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The Influence of Adrenalectomy on the Concentration of High Energy Phosphate and of Electrolytes in Cardiac and Skeletal Muscle

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THE INFLUENCE OF ADRENALECTOMY ON THE CONCENTRATION OF HIGH ENERGY PHOSPHATE AND OF ELECTROLYTES IN CARDIAC AND SKELETAL MUSCLE

by

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APPROVAL SHEET

The thesis submitted by Allen L. Wright has been read and approved by three members of the faculty of the Graduate School.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

January 13, 1956
Date

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Signature of Adviser

LIFE

Allen L. Wright was born in Chicago, Illinois on April 15, 1922.

He graduated from Jean Baptiste Pointe DuSable High School in 1939 and from Woodrow Wilson Jr. College in 1942. In 1943, he received a Bachelor of Science degree from the Loyola University College of Arts and Science. He was graduated from the Loyola Medical School in 1947, and interned at Cook County Hospital in 1947 and 1948. Since that time he has engaged in the general practice of medicine.

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INTRODUCTION

The high energy phosphate compounds have been shown to play an important role in energy transfer mechanisms in the living cell. The chemical reactions of the anaerobic glycolysis as well as the Krebs cycle are directed at the synthesis of these compounds. These compounds are utilized in physical processes, such as muscle contraction, nerve conduction, glandular secretion, and sperm motility. A recent finding of great importance is the combination of adenosine triphosphate (ATP) with actomyosin, the contractile element of muscle. The reaction evolving from the association of ATP and muscle protein suggests that ATP is the substance directly utilized as the energy source in muscle contraction.

Control of the metabolism of the high energy compounds in the organism represents a profoundly important and complex problem. Possible influential mechanisms of control are the nervous system, hormones, parahormones, electrolytes. In contributing to the elucidation of this problem it was considered of value to determine whether the adrenal hormones in any way affect the level of high energy phosphate compounds in muscle tissue. Also, since these hormones influence numerous metabolic processes, it would be quite interesting to observe whether changes in high energy phosphate may precede these other metabolic changes.

The influence of the adrenal hormones on metabolism may be investigated in several ways. It is probably best to study the influence of adrenalectomy initially. If an effect is not observed, it would indicate that injection experiments with specific hormonal substances may not be fruitful. If a positive result is observed a determination of the particular hormone or hormones involved may be carried out systematically. For example, it would be necessary to delineate cortical from medullary influences. The purpose of this thesis, therefore, will be to investigate the influence of adrenalectomy upon the metabolism of high energy phosphates in skeletal and cardiac muscle in the albino rat. A second aim will be to study associated changes in the sodium and potassium ions.

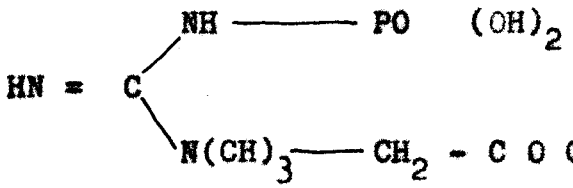
LITERATURE

If a hormone is observed to cause an alteration in the concentration of a given metabolite in the whole organism, it is possible for the primary change to take place at one of a number of different sites in the whole body. Starting from the external environment the transfer of the basic organic food-stuffs, which are the precusors to the metabolite involved across the intestinal epithelium needs to be considered. Furthermore, if the concentration of material is determined, the events which degrade the substance need to be considered. The review of the literature will, therefore, attempt to include briefly all of these

possibilities as they relate to phosphorus and its cellular metabolism in energy requirements of muscle tissue.

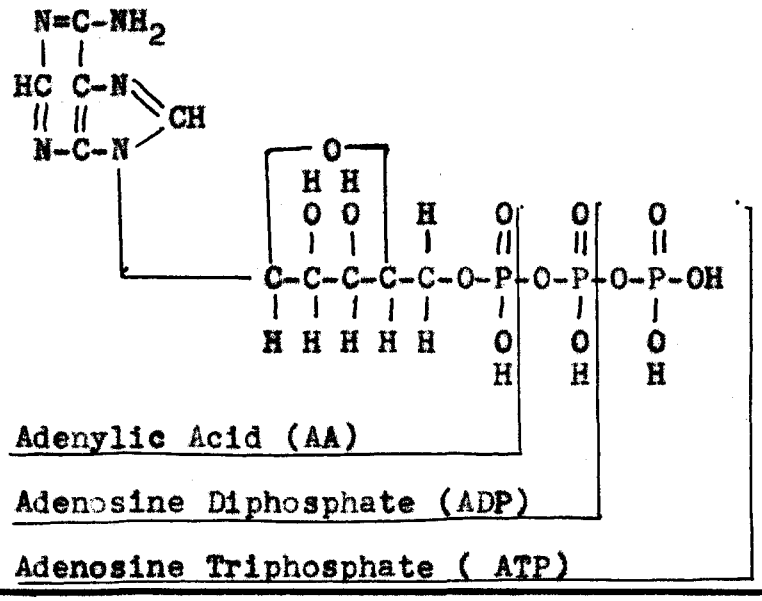
Role of Phosphorus in Muscle Metabolism

On of the earliest known facts of human biochemistry was the importance of phosphorus in the living organism. In 1893, W. B. M. Davidson declared that "if the biographies of the elements could be written, that of phosphorus would be the most interesting of all." Since no other element exerts such a profound influence on life phenomena this above quoted statement may be considered a masterful prophecy. Considerable information about many biological roles of phosphorus has been accumulated in the past fifty years. Harden's (1908) demonstration of the need for phosphates to produce fermentation of glucose by yeast extract and for the breakdown of sugar that takes place in active muscle extracts was an important early development in our knowledge of enzymatic degradation of carbohydrates. Many of the steps in the metabolism of carbohydrate by muscle have been worked out by Meyerhof, Parnas, Lohmann and Cori in their laboratories, but it was not until 1927 that the role of phosphate metabolism was definitely demonstrated by Eggleton and Eggleton in England and Fiske and Subbarrow in America. The Eggletons called their labile phosphate substance phosphocreatine (PC) and determined its structure to be:



They also demonstrated the breakdown and reformation of phosphocreatine in active muscle. It was not until Lundsgaard (1930) demonstrated that PC supplied energy for muscle contraction that the significance of this labile phosphate was recognized. He also demonstrated that the hexosephosphate present was formed by a transfer of a phosphate group from the phosphocreatine to a hexose molecule.

The breakdown of a phosphate compound, adenosinetriphosphate (ATP), after muscle activity was demonstrated by Lundsgaard in 1934. In 1935 Lohmann determined the chemical structure of this important phosphate and found it to be composed of adenine, ribose, pyrophosphate and phosphate. The structural formula of this adenylic acid compound is shown below:



Adenylic Acid (AA)

Adenosine Diphosphate (ADP)

Adenosine Triphosphate (ATP)

Theories of Muscle Metabolism

Lundsgaard (1938) proposed the hypothesis that the energy for muscle contraction came immediately from creatine phosphate breakdown and glycolysis served to reconstitute the ATP for metabolic energy both in glycolysis and synthesis of phosphocreatine. The dominant process was clearly established, however, when Engle-hardt (1942), produced myosin threads possessing elastic properties that reacted when suitably weighted to the application of ATP in the presence of various organic and mineral substances. ATP added to the muscle fibril preparation caused elongations that were reversible upon removing the ATP. The work of Kenneth Bailey (1942), demonstrated that the substance myosin was an enzyme and was so closely associated with the adenosinetriphospha-tase (ATPase) that we can regard them as identical. Myosin is the contractile protein found in the form of folded chains lying roughly parallel to the axis of the fibre. The reaction, according to Bailey, which is capable of supplying free energy for contraction is the breakdown by this ATP-ase of ATP to ADP and phosphate. Bailey also has shown the ATP-ase is specifically activated by calcium ion. Calcium is necessary for muscle contraction and magnesium cannot be substituted for calcium ion. The factor therefore, which limits the supply of energy to the contractile elements is the rate of ATP resynthesis.

Szent-Gyorgi (1950) demonstrated that the fibrils produced by Englehardt consisted of two proteins, actin and myosin, previously mentioned. He demonstrated that these threads formed from these two proteins may be made to vary their length by altering the concentration of ATP, potassium and magnesium. These findings seem to prove that ATP was the substance supplying energy for either contraction of muscle fibers or restoration of fibers to its resting state. Munch-Peterson (1953) reported that dephosphorylation of ATP to ADP is the metabolic process most intimately connected with muscle contraction, but it is uncertain when dephosphorylation occurs in the activity cycle. Muscles fixed in the rising phase of a twitch show an increase of 6-7% in quantity of ADP. This indicated that phosphorylation occurs at the initial phase of contraction in a magnitude proportional to heat production of a twitch. ATP according to Munch-Peterson showed no change in short tetanic contractions, but was broken down in tetanus of long duration. This would seem to cast doubt as to whether ATP is decomposed in muscular contraction. Fleckenstein, Janke et.al., (1954) reported on isolated muscularis recti of female Rana Temporaria that were frozen in liquid nitrogen during the rise in the tetanic contraction curve 1.0-1.2 seconds after the start of stimulation. These muscles were compared with paired resting muscles. Total acid-extractable phosphorus and

creatine phosphate were measured directly; ATP and ADP were chromatographed on paper and measured under ultraviolet light. At 0° the muscles show greater fusion of twitches and greater shortening at 2-5 stimuli per second than at 20°. The ATP and ADP content of muscle was unchanged by temperature (0-20°) or by or by stimulation. The tetanus tension was not related to the ATP content of the fiber. During stimulation at 20° there was a decrease in creatine phosphate but at 0° no decrease was observed. How to resolve the Janke findings in the light of the aforementioned findings of Englehardt and Szent-Gyorgi presents a problem.

Sources of Fuel for Metabolism of Muscle

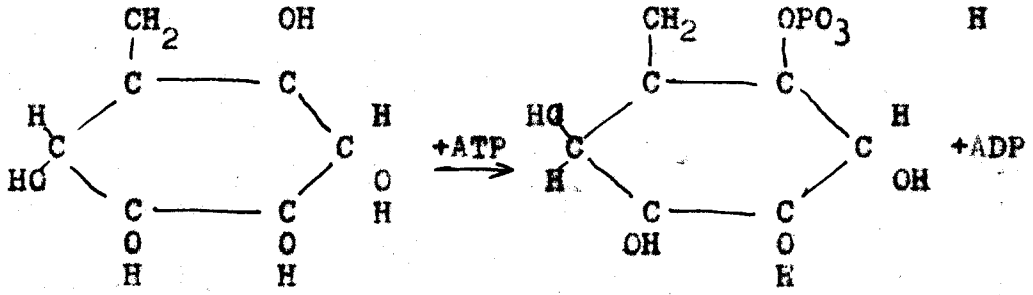
The most efficient fuel for muscular activity is carbohydrate, although energy may be derived from protein or fat substances. The carbohydrate of the body is largely in the form of glycogen in the skeletal, cardiac and smooth muscle, as well as the liver. These reserve depots allow for rapid mobilization of emergency fuel. Other carbohydrate forms are present in minor amounts and are not primarily used as fuel such as galactolipins of nervous tissue (Peters, 1931), the pentoses associated with nucleoprotein (Sevag, 1941) and the glucose in the glycoproteins such as mucin in salivary secretion. Meyerhof's (1941) work demonstrated the presence of several intermediate metabolites of glucose that are caught in transit as the glucose is utilized.

The primary importance of carbohydrates were evidenced by the increase in efficiency of animals on carbohydrate diets, the potentiation of muscular effort after carbohydrate ingestion, the hypoglycemia produced by muscular exercise and the production of lactate during severe exercise (Gemmill, 1942).

Entrance of Phosphate into Carbohydrate Metabolism

It, therefore, became necessary to establish the site of entrance of inorganic phosphate into the glycolytic cycle. Cori, et.al., (1934) indicated that hexosemonophosphate was produced after esterification of carbohydrates with inorganic phosphate. The Cori experiment demonstrated that injections of epinephrine into anaerobic muscle produced a decrease in inorganic phosphate which was due to esterifications. There was no significant change in PC or ATP in these investigations.

The work of Kalckar (1939) illustrated that the first step in the series of reactions by which sugar enters the metabolic cycle of the cell is the addition of phosphate to the sixth carbon atom of the glucose molecule. The enzyme, hexokinase, responsible for this probably activates the glucose molecule in such a way that it can receive phosphate from a suitable source. The phosphate donor and also the coenzyme of this phosphorylation reaction in this case is ATP. The chemical notation of the reaction may be represented as follows:



The amount of ATP present in the cell at any given time is small so ADP and AA must be continuously converted to ATP so that it can act as a phosphate donor. M.P. Hele, (1953) reported that different sugars are phosphorylated at varying rates. The hexokinase-reaction reveals different patterns according to the effect of changes in sugar concentration on the phosphorylation rates. When the sugar concentration is not the limiting factor, phosphorylation varies with the concentration of ATP. Phosphorylation was different for glucose and fructose probably because of existence of different hexokinases. The rate of hexokinase reaction can account for the phosphorylation of at least 50% (probably 70-100%) of the carbohydrate consumed by a normal 150 gram rat during 24 hours.

Verzar and Luthy (1954) measured ATP-ase and hexokinase in the epithelium of the small intestine in normal and adrenalectomized rat. In adrenalectomized rats both ATP-ase and alkaline phosphatase activities of the intestinal mucosa decrease considerably and both enzyme activities can be restored within a short time by treatment with corticosteroids. However, there

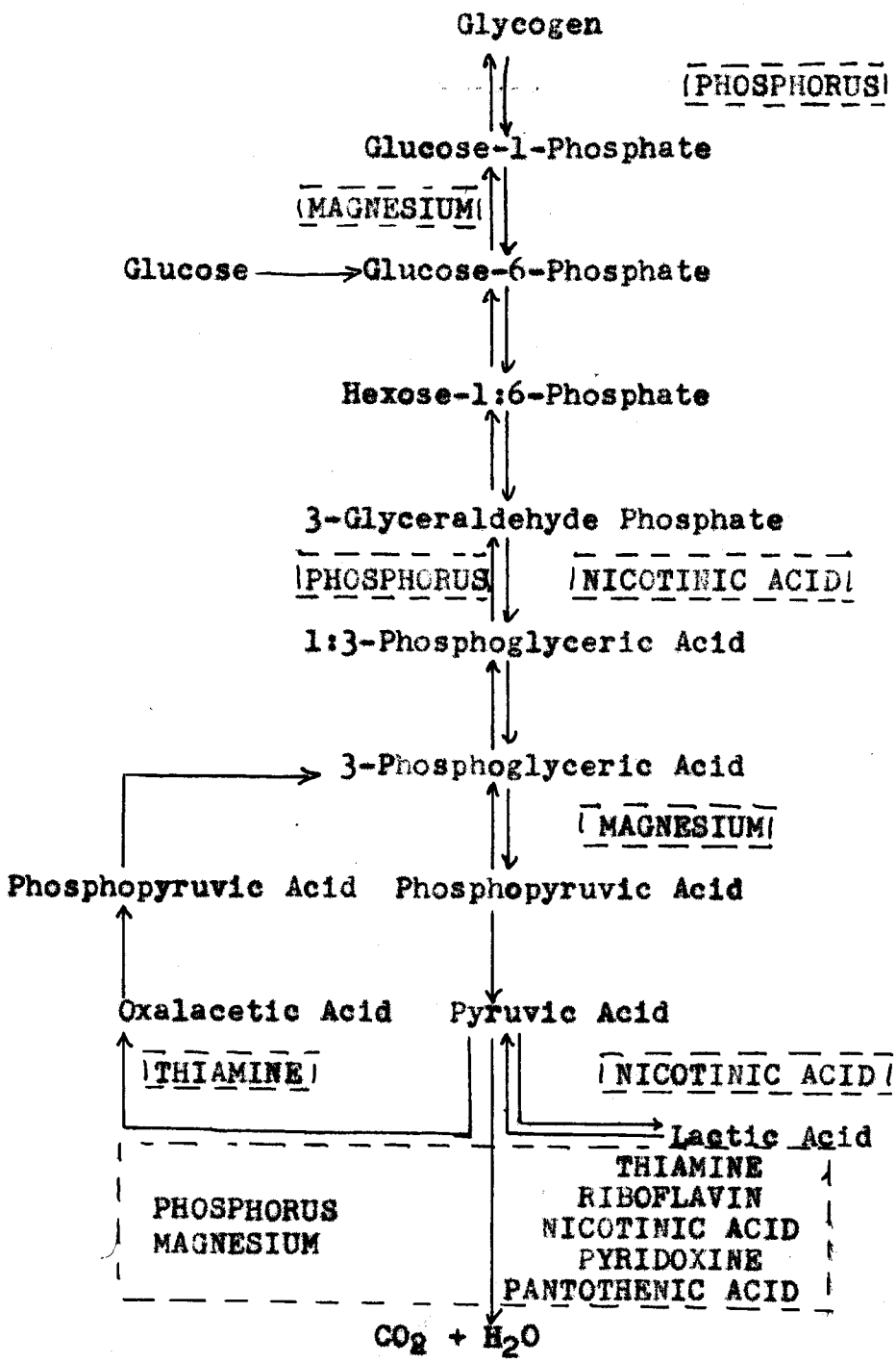
was no difference found in hexokinase activity following adrenalectomy.

Factors Influencing Fuel Utilization

Adequate carbohydrate intake will not insure its availability for fuel utilization because carbohydrate metabolism is dependent upon specific co-factors and enzymes. An intregal part in carbohydrate metabolism is played by vitamin B complex, thiamine, phosphorus, magnesium and nicotinic acid. All the factors required for a particular reaction are necessary for synthesis and utilization of metabolites. The formation of carbon dioxide and water from carbohydrate metabolism is the basic reaction demonstrated in these reactions which supply energy to support the various functions of living cells. In this oxidative process it was demonstrated that carborn dioxide largely arose by splitting off of carboxyl groups from lower metabolic intermediates. Until recently, carbon dioxide was regarded as purely an end product of metabolism. The main reaction in animals involving carbon dioxide with biotin playing a catalytic role is a carboxylation, or fixing of the carbon dioxide as the carboxyl group of dicarboxylic acid. This refutes a notion that carbon dioxide is completely a waste product of metabolism.

The following diagram (Soskin, 1952) illustrates the points

of action of vitamins and minerals in carbohydrate metabolism:



Factors that Regulate Metabolism in the Living Cell

Regulation of metabolism is an essential characteristic of living cells and in all probability no single effect regulates total metabolic activity. Enzyme systems are numerous and they demand the participation of hormones for regulation. Hormone activity will require extensive study to enable biochemist and physiologist to completely understand liberation and transfer of energy derived from carbohydrate metabolism. All hormones that are currently known can be shown to have some influence on carbohydrate metabolism. These hormonal substances appear to influence metabolism without interaction with the substrate. Among the hormones chiefly concerned with metabolism, we recognize insulin to be a protein as are also the thyroid gland and anterior pituitary hormones. The posterior pituitary gland secretions are polypeptide in nature. While the hormone of adrenal medulla is a protein derivative, the adrenocortical and gonadal secretions are steroids.

It is beyond the scope of this discussion to give a complete description of allthe mechanisms involved in hormone action on metabolism,so only those known physiologic effects can be presented to illustrate the manner of hormonal metabolic control. The oxygen consumption of an animal is influenced by the thyroid hormone concentration in the blood. The rate of glucose phos-

phorylation is influenced by insulin (Soskin and Levine, 1940). Cori in <u>in vitro</u> experiments has shown that insulin is an inhibitor of the adrenal cortical or anterior pituitary inhibition of hexokinase in the conversion of glucose to glucose-6-phosphate in the presence of adenosine triphosphate. This has not been completely confirmed but is an important possibility. Regulation of carbohydrate storage is influenced by both the pituitary gland and the adrenal cortex. The findings indicate that the anterior pituitary regulates the rate of carbohydrate utilization and the adrenal cortex regulates the rate of gluconeogenesis. The influence of the adrenal gland on carbohydrate metabolism was for a long time attributed to the influence of the adrenal medulla. Porges, in 1909, described hypoglycemia in Addison's Disease, which was then recognized as a cortex disorder. He also demonstrated low carbohydrate levels by bilateral adrenalectomy in dogs. It was not until 1930, that any further advance in knowledge of carbohydrate functions of the adrenal cortex was obtained by Britton, who emphasized the fact that death in adrenalectomized animals was associated with hypoglycemia and low glycogen levels in addition to the effect on serum sodium and potassium levels. The inter-relationship of mineral and carbohydrate effects of the adrenals then became apparent.

Extractions of the cortical hormones has made it evident

that the adrenal gland cannot be regarded as containing a single active substance. Potent steroids separated from the extracts by Reichstein (1943) and by Kendell (1942) made possible accumulation of data on each aspect of function of the adrenal steroids independent of each other. Hartman (1940) described two adrenal cortical factors which have separated actions, yet they potentiate each other. One maintains the sodium levels of tissues and the other factor preserves life, appetite, and weight and normal behavior.

In view of the effect of glycogen depletion that is known to occur in adrenalectomized animals, it was considered important to determine whether there was an associated decrease in the phosphate compounds that supply energy for skeletal and cardiac muscle activity. A search of the literature failed to reveal any previous attempts at specifically determining quantitative changes in the inorganic and high energy phosphate compounds following adrenalectomy. The effects of hypophysectomy, castration and testosterone therapy on certain phosphorus fractions in skeletal muscle was determined by Kare (1953). The concentration of IP was decreased in some skeletal muscle following hypophysectomy and gonadectomy. The IP was increased with testosterone therapy. The concentration of the acid soluble organic fractions was indifferent to alterations in the hormone environment.

Harrison and Darrow (1938) originally reported the changes in concentrations of sodium and potassium ions after adrenalectomy, thereby, indicating the influence of these hormones. A positive influence of loss of adrenocortical hormones on the quantity of phosphate must first be established before any attempt should be made to systematically evaluate the relationship of particular cortical hormones on HEP. This constitutes the basis for the investigation herein reported.

METHODS AND MATERIAL

Procedure

Albino rats were obtained from the Hormone Assay Laboratories, Chicago. The animals were housed in a constant temperature room and were given food and water at libitum. No salt was added to this food supply since the main interest was concerned with the development of a progressive insufficiency. In a usual series of studies, adrenalectomized rats were sacrificed daily, starting from the first post adrenalectomy day. No animals were observed to die until about the sixth or seventh day. Only a few animals were carried this length of time, so that the incidence of deaths cannot be accurately assessed. Occasionally, an adrenalectomized rat seemed to survive for at least two weeks without significant change in weight. In these instances, it can only be concluded that adrenalectomy was incomplete or that aberrant tissues were present.

The animals were initially sacrificed by stunning them with a blunt instrument, but this procedure was abandoned because it was believed that the massive sympathetic responses prompted by the trauma of the method might influence the chemical results. Therefore, the animals were anesthetized by using Pentobarbital Sodium (Abbott) and urethane. The dosage was 20 mg/kg for each drug.

The thoracic cage was opened immediately upon procuring com-

plete relaxation of the animal and the heart was removed by severing the great vessels at the base of the heart. The entire heart was then quickly frozen in an ether-carbon dioxide snow mixture. The gastrocnemius muscle was then removed as rapidly as possible and also quickly frozen. The time taken for the removal of the heart was less than two seconds from the time of the opening of the thoracic cage. The gastrocnemius muscle was frozen approximately six seconds later.

Preparation of TCA Extract

A trichloroacetic acid (TCA) extract was prepared for the analysis of phosphate in the following manner. The weight of cold tare was obtained and the pulverized frozen tissue was added to the cold tare. The difference between the weights gave the weight of the tissue. The pulverized tissue was ground in a precooled porcelain mortar with a small portion of twelve volumes of cold five per cent trichloroacetic acid. The ground tissue was then extracted with the remainder of the 5% TCA for 15 minutes. Gentle stirring assured adequate contact of the 5% TCA with all portions of the pulverized tissue. The extract was then filtered into a cold graduated cylinder using #41 Whatman Filter paper. One drop of phenolphthalein indicator was added to the filtrate and the extract was neutralized with six normal sodium hydroxide to the appearance of a pink color in the extract.

Analysis of Phosphate Compounds

The method used by Wollenberger (1947) was followed for the estimation of the inorganic phosphate, phosphocreatine and adenosine polyphosphate concentrations. The method is essentially a composite of the inorganic phosphate determination by Fiske and Subbarrow (1929), phosphocreatine determination by Fiske and Subbarrow (1929) and the adenosine triphosphate determination by Lohmann, (1934). The latter two compounds are determined by difference.

Flame Photometer Analysis for Sodium and Potassium

Approximately one gram samples were taken from the tissues and weighed, care being taken to remove vessels, valves, fat, etc. The weights were recorded and the samples placed into marked digestion tubes. Approximately 4 cc of concentrated nitric acid were added to each tube. One clean glass bead was added to each tube to prevent bumping. The tubes were digested on the digestion rack until only 1 cc or less of the acid remained. Whereupon the contents were quantitively transferred to 100 cc volumetric flasks. One cc of two per cent lithium nitrate solution was pipetted into each flask, and the flask contents brought to volume with distilled water. Sodium and potassium concentration was then determined by the internal standard method, using the Perkin Elmer Flame Photometer.

RESULTS

The information presented in the following tables and graphs was obtained from ventricles and gastrocnemius muscles of fourteen normal, eight sham adrenalectomized and thirty-four adrenalectomized albino rats.

The Influence of Adrenalectomy on Inorganic and High Energy Phosphate in Rat Ventricle and Gastrocnemius

As indicated in Table I the concentration value of high energy and inorganic phosphate of the ventricle of normal albino rats show considerable variation. This is perhaps mainly due to the fact that we are dealing with extremely labile compounds in a very active tissue. Compared to the phosphate values for dog ventricles, which have been reported by Wollenberger, the values in Table I are higher by 6.3 mg % for inorganic phosphate (IP); lower by about 12.9 mg % for phosphocreatine, (PC); and lower by 15.3 mg % for adenosine polyphosphate, (APP). The phosphocreatine content of mammalian cardiac muscle was reported in 1950 by Fawaz and Hawa. Approximate average resting value for PC content of ventricles removed under pentobarbital anesthesia with artificial respiration were in mg % fresh tissue: dog, 19; cat, 15; rabbit, 14; and rat, 9. Even lower values are reported in the literature because the substance is decomposed rapidly.

The appearance of negative values in the column of PC analysis is a result of the analytical method. Phosphocreatine con-

centration is calculated as the difference between the apparent inorganic phosphorus (including phosphocreatine) and the true inorganic phosphorus, i.e., the difference between phosphorus present after thirty minute molybdic acid hydrolysis at room temperature and the phosphorus which is precipitated by calcium at PH 8.8. It is believed that a high "true" inorganic phosphorus value is attained since some APP is co-precipitated in the calcium procedure,(Kerr, 1935). The amount co-precipitated is not considered to be an extremely large percentage of the APP but is evidently a large fraction of the PC.

The gastrocnemius muscle contains a higher quantity of phosphorus than the ventricles. The primary difference being the relatively greater amount of high energy phosphorus that is present. This may be related to the relative inactivity of this tissue.

It was considered important in this investigation to evaluate the effect of the surgical procedure upon the phosphorus content so that sham operated rats were included in the studies. Kramer in (1935) reported that after sham adrenalectomy capillary resistance changed in a typical way, showing a pattern with 4 successive phases: (1) a more or less pronounced increase in the first days (2) a critical drop at the end of the first and the course of the second week (3) a state of pathologically low resis-

tance lasting about 2 weeks (4) a period of recuperation. The entire phenomena, capillary crisis, takes about one month. After adrenalectomy only the second and third stages occurred and resistance stayed at low levels indefinitely unless cortisone administration was instituted. This quickly restored capillary resistance. Inspection of Table 2 shows that 3,4,5, and 6 days after the sham adrenalectomy there was no variation of any significant degree from the normal, as far as the ventricle results are concerned. Any variation which may be seen in the hearts of adrenalectomized rats is then not directly attributable to the surgery itself. On the other hand, phosphate changes took place in the gastrocnemius muscle. This indicates that some caution is required in the interpretation of these results.

As noted in the previous table a factor of time has been introduced into this project. It was necessary to consider this factor because of the possibility that retention of certain cortical hormones may occur after adrenalectomy. Table 3 is composed of values obtained in the time course study of the concentration of phosphate compounds in the rat ventricle. When compared to the normal value of 35 mg %, it would appear that more IP was present 1 day after the operation and less was present at the sixth post-operative day. The average values of inorganic phosphorus then appear to decrease from a value of about 42 mg % to

about 28 mg % from the first to the sixth post-operative day.

Phosphocreatine seems to be slightly less than normal on the first post-operative day and increases to a value above the figure for the normal rat. The change is so small and the standard error is relatively large, that it seems best to consider that no definite change has been observed in the present study. Since the PC content values are low, it may be considered that PC remains close to zero in all the experiments.

The adenosine polyphosphate content is apparently lower than normal during the first five days post-operatively, but on the sixth day the values are very close to the normal value. It may be suggested that the animals which survive for as long as six days, may not have been completely adrenalectomized. The data for the ventricles may be summarized by noting that adenosine polyphosphate decreases insignificantly, inorganic phosphate first increases and then decreases, and phosphocreatine which is close to zero concentration normally shows little significant change following the removal of the adrenals.

In the gastrocnemius muscle, inorganic phosphate is increased above the normal following adrenalectomy, reaching a peak on the fourth post-operative day as shown in Table 4. Phosphocreatine concentration is lowered by the adrenalectomy, but must be considered due primarily to the surgery since the values are about the same as the sham operated rat. The adenosine poly-

phosphate content seem to drop slightly below the normal level. Here, too, the decreased values appear to be attributed to causes other than the loss of the adrenals, per se. In general, there appears to be a tendency for high energy phosphate to remain unchanged from the sham value and inorganic phosphate to increase in the gastrocnemius muscle after adrenalectomy. In all three cases, the values on the fifth and the sixth post-operative day appear to return towards normal values. It is interesting to note that the IP in heart muscle tends to decrease after the first post-operative day, while the IP values in skeletal muscle rises with each successive post-operative day. This may be related to the fact that the more active tissue may tend to lose phosphate more readily from the intracellular space.

The Influence of Adrenalectomy on Potassium and Sodium Content in Rat Gastrocnemius

The results of the investigation of sodium and potassium ion content in the normal, sham operated and adrenalectomized albino rat in this study are limited to gastrocnemius muscle values due to the fact that the entire ventricular tissue was utilized for study of the phosphate content. Approximately one gram of tissue was necessary to make accurate studies of ion content and the total rat ventricle weight seldom exceeded 0.6 gram in the young rats used in this study.

The average normal values as shown in Table 5 was 101 meq/L

for K and 29 meq/L for Na. Sham values illustrated by Table 6 are practically within one millequivalent per liter of the normal figures and thus indicated no influence of operative procedures on electrolyte content.

A consistent influence of adrenalectomy in our hand is evidenced by the time course study of the values of the two electrolytes. Table 7 reveals a gradual depletion of sodium ion and comparable increase in the potassium content of the cell. The change in potassium content appears to be the most marked after the fourth day when it is noted that a large upswing seems to take place.

In order to allow a comparison of the cation and phosphate changes, the data has been summarized graphically in Figure 1. Due to the large spread in the phosphate figures precise changes cannot be indicated. It is evident however, that the absence of the adrenal glands has caused marked metabolic alterations. The primary changes seem to be an increase in potassium and a loss in sodium in the gastrocnemius muscle, but there is no significant change in the high energy phosphate content in cardiac tissue.

Statistical Analysis of HEP, Na and K variation in Ventricle and Gastrocnemius

The result of the high energy phosphate findings were subjected to statistical analysis to determine whether the variations noted were significant in certain selected cases. The

comparison of the sham and the adrenalectomized animals indicated that the two groups were not significantly different from each other.

The ventricular IP, and the ATP on the 4th, 5th, and 6th post-operative days show no significant variations from the normal. Gastrocnemius PC 4 days post-operatively was statistically insignificant, but IP and ATP had P values less than 0.01. However, compared to the sham values, these changes were not significant. All the variations in Na and K ions are statistically significant.

DISCUSSION

This investigation was instituted to determine the influence of adrenalectomy upon the content of high energy phosphate compounds in skeletal and cardiac muscle. This study has demonstrated that adrenalectomy results in no significant change in high energy and inorganic phosphate content in cardiac muscle and an increase in inorganic phosphate in gastrocnemius muscles. In addition, an increase in potassium ion concentration and a decrease in sodium ion concentration were observed in skeletal muscle, as reported initially by Harrison and Darrow (1938).

Influence of adrenalectomy on sodium and potassium exchange in muscle has been reported by Fluckiger and F. Verzar, using radioactive ions. Na and K permeability of the normal and adrenalectomized rat diaphragm was determined after immersion for 1 hour in Ringer solution containing Na^{24} and K^{42}. The muscle of adrenalectomized animals took up less Na^{24} and more K^{42} than that of normal rats. Glucose reduced the Na^{24} uptake in the muscles for normal and adrenalectomized animals, but did not influence significantly the uptake of K^{42}. The Na/K ratio in meq is 3 in the muscle of normal animals and 2.4 with glucose. These findings are in accord with changes in content observed by us and by Harrison and Darrow.

The decrease in sodium ion concentration in the gastrocnemius muscle should perhaps have early consideration in the discussion

of the results. Sodium depletion is a well known concomitant of adrenal deficiency. Since sodium is the major anion of extracellular fluid, it may be pointed out that the volume of extracellular fluid may be depleted under these conditions. It, therefore, appears to be a fair assumption that the concentration of tissue substances is increased in terms of wet weight of tissue since less water is available from the extracellular space. The change in tissue potassium may not be an intracellular change but an indirect result of extracellular fluid loss. Unfortunately, the expression of these results in other terms, such as, milligrams of nitrogen, was not carried out.

If this assumption is drawn for both tissues it would appear that in the ventricle of the adrenalectomized animal, high energy phosphate and inorganic phosphate would have been higher than the sham values. Since the recorded values were not higher, this appears to support an increased utilization of these compounds. In the case of the gastrocnemius muscle, the tendency appears to be the same. Inorganic phosphate does increase and the high energy phosphate content remains essentially unchanged.

To explain a possible decrease in high energy phosphate content, two general possibilities need to be considered, viz., a decrease in the rate of formation or an increase in the rate of utilization. From information which is available in the literature, it seems that the major impairment to carbohydrate meta-

bolism is a decrease in gluconeogenesis (Soskin and Levine,1952). Despite this change, the adrenalectomized animal does not exhibit signs of hypoglycemia unless fasted. Since the animals in the present study were fed, it is quite likely that these animals had sufficient substrate as far as availability in the extracellular environment is concerned. With little information available with regard to a specific hormonal influence on enzyme systems, it is difficult to suggest that a specific lesion may exist in the tricarboxylic or glycolytic cycles. The observation that the oxidation of labeled glucose to carbon dioxide is the same in adrenalectomized rabbits and normal rabbits given C_{11} oxysteriods (Wick, Drury, and Mackay, 1951) is presumptive evidence that there is not drastic change in cellular oxidation of carbohydrate.

From a gross view point, there appears to be no general indication that the rate of utilization of high energy phosphate is increased in the adrenalectomized animal. The adrenal deficient animal shows decreased activity, appears listless and is easily fatigued. Thus, there does not appear to be an increase in energy demand. It is quite possible, however, that the degradation of high energy phosphate into waste heat energy may be increased by certain enzymes, i.e., adenosine triphosphatase. There does not, however, appear to be any direct evidence indicating that the rate of utilization of adenosine tri-

phosphate is increased under these conditions.

The fatigability of the skeletal muscle of the adrenalectomized animal which was first explained on the basis of hypoglycemia (Ingle and Lukens, 1941) has been recently reviewed and shown to be due to a drop in blood pressure (Goldstein, Ramey and Levine, 1950). The loss of sodium results in a decrease in extracellular fluid causing a decrease in blood volumn and hypotension. This may mean that the high energy phosphate content declines simply as the circulation changes. In other words, there may be a lack of oxygen supply as circulation decreases, so that the low rate of oxidative processes may be responsible for the decreased high energy phosphate content. It is not impossible that these conditions may increase adenosine triphosphatase activity as well. This appears to be rather unlikely since the change in high energy phosphate content is not a terminal change, i. e. after about the second post adrenalectomy day the chemical changes appear to have taken place.

The increased cell potassium which is observed in the untreated adrenalectomized animal may also have a possible influence on the high energy phosphate content. Potassium is required in many enzyme systems. However, it is difficult to single out any particular step as being influenced by high concentration of potassium, if this change is considered to be real.

The increased extracellular potassium known to be present may have some influence as well. Heart functions stop in the presence of high extracellular potassium. Experiments on the isolated rabbit heart have shown no significant influence of high extracellular potassium on high energy phosphate content (Cowan, 1954).

The finding of no significant alteration in high energy phosphate content after adrenalectomy seems to indicate that the metabolism of these compounds may not be directly under control of the cortical hormones as is the case with the sodium and potassium ion. At the present stage it seems that further work is necessary in order to clarify the relationship between cell potassium and high energy phosphate metabolism.

SUMMARY

1. Normal rat cardiac and skeletal muscle values for inorganic phosphate, phosphocreatine and adenosine polyphosphate were determined and compared with these in sham operated and adrenalectomized rats in a time course study. Normal sodium and potassium values for these tissues were also obtained and compared with the changes occurring after adrenalectomy.

2. The results in the ventricle indicate that the inorganic phosphate, phosphocreatine and adenosine triphosphate values did not vary significantly from the normal after sham adrenalectomy.

3. With respect to high energy phosphate in the gastrocnemius muscle, the changes were no greater than the values in the sham operated rat. Inorganic phosphate, however, increased in the skeletal muscle. In addition, there was a definite increase in potassium ion in the muscle after adrenalectomy and a comparable decrease in sodium content.

4. The explanation of these findings is not simple and several possibilities were discussed. The relationship between elevated cell potassium and energy-rich phosphate content appears to deserve further study.

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TABLE I

Concentration (in mg %) of High Energy and Inorganic Phosphate in Ventricle and Gastrocnemius Muscle of Normal Rat

Exp. No.	Ventricle IP	PC	APP	Gastrocnemius IP	PC	APP
8	34.4	-1.2	17.2	62.2	-1.0	41.8
10	35.2	-3.8	15.8	71.8	7.2	49.5
12	36.8	-3.8	31.1	27.4	49.2	49.7
13	30.0	-3.4	12.8	28.0	34.8	37.2
14	35.0	-3.4	12.8	33.5	49.7	23.1
15	36.2	-1.4	15.8	28.2	46.0	26.7
16	19.5	6.5	28.0	26.5	43.8	34.7
17	39.8	-2.7	25.4	27.7	40.7	37.2
19	41.9	1.7	9.6	36.4	26.3	38.6
20	38.3	0	11.9	59.0	7.4	19.4
28	44.2	-1.5	15.5	33.0	24.3	30.5
34	26.7	3.3	34.6	90.6	34.2	47.4
43	37.8	0.0	17.5	48.6	15.6	36.6
47	27.2	3.6	26.8	60.6	-0.1	31.2
Mean	34.5	1.4	19.9	38.1	34.8	35.9
S.E.of Mean *	1.8	±1.0	±2.1	±5.3	±5.2	±2.5
Range	19.5 to 44.2	(-3.8) to6.5	9.6 to 34.6	26.5to 90.6	(-1.0) to90.6	19.4 to 49.7

TABLE 2

Concentration (in mg %) of High Energy and Inorganic Phosphate in Ventricle and Gastrocnemius Muscle of Sham Operated Rat

Exp. No.	P.O. Day	Ventricle IP	Ventricle PC	Ventricle APP	Gastrocnemius IP	Gastrocnemius PC	Gastrocnemius APP
7	3	33.0	-1.9	17.7	24.6	45.3	34.1
46	3	34.2	-3.8	24.6	37.0	5.4	37.2
10	4	35.2	-1.0	14.0	22.6	45.4	31.8
21	4	35.5	-0.7	9.1	60.0	-5.2	35.4
36	4	27.2	1.4	26.9	33.0	37.8	42.0
41	5	40.0	-1.3	15.1	73.2	-3.0	11.4
51	5	28.2	3.8	15.0	62.4	22.8	39.6
42	6	22.4	4.1	26.8	75.0	1.2	30.6
Mean		31.9	1.4	18.7	48.4	22.2	32.8
S.E. of Mean		±1.8	±1.0	±2.3	±7.6	±7.8	±3.3
Range.		22.4 to 40.0	(3.8) to 4.1	9.1 to 26.9	22.6 to 75.0	(-5.2) to 45.4	11.4 to 42.0

TABLE 3

Concentration (in mg %) of High Energy and Inorganic Phosphate in Ventricle of the Adrenalectomized Rat

Exp.	P. O. Day	IP	PC	APP
17	1	29.4	0	18.0
18	1	44.6	-1.6	11.1
27	1	45.2	-1.7	11.3
35	1	46.5	-0.2	12.3
Mean		41.6	-0.7	13.1
S.E. of Mean		±4.0	±0.6	±1.8
9	2	43.8	0.3	6.6
18	2	32.0	-2.1	16.8
20	2	45.6	6.4	3.6
21	2	44.2	1.7	8.9
45	2	23.1	4.7	28.3
Mean		37.7	2.7	12.8
S.E. of Mean		±4.4	±1.5	±4.4
9	3	28.9	0	22.2
11	3	35.2	4.8	4.1
19	3	32.4	3.3	12.0
22	3	44.4	2.8	10.5
23	3	33.1	2.1	12.8
29	3	46.2	2.9	7.4
30	3	40.4	1.0	9.8
31	3	21.8	3.0	32.3

(continued)

TABLE 3(cont.)

Concentration (in mg %)of High Energy and Inorganic Phosphate in Ventricle of the Adrenalectomized Rat

Exp.	P.O. Day	IP	PC	APP
37	3	45.8	0.9	9.6
49	3	29.1	1.9	23.3
40	3	26.9	0.9	24.9
Mean		34.9	2.4	15.4
S.E. of Mean		±2.9	±0.5	±2.6
7	4	35.0	0.2	9.2
12	4	27.8	-2.2	17.8
22	4	36.0	0.9	6.5
24	4	22.6	6.2	26.2
33	4	36.6	0.9	6.5
39	4	51.2	-0.8	2.9
40	4	31.6	-0.6	24.1
52	4	22.0	4.8	29.8
53	4	36.8	1.8	10.6
Mean		33.3	2.0	16.5
S.E. of Mean		±3.0	±1.0	±3.1
P		0.9		0.6
26	5	19.5	5.7	21.2
44	5	41.3	3.1	8.5
15	6	32.0	-6.4	21.6
54	6	29.5	7.6	21.1
55	6	17.8	4.0	33.7
Mean		28.0	3.5	21.2
S.E. of Mean		±4.3	±2.2	±2.8
P		0.5		

TABLE 4

Concentration (in mg %) of High Energy and Inorganic Phosphate in Gastrocnemius Muscle of the Adrenalectomized Rat

Exp.	P.O. Day	IP	PC	APP
1a	1	36.2	29.0	41.0
2	1	53.0	11.5	40.5
17	1	100	2.0	56.0
18	1	23.6	35.2	24.8
27	1	37.5	15.0	32.5
35	1	40.8	28.2	43.2
Mean		48.2	20.2	39.6
S. E. of Mean		±11.6	±5.1	±4.3
1b	2	76.4	0.2	28.4
4	2	29.4	41.4	29.0
5	2	23.6	46.2	38.2
9	2	99.3	16.7	22.0
18	2	77.1	21.9	35.8
20	2	45.1	8.8	25.5
21	2	40.0	37.4	25.7
45	2	56.4	4.5	39.2
Mean		59.7	22.3	30.5
S. E. of Mean		±2.9	±6.2	±2.3
9	3	89.6	54.4	27.0
11	3	73.4	-2.6	34.2
19	3	58.6	8.6	29.2

(continued)

TABLE 4 (cont.)

Concentration (in mg %) of High Energy and Inorganic Phosphate in Gastrocnemius Muscle of the Adrenalectomized Rat

Exp.	P.O. Day	IP	PC	APP
22	3	139.1	15.5	27.7
23	3	107.2	31.1	33.5
29	3	46.2	2.9	7.4
30	3	40.4	1.0	9.8
31	3	21.8	3.0	32.2
37	3	99.0	34.2	35.4
38	3	30.6	43.2	42.0
49	3	100.2	11.4	36.6
50	3	73.2	1.2	32.4
Mean		73.3	18.6	28.9
S. E. of Mean		±9.7	±5.8	± 2.9
P				0.01
12	4	89.6	1.9	16.5
22	4	62.4	2.8	30.8
24	4	119.2	19.5	35.8
32	4	102.7	34.6	6.0
33	4	108.5	33.6	38.4
39	4	88.8	-3.0	32.4
40	4	87.6	4.2	46.8
52	4	43.2	28.2	35.4
53	4	47.4	28.2	39.0
Mean		48.6	28.0	31.6

(continued)

TABLE 4 (cont.)

Concentration (in mg %) of High Energy and Inorganic Phosphate in Gastrocnemius Muscle of the Adrenalectomized Rat

Exp.	P. O. Day	IP	PC	APP
S. E. of Mean		±8.9	±4.4	±4.1
P		0.02	0.7	
14	5	12.6	16.0	30.8
26	5	85.8	41.0	27.2
44	5	47.4	27.0	36.0
Mean		48.6	28.0	31.6
S. E. of Mean		±20.0	± 7.9	±2.3
15	6	52.0	22.2	24.1
54	6	50.4	22.5	39.0
55	6	38.4	35.4	41.4
Mean		46.9	26.7	34.8
S. E. of Mean		±4.2	±4.3	±5.4

TABLE 5

Concentration (in Meq/L) of Potassium and Sodium in the Gastrocnemius Muscle of the Normal Rat

Exp. No.	Na	K
8	28	99
10	34	95
20	24	87
3	35	94
6	30	99
14	30	104
15	31	110
16	30	102
17	30	109
19	36	96
28	29	96
34	27	97
43	30	106
47	30	99
48	28	102
Mean	31	101
S.E. of Mean	± 1	± 2

TABLE 6

Concentration (in Meq/L) of Potassium and Sodium in the Gastrocnemius Muscle of The Sham Operated Rat

Exp. No.	P.O. Day	Na	K
46	2	34	102
7	3	32	93
36	3	25	99
10	4	28	96
21	4	24	89
41	4	32	107
13	5	26	97
42	6	29	105
Mean		30	100
S. E. of Mean		± 1	±2

TABLE 7

Concentration (in Meq/L) of Potassium and Sodium in The Gastrocnemius Muscle of the Adrenalectomized Rat

Exp.	P. O. Day	Na	K
2	1	23	112
17	1	24	101
18	1	35	96
27	1	30	94
35	1	25	104
Mean		28	102
S. E. of Mean		± 2	± 3
4	2	22	102
5	2	23	105
9	2	20	111
20	2	23	86
21	2	24	89
45	2	21	113
Mean		23	99
S. E. of Mean		± 0	± 4
11	3	22	110
19	3	35	96
22	3	36	96
23	3	19	103
29	3	23	100
30	3	22	101

(continued)

TABLE 7 (cont.)

Concentration (in Meq/L) of Potassium and Sodium in The Gastrocnemius Muscle of the Adrenalectomized Rat

Exp.	P. O. Day	Na	K
31	3	23	105
37	3	21	111
38	3	21	104
49	3	22	110
50	3	22	112
Mean		23	105
S. E. of Mean		± 0	± 2
7	4	25	122
12	4	14	111
22	4	21	97
24	4	19	105
32	4	21	104
33	4	21	111
39	4	19	116
40	4	20	115
52	4	20	115
53	4	22	112
Mean		20	111
S. E. of Mean		± 0	± 2
P		0.01	0.01

(continued)

TABLE 7(cont.)

Concentration (in Meq/L) of Potassium and Sodium in the Gastrocnemius Muscle of the Adrenalectomized Rat

Exp.	P. O. Day	Na	Ka
14	5	17	107
26	5	21	107
44	5	22	127
15	6	23	102
54	6	19	120
55	6	20	121
Mean		21	119
S. E. of Mean		±1	±4
P		0.01	0.02

FIGURE I

Influence of Adrenalectomy on the Concentration of Phosphates and Cations in Skeletal and Cardiac Muscle