Nucleotide Metabolism in Cardiac Activity

I. Methods and Initial Observations

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The application of ion-exchange chromatography for the determination of adenosine nucleotides in mammalian hearts is described. It is found that 1 gram ventricular muscle contains nearly $5 \times 10^{-6}$ mole of adenine nucleotides, presumably present as ATP in the relaxed heart in vivo. After isolation of the heart, breakdown of ATP takes place especially under anaerobic conditions, and the reaction products AMP and IMP diffuse into the medium. Effects upon these reactions of glucose, glutamic acid and excess potassium are suggested by preliminary experiments.

THE PRESENT SERIES of investigations is devoted to the elucidation of the role of adenosine triphosphate (ATP),* in the contraction of the myocardium. In this first paper, we present an outline of our analytic technic, together with a number of observations designed to illustrate the applicability of the method, to determine the amount of adenine nucleotides present, and to obtain information about their behavior under some experimental conditions.

METHODS

Extraction of Tissue. Experiments were usually performed on the ventricular parts of feline hearts. After their isolation from the body and exposure to experimental conditions, they were weighed† and rapidly disintegrated in a Waring blender in 100 ml. of cold 0.5 N perchloric acid.‡ The extracts were filtered in the cold, rapidly neutralized with potassium hydroxide, and freed of potassium perchlorate by renewed filtration. The filtrates were stored in the icebox for brief periods until analyzed.

Chromatographic Analysis. The procedure is based on the method of Cohn and Carter for the chromatographic separation of nucleotides. An aliquot of a heart extract, frequently 10 ml., was adjusted to pH 9.5 to 10.0, and was applied after dilution with water to 50 ml. to a column (3 cm. high, 1 cm. diameter) of the anion exchange resin Dowex—1 in the chloride cycle. Retention of the nucleotides, as tested by measurement of the ultraviolet absorption of the filtrate, was practically complete (95 to 100 per cent). The nucleotides were then stepwise eluted with a series of three solvents: (A) 0.003 N hydrochloric acid; (B) 0.01 N hydrochloric acid, 0.02 M sodium chloride; (C) 0.01 N hydrochloric acid, 0.2 M sodium chloride. These solvents elute AMP, ADP and ATP respectively, but by the use of larger volumes (250 ml. of each, as a rule), we searched for additional components. The eluates were collected in fractions of 10 to 15 ml., for each of which the extinction coefficient was determined at 258 m\(\mu\)l.

Individual nucleotides appear as consecutive peaks, each of which was further identified by its absorption spectrum and, in critical instances, by chemical analyses. In those cases, nitrogen was determined by the micromethod of Lanni and associates, ribose according to Mejbaum, and phosphate according to Berenblum and Chain.

† We are aware that due to incomplete removal of enclosed fluids, and sometimes because of edematous swelling, this determination is likely to overestimate the tissue mass. This circumstance will not affect those of our experiments in which primarily the proportions of the individual nucleotides were determined, but must be taken into account in case of the determinations of the total nucleotide.

‡ Perchloric acid was used as a protein-precipitant, because it does not absorb in the ultraviolet, and because upon neutralization with potassium hydroxide it is removed as an insoluble perchlorate.

§ For quantitative measurements, we accepted a value of $1.6 \times 10^4$ for the molar extinction coefficient of adenosine phosphates (Kalckar). The slight uncertainty of this value in our elution media does not affect the outcome of the measurements.
RESULTS

Freshly Excised Myocardium. As an example, figure 1 brings a chromatogram of the nucleotides in the ventricles of a cat's heart which was removed from the body without special precautions, washed in physiologic saline in which it performed a few contractions, and extracted as described. According to the chromatogram, the organ contained a small amount of AMP, no IMP, a larger fraction of ADP, and a preponderant amount of ATP. These identifications were made on the basis of the early appearance of the respective fractions after the application of each of the three eluting solvents, by their ultraviolet absorption spectra and, in the case of ADP and ATP, by the following chemical analyses (table 1).

![Fig. 1. Nucleotide chromatogram in "relaxed" heart.](image)

Total Amount and Distribution of Nucleotides.
We have not attempted to evaluate the nucleotide contents of extracts by direct spectrophotometry, since additional ultraviolet-absorbing substances may occur. Instead, the total content of adenine nucleotides was obtained by summation of the total amounts of each of three fractions.

In table 2 we give such analyses for left and right ventricles of cat's hearts, and in table 3 for entire ventricles of cats and dogs. These absolute values are uncertain due to the circumstances alluded to earlier.* With some reference to this, and assuming that in diastole all the nucleotide is in the form of ATP, we may accept the estimate, in round numbers, that the ventricle contains nearly 2.5 mg. or 5 × 10⁻⁶ mole ATP per gram net weight (with some species differences), and that the left ventricle contains one-third more ATP than the right ventricle.

Hearts Stimulated in Vitro. One ventricle of a cat's heart was directly analyzed, the other

* See last footnote (†), first column, page 8.
periments, furthermore, the aliquots taken to be analyzed were not identical, so that various total amounts of nucleotide appear in the chromatograms. In aerobic experiments, a mixture of 95 per cent oxygen, 5 per cent carbon dioxide was led through the medium through a sintered glass disc in the bottom of the vessel; for anaerobic experiments, oxygen was replaced by nitrogen. Variations in the composition of the bathing fluid included the presence or absence of glucose and of glutamic acid, and elevation of the potassium content over the normal. Representative results are given in figure 2. In these cases, the left ventricles served as controls; more breakdown of ATP was noted in such controls than in the freshly excised heart, due to the additional manipulations in these experiments. The analyses of the stimulated right ventricles and of the bathing fluid revealed breakdown of the ATP into ADP, AMP and IMP (table 4). The latter appeared as a second peak eluted by solvent A after the AMP, and was identified by its absorption spectrum (maximum at 250 μm) and its chemical composition (table 1).

Of these substances, ATP and ADP always remain confined to the tissue, but the monophosphates are lost into the bathing fluid (table 5). This breakdown of ATP, and exosmosis of the reaction products, are pronounced under anaerobic conditions. Oxygenation reduces the breakdown of ATP.

Single experiments (tables 4 and 5) suggest effects of glucose and of glutamic acid, which, however, need further confirmation. Even in the aerobic experiments, oxygenation was certainly not complete, due to the dimensions of the tissue even in the case of the thinner right ventricle; however, we found that aerobically after five hours the preservation of the adenosine polyphosphates is better than after one and one half hours of anaerobiosis. With an excess of potassium (50 mg. potassium added in the form of potassium chloride to 100 ml. of medium), in which case, notwithstanding regular stimulation at a higher intensity, only few effective contractions took place, the breakdown and exosmosis of nucleotides was nearly as pronounced as under anaerobic conditions.

These results are only exploratory, and no conclusions are drawn from some of the finer differences.

**Discussion**

We wish to emphasize that our method of analysis is inclusive in the sense that the total amount of adenine nucleotides is estimated, regardless of the effectiveness with which they are maintained, in the tissue or during the
manipulations, in fully phosphorylated form. After removal of the heart from the body, dephosphorylation takes place, whereas, in the living relaxed heart all the nucleotide is ATP. Determinations of, for example, the easily hydrolyzable phosphate as a measure of ATP, as usually practiced, would, therefore, tend to underestimate the amount of this nucleotide potentially present. Correspondingly, our ATP estimates are high, about $5 \times 10^{-6}$ moles per gram, which is the same as in skeletal muscle. Previous investigators have sometimes but not always obtained such high values. In confirmation of other work we have found more nucleotide per gram in the left than in the right ventricle.

Whereas, in the fresh relaxed heart ATP occurs predominantly or exclusively, extensive breakdown occurs to ADP, AMP and IMP after isolation. Presumably, the following reactions occur in prolonged activity:

$$\text{ATP} = \text{ADP} + \text{phosphate (contractile proteins)}$$

$$2\text{ADP} = \text{ATP} + \text{AMP (myokinase)}$$

$$\text{AMP} = \text{IMP} + \text{NH}_3 \text{ (adenylate deaminase)}$$

Except for a new nucleotide to be described subsequently, no other substances were encountered. Thus, like Lohmann, we could find no evidence for the diadenosine pentaphosphate which has frequently been regarded as a characteristic cardiac nucleotide. We believe that such reports were based upon the simultaneous isolation of approximately equimolar amounts of ATP and ADP from hearts in which a certain decomposition had occurred.

**Summary**

1. Heart muscle has been analyzed for adenine nucleotides by extraction with perchloric acid, ion-exchange chromatography, and photometric determination of the fractions.

2. The mammalian myocardium contains about 2.5 mg. or $5 \times 10^{-6}$ mole of nucleotide per gram, mainly ATP. The left ventricle contains more than the right ventricle.

3. Upon prolonged stimulation in vitro, far-going breakdown of the nucleotides takes place, with adenylic and inosinic acids as the end products, which diffuse into the medium. This is more pronounced in anaerobiosis.

**References**

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