting potential and action potential duration) those produced by cold-storage (Table 8). If the reduction in the contractile force of the cold-stored atria was caused by the reductions in these parameters of the transmembrane potential, it would be expected that fresh atria incubated in 12 mM K^{+} Krebs would have a contractile force of a similar magnitude. However, this was not the case. The contractile force of the fresh atria incubated in 12 mM K^{+} Krebs was over 2.5 times that of the cold-stored atria (Table 8). It thus seems likely that the reduction in the contractile force of the cold-stored atria can not be explained solely by the reduction in the various parameters of the transmembrane potential. The only possible argument against this is that while high K^{+} caused reductions in the resting potential, action potential height, duration, area and depolarization rate, the reduction in the contractile force that would have been produced by these changes was offset by other effects of high K^{+} on the atrium.

The experiments performed on fresh atria at 10° C provide further

evidence that the electrophysiological changes could not account for the entire reduction in contractile force caused by cold-storage. In this case, the resting potential and action potential height and depolarization rate of the atria at 10° C stimulated with 50 V were markedly lower than those values of the cold-stored atria. In spite of this, the force of contraction of the atria at 10° C was over 2 times that of the cold-stored atria (Tables 2 and 4). Even greater reductions in the values of these parameters of the transmembrane potential were required before the contractile force of the atria at 10° C approached that of the cold-stored atria (Tables 2 and 8). However, the action potential duration and area of those atria incubated at 10° C, were significantly greater than those of the cold-stored tissue (Table 8). It thus could be argued that the increases in these parameters compensated for the reductions in the resting potential and action potential height and depolarization rate. However, if one examines the evidence from the effects of low K^+ on cold-stored atria (Fig. 6) as well as the comparisons of the fresh atria in the cold and in high K^+ with the cold-stored tissues (Table 8), it seems reasonable to conclude that the electrophysiological changes could not account for the entire reduction in force produced by cold-storage.

The experiments performed in 12 mM K^+ Krebs or at 10° C further illustrate the relationship between the transmembrane potential and the contractile force. It would appear from these results that the action potential can be greatly altered without affecting the contractile force to any large extent. The atria incubated in 12 mM K^+ exhibited a significantly reduced resting potential and action potential height, duration, area and depolarization rate; they were 80%, 63%, 78%, 55% and 17%, respectively, of

those values in 6 mM K^+, and yet the contractile force was 85% of that in 6 mM K^+ Krebs (Table 7). The results from the atria incubated at 10° C were even more striking. In the first place, the atria were unable to exhibit action potentials with the usual characteristics. The lack of the "all or none" phenomenon, as shown by the voltage dependency of these potentials (Table 4) and the similarity between the depolarization and repolarization rates seem to indicate that these potentials are more like graded depolarizations than true action potentials. These potentials were, however, most likely caused by the influx of Na^+ as both the action potential height and the contractile force were reduced by TTX (Table 5), a known inhibitor of the Na^+ conductance during the action potential (Kao, 1966). In the second place, although the contractile force was 70% of control, the resting potential and action potential height and depolarization rate were markedly reduced (they were 75%, 48% and 1%, respectively, of those values at 30° C) and the action potential duration and area were significantly larger (they were 378% and 230%, respectively, of those values at 30° C) (Tables 2 and 4). It thus seems difficult to correlate changes in the transmembrane potential with changes in the contractile force. It should be noted, however, that the reduction in the action potential height and area by a decreased stimulating voltage (Table 4) or by TTX (Table 5) in those atria at 10° C resulted in the reduction in the contractile force. It does seem, therefore, that the reduction in some parameters of the transmembrane potential can result in the reduction of the contractile force. Finally, it should be noted that, as the contraction of the cardiac muscle is induced by the entry of Na^+ into the cells (Langer, 1968) and probably not by the electrical activity <u>per se</u>, it is understandable that the configuration of the action potential could be

changed without the significant alteration of the contractile force.

Since the electrophysiological changes do not account for the entire reduction in contractile force produced by cold-storage, other factors are likely to be responsible for the decrease in contractile force. It is well known that red blood cells lose ATP when stored for prolonged periods of time in the cold and that this loss is accompanied by a decrease in viability (Nakao et al., 1959; Bishop, 1961; Nakao et al., 1962; Simon, 1967). Calman et al., (1970) studied stored whole rat hearts and found that the ATP levels correlated with the "percentage survival" of the hearts. It thus seemed not unlikely that the reduction in the contractile force of stored atria might be due to a decrease in ATP content. It was, in fact, found that the reduction in force after cold-storage was accompanied by a reduction in the ATP content (Fig. 11). A positive correlation between the ATP content and the contractile force was quite consistent except in the case of those atria which were stored for 24 hours (Figs. 13 and 14). The reason for this discrepancy is difficult to explain if the assumption is made that the reduction in force was due to the decrease in ATP content. However, it is possible that in this series of experiments, at least, other factors were responsible for the increased contractile force or low ATP content of these atria. As the energy for the contraction of the heart muscle comes from the hydrolysis of the high energy bonds of ATP (Furchgott and Lee, 1961), it seems likely that the reduction in ATP stores was a major factor in causing the low contractile force of the cold-stored atria. There may also be an interrelationship between the ATP content and the electrophysiological changes. The cold-stored atria exhibited an inability to extrude the excess Na^+ or accumulate K^+. This may indicate a defect in the Na^+-K^+ pump. As ATP is the energy source for this pump

(Langer, 1968), it is possible that the reduction in ATP content was the factor responsible for the inability of the cold-stored tissues to restore the ionic balance to normal.

If the reduction in contractility were at least in part due to a decrease in the ATP content, one would expect that the restoration of the ATP level would also restore the contractility. It is well established that the incubation of cold-stored red blood cells with adenosine or adenine increases the viability as well as the ATP content of the cells (Nakao et al., 1959, 1960; Simon, 1967; Lacelle, 1969). In the present experiments, the incubation of fresh and cold-stored atria with adenosine led to slight and approximately equal increases in the contractile force. However, the incubation of fresh tissues with adenosine led to a large (almost 4 times) increase in ATP content; but in the case of the cold-stored atria, the increase in ATP content was not quite significant ($0.1>p>0.05$) (Fig. 12). After incubation with adenosine, the ATP content of cold-stored atria was approximately the same as that of the control fresh atria before adenosine incubation (Fig. 12). However, the restoration of the ATP content of the cold-stored atria to values close to that of fresh control atria did not lead to the restoration of the contractile force to that of fresh atria. Furthermore, the increases in contractile force in the fresh and cold-stored atria after incubation with adenosine were similar, even though the increase in ATP content of fresh atria was far greater than that of the stored atria. It is possible that the heart cells under physiological conditions are operating with the optimum level of ATP and any further increase would not change the contractile force. However, this does not explain the lack of increase in the contractile force of the cold-stored tissues when the ATP levels were raised. It would therefore appear that either the reduction

in ATP content after cold-storage is not responsible for the reduction in force or that the newly synthesized ATP is unable to be utilized by the contractile mechanism. If the latter is the case, the atrial cells behaved differently than the red blood cells whose viability is restored by incubation with adenosine. However, it should be pointed out that the red blood cell is a much more simple structure than the cardiac cell and does not possess any contractile mechanism. While increases in ATP might restore the viability of the red cell, it does not follow that it would necessarily do so in the heart. This is likely to be so as the red cell does not possess a contractile mechanism which is the very structure whose function may be affected by the low ATP levels in the heart. The relatively smaller increase in ATP content of cold-stored atria after incubation with adenosine compared to fresh tissues indicates possible impairment in either the biosynthetic pathway for the formation of ATP from adenosine or in the storage mechanism for ATP in these tissues.

Electron microscopic studies were performed to detect any structural changes which may account for the low contractile force in the cold-stored atria. Other workers have found structural changes in perfused whole dog hearts after 24 hours of storage; these authors found mitochondrial swelling, angulations in the mitochondrial cristae, loss of glycogen and changes in the sarcoplasmic reticulum which correlated with the loss of function (Ferrans et al., 1970; Trunkey et al., 1970). However, in the present work, no changes in the extracellular space (Fig. 16) or histological changes (Figs. 17, 18 and 19) could be detected in atrial muscle after either 24 or 48 hours of cold storage, except for a loss of glycogen. The decrease in ATP content was unaccompanied by any noticeable changes in the mitochondria and no changes were seen in

the plasma membrane to account for the altered ionic balance of the cold-stored atria. Thus the decrease in contractility of the cold-stored atria does not seem to be explained by any structural changes which could be detected by electron microscopy. The discrepancy between the present findings and those of the above authors may be due to the different preparation involved and different methods of storage. Considerable difficulty has been encountered during the perfusion of hearts for the long periods of time required for cold-storage, especially with microemboli (Webb, 1968; Childs and Lower, 1969). It is thus possible that inadequate perfusion could account for the more extensive damage in the experiments with the storage of whole hearts.

The low contractile force in the cold-stored atria does not seem to be due to a deficiency of Ca^{++} as this agent did not increase the contractile force to a greater extent in the stored than in the fresh atria (Fig. 23).

It is of interest that ouabain did not increase the force of contraction of the cold-stored atria to a greater extent than that of the fresh atria (Fig. 21). Cardiac glycosides are known to have a much greater effect both on pathologically and experimentally induced failed hearts than they have on non-failed hearts (Lee and Klaus, 1971). This "extra" increase in failed preparations can occur in heart muscle whether the ATP content is normal or reduced (Furchgott and Lee, 1961). However, in the case of failure produced by prolonged storage at low temperatures, both when the ATP content was reduced or when it was restored to normal values by incubation with adenosine, ouabain was no more effective than in the fresh preparations (Figs. 21 and 22). On the other hand, when failure was induced in fresh preparations by high concentrations of noradrenaline, ouabain was much more effective than it was in

the fresh controls. This indicates that the type of myocardial failure is different in cold-stored atria than in other situations, or that cold-storage produces certain other changes which prevent ouabain from exerting its usual effect on failed hearts.

B. SPONTANEOUS RATE AND COLD-STORAGE

The experimental results showed that spontaneously beating cold-stored atria were able to contract at the same frequency as fresh tissues even after 72 hours of cold-storage (Fig. 25), despite the large reduction in contractile force. These results are consistent with the findings of Kamiyama et al. (1970) who found no change in rate after the storage of whole rat hearts for 48 hours in the cold.

Hoffman and Cranefield (1960) have pointed out that rate can be altered by 3 mechanisms: 1) a change in the resting potential; 2) a change in the threshold potential; 3) a change in the slope of phase 4 of the action potential. In the present experiments, the resting potential of the left atrial cells was found to be reduced, which indicates that there should have been an increase in the rate. There are two possibilities for this lack of change in the spontaneous rate of cold-stored atria. The first is that although the resting potential was decreased by cold-storage, the threshold potential was also changed in the same direction and to the same degree. The second is that the pacemaker cells of the muscular tissue from which the measurements were taken were more resistant to cold-storage than the muscle cells. The second alternative seems more plausible, even though the potentials of the pacemaker cells were not measured. It is known that the pacemaker cells are more resistant to the depolarizing actions of high K^+ than those of the

muscular tissue; little change is seen in the resting potential even after marked depolarization has occurred in the rest of the atrium (Hoffman and Cranefield, 1960). Furthermore, incubation of fresh atria in 12 mM K^+ Krebs produced resting potentials equivalent to those found after 48 hours of cold-storage, but did not change the rate (Table 10). However, the depolarizing actions of cold-storage and those of high K^+ were due to different causes and therefore the comparison is only suggestive and does not prove that cold-storage affects the resting potential of only the non-pacemaker cells.

It is also of interest that the spontaneous rate of cold-stored atria was the same as that of the fresh atria despite a large reduction in the ATP content (Table 11). This is in contrast to the findings of other workers who have studied the effect of metabolic inhibitors on the spontaneous rate and on the ATP content. Gardner and Farah (1954) found that anoxia or fluoroacetate prcduced a decrease in the spontaneous rate of rabbit atria. These treatments have also been shown to reduce the ATP content of heart muscle (Furchgott and Lee, 1961). Harry et al. (1967) and Seraydarian et al. (1968) found that the reduction in ATP levels in cultured rat heart cells produced by various metabolic inhibitors was invariably accompanied by a reduction in rate or loss of spontaneous contractions altogether. The reduction in the ATP content in the present experiments was much greater than what the above authors found was necessary for a reduction in rate in cultured heart cells. The authors attributed the reduction in rate to the decrease in ATP stores. It seems likely, however, in view of the present experiments, that the reduction in rate produced by metabolic inhibitors or anoxia is due to other factors than the reduction in ATP content per se. These results also show that the ATP content has little or no effect on the spontaneous rate of rat

heart.

Fresh and cold-stored atria responded in a similar manner to the chronotropic effects of noradrenaline and Ca^{++} (Figs. 27 and 28). Fresh and cold-stored atria also resumed spontaneous contractions at the same temperature when rewarmed from 2^{o} C (Fig. 2). These results indicated that the mechanisms for changing rate were unaltered by cold-storage. However, cold-stored atria showed an increased sensitivity to high K^{+} when compared with the fresh tissues (Fig. 26). However, Hoffman and Cranefield (1960) pointed out that high K^{+} causes a cessation of spontaneous contractions by blocking the impulses from the pacemaker cells by its depolarizing actions rather than by affecting the pacemaker cells themselves. In all probability, the increased sensitivity of cold-stored atria to high K^{+} was due to a block in conduction as the muscle cells were already partially depolarized by the cold-storage. It thus seems likely that cold-storage has little effect on the ability of the pacemaker cells to "fire" or on their ability to alter the rate of "firing".

In conclusion, both the electrophysiological changes (reductions in the resting potential, action potential height, duration, area and depolarization rate) and the reduction in the ATP content seem to be involved in the decrease in contractile force produced by cold-storage. Other factors, which were not studied in this work, may also be involved. The decreased ATP content of the cold-stored atria may be responsible for the electrophysiological changes. The rate of spontaneous contractions of rat atria seems to be unaffected by the decrease in the resting potential of the non-pacemaker cells and the reduction in the ATP content produced by cold-storage.

SUMMARY

1) Storage of rat atria at 6° C for 8-72 hours produced a decrease in the contractile force without any change in spontaneous rate.

2) Cold-stored atria had reduced intracellular concentrations of K^+ and increased intracellular concentrations of Na^+. Contents of these two ions were the same at 6° C as at 30° C indicating that the cold-stored atria were unable to reaccumulate K^+ and extrude the gained Na^+.

3) Cold-stored atria had a reduced resting potential, action potential height, duration, area and depolarization rate, as measured with intracellular microelectrodes.

4) Incubation of atria in 1.2 mM K^+ Krebs resulted in an increase in contractile force of the cold-stored atria but not of the fresh atria. The force of contraction of the cold-stored atria in 1.2 mM K^+ Krebs was only 58% of that of the fresh atria. These results indicated that the reduced K^+ gradient and consequent alterations in the action potential were, in part, responsible for the decrease in contractile force produced by cold-storage.

5) Fresh atria incubated in 12 mM K^+ Krebs had a resting potential, action potential height, duration, area and depolarization rate similar to or lower than those values of the cold-stored atria but had a contractile force over 2.5 times that of the cold-stored atria. Fresh atria at 10° C had a contractile force comparable to that of cold-stored atria, but had a resting potential and action potential height and depolarization rate much lower than those values in the cold-stored atria. These results indicated that the decrease in contractile force could not be entirely explained by the electrophysiological changes produced by cold-storage.

6) Fresh atria incubated at 10° C exhibited abnormal action potentials. The height of the action potential as well as the contractile force was stimulus strength dependent. This indicated that the potentials recorded at 10° C were more like graded depolarizations than true potentials. However, these depolarizations were blocked by tetrodotoxin.

7) The ATP content was reduced by cold-storage and a correlation between the decrease in ATP content and the reduction in the contractile force was found in atria stored for various periods and under different conditions. These results suggested that low contractile force of cold-stored atria may be partly due to the decrease in ATP content.

8) Incubation of fresh and cold-stored atria with adenosine resulted in the increase in ATP content but not in the contractile force. It was suggested that the contractile mechanism could not use this newly formed ATP. ATP accumulation after adenosine incubation was much greater in the fresh than in the cold-stored atria. These results indicated that the biosynthetic pathway for the formation of ATP from adenosine or the storage mechanism for ATP was impaired by cold-storage.

9) Electron micrographs of atria stored for 24 and 48 hours revealed no histological changes other than a decrease in the amount of glycogen. There was no difference in the extracellular space between the fresh and cold-stored atria.

10) Storage of atria in Krebs containing up to 18 mM K^+ did not prevent the decrease in contractile force produced by cold-storage.

11) Noradrenaline, ouabain or Ca^{++} produced comparable increases in the contractile force of fresh and cold-stored atria. The inotropic effect of ouabain on stored atria was not altered by the prior incubation of the atria with adenosine.

12) Noradrenaline or Ca^{++} produced comparable increases in the spontaneous rate of fresh and cold-stored atria.

13) Cold-stored atria were more sensitive to the negative inotropic and chronotropic effects of high K^{+} than were the fresh atria.

14) Cold-storage for periods up to 72 hours produced no change in the spontaneous rate of rat atria despite the decreases in the resting potential of the non-pacemaker cells and reductions in the ATP content.

15) High K^{+} Krebs produced a similar change in the resting potential as did cold-storage but caused no change in the spontaneous rate. It was suggested that the pacemaker cells were more resistant to the effects of cold-storage than were the muscle cells.

CONTRIBUTIONS TO ORIGINAL RESEARCH

The following findings are original contributions to knowledge as far as the author is aware.

1) A systematic study has been performed to determine the cause of the reduction in the contractile force of atria stored in the cold; no such study is reported in the literature.

2) An investigation has been made to determine the reasons for the lack of change in the spontaneous rate of rat atria after prolonged cold-storage; no previous work of this type is reported in the literature.

3) It was found that cold-stored atria were unable to recover lost K^+ and extrude the gained Na^+ upon incubation at warm temperatures.

4) It was found that the contractile force of cold-stored atria was increased by low K^+ to a greater extent than that of the fresh atria.

5) It was found that cold-stored atria were more sensitive to high K^+ than were fresh atria.

6) The characteristics of potentials recorded at low temperatures (10° C) from fresh atria were elucidated.

7) It was found that the increase in ATP content after incubation with adenosine was greater in fresh atria than in cold-stored atria.

8) It was found that the increase in ATP content of cold-stored atria to values seen in the fresh atria did not restore the contractile force to normal values.

9) It was found that the increases in rate and contractile force to noradrenaline, ouabain or Ca^{++} were similar in fresh and cold-stored atria.

10) It was found that the spontaneous rate of rat atria did not change

after storage in the cold for 8-72 hours despite reductions in the resting membrane potential and ATP content.

11) It is suggested that the electrophysiological changes produced by cold-storage are in part responsible for the reduction in the contractile force.

12) In view of the positive correlation between the decrease in contractile force and the decrease in ATP content produced by cold-storage, it is suggested that the reduction in ATP content is, in part, responsible for the reduction in contractile force.

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