# ACTION OF VARIOUS PHOSPHORIC ACID COMPOUNDS ON THE HEART

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The actions of various organic phosphoric acid compounds, especially of adenine compounds, on the heart have been studied for some times past. Among the pioneers of this type of work are Drury and Szent-Györgyi[1] who observed the cardiac action of adenosine-5-phosphoric acids (A5MP) extracted from cardiac muscle of the bullock, and Rothmann[2], Lindner and Rigler[3] who studied the therapeutic drug for angina pectoris, Lacarnol. Since then there have been numerous reports on this subject, including the noteworthy investigations of Green and Stoner[4] published recently. However some of these reports assert cardiac stimulation, others a functional inhibitory action, and do not agree regarding the action. On the other hand, in recent years there has been marked progress in investigations on the biochemistry of energy-rich phosphate compounds, especially of adenosine-5-triphosphate (ATP), ending in the discovery of the central role played by these compounds in biological function. Interested in the actions of these substances on the heart, the writers also undertook investigations with the isolated heart. Together with the adenine group of energy-rich phosphate compounds, phosphocreatine (PC) has a close relation with muscular contraction, but its pharmacological action is almost unknown, so we synthesized this compound and observed its action on the heart. Recently in France, thiaminetriphosphoric acid (TTP) has been synthesized and its powerful action on the metabolism of cardiac muscle has been demonstrated, and Plotka *et al.*[5] observed the pharmaological action of this compound and found it to be a strong cardiac stimulant. We also made an investigation regarding this.

## METHOD

As heart of cold blooded animals, those of the frog and toad were used. The heart was isolated by Hartung's method and perfused with Ringer's solution. In the case of the frog the minute volume propelled and amplitude of movement of the ventricle, and in the case of the toad the amplitude, were recorded. The drug was dissolved in Ringer's solution and poured into the perfusing fluid. In many cases the isolated heart contracted not powerfully by the influence of the operation. In such preparations a stimulating action of drugs was found definitely. On the other hand when the contraction of the heart was powerfull after isolation, the weak action of small amounts of drugs could not be shown

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clearly. In such cases the Ca/K content of the perfusing Ringer's solution was reduced and the amplitude and volume of propelled blood were lowered to the necessary degree before the drug was added. In this case the drug was dissolved in the Ca/K reduced Ringer's solution in order to prevent the changes arising from the difference in the solvent. It was found that when the perfusing fluid was normal or was reduced in the Ca/K content there resulted no difference in reactivity of the organ to the drug. The constituents of the normal Ringer's solution used in the cases of cold blooded animals were as follows: NaCl 0.6%, $CaCl_2$ 0.01%, KCl 0.0075%, $NaHCO_3$ 0.01%.

The cat was used for experiments of a warm blooded animal. The heart was isolated by Langendorff's method, and the ventricular amplitude and the volume of blood in the coronary circulation were recorded. The constituents of the Ringer's solution were as follows: NaCl 0.92%, $CaCl_2$ 0.024%, KCl 0.042%, $NaHCO_3$ 0.01%, Glucose 0.1%.

ATP was extracted from the muscles of the cat and rabbit as the barium salt, according to the method of Le Page,[6] and before use was changed into the Na salt. Adenosine-5-diphosphate (ADP) was prepared from the above described ATP by Le Page's method,[6] stored as the Ba salt, and changed into the Na salt before use. In every case the amounts of labile hydrolysable phosphorus and pentose in the drug solution were estimated and the actual amounts of drug used were quantitatively determined. A5MP was a product of General Biochemicals Inc. (USA), adenosine-3-phosphoric acid (A3MP) of Bayer Co. (Germany), while adenine and adenosine were prepared by the Faculty of Science, Nagoya University. PC was synthesized from creatine and $POCl_3$ according to the method of Zeile and Fawaz,[7] while TTP was a product of Takeda Research Laboratory.

## RESULTS

*Isolated frog and toad's heart:* Heart stimulation appeared with small amounts of ATP (fig. 1); namely, after the administration of ATP ($10^{-8}$–$10^{-7}$) there appeared immediately increases in amplitude of contraction and volume propelled. With increase in the amount of the drug there appeared marked cardiac stimulation, and with amounts above $10^{-6}$–$10^{-5}$, there occurred immediately marked increase in amplitude, followed by a transient inhibition (fig. 2). With greater amounts the inhibitory action was also enhanced, and there appeared slowing of heart rate which sometimes reached a state of heart block ending in standstill of the ventricle in a state of diastole. However, the heart started to beat again more strongly than normal for a long time (100 minutes or over), and the rhythmic irregularity seen occasionally before administration of the drug disappeared in some of the cases (fig. 3). Next, in order to find out if the block appeared *per* the parasympathetic or not, atropinization was conducted and a large amount of ATP was administered, but block appeared also just as without atropinigation. During the occurrence of block the ventricle was stimulated electrically and there resulted an excitatory contraction. This should be interpreted as being due not to the lowering in excitability of the ventricle, but rather to interference in the conduction of impulses from auricle

to ventricle. The reaction to ADP did not differ much from that obtained with ATP (fig. 1). There was cardiac stimulation with small amounts, and there was greater stimulation with larger amounts with however, the occurrence of transitory inhibitory action as was seen in the case with ATP. We attempted to investigate the quantitative relationship of ATP and ADP to cardiac action but with the methods employed it was not possible to obtain definite results. There was no action at all when small amounts of A5MP was applied to the isolated toad's heart, but when amounts greater than $5 \times 10^{-6}$ were applied, there was seen some cardiac stimulation. However, on the same heart there was seen an effect even with very small amounts of ATP (fig. 4). Hence the drug used was examined for phosphoric acid hydrolyzed at 100° C in 1 N HCl for 7 minutes, and it was found that there existed labile hydrolyzable phosphorus equivalent to 0.005 mg of ATP or 0.01 mg of ADP in 1 mg of the drug. With large amounts of A5MP there resulted slowing of pulsation of the toad's heart, so that there exists an inhibitory action similar to that noted in ATP and ADP. A3MP had no stimulatory action at all on the isolated frog and toad hearts, and at very high concentrations there was seen an inhibitory action. However this inhibitory action was weaker than that seen with A5MP and other muscular adenine compounds. Adenosine showed no cardiac stimulatory action, and at concentrations below $2 \times 10^{-6}$ there was seen no action at all. At higher concentrations there was diminution of both amplitude and volume propelled (fig. 5). The heart rate decreased and in marked cases there was complete block, with stoppage in a state of diastole. This action was marked as the amount was increased, and resembled the inhibitory action seen when large amounts of ATP, ADP and A5MP were administered. Adenine showed neither stimulatory nor inhibitory action in concentrations up to $5 \times 10^{-6}$, but when the amounts were increased further, there resulted decrease in amplitude and volume propelled. In the frog PC, in concentration below $10^{-6}$ showed no action, but depending on the amounts increased, there was seen increase in amplitude and volume propelled, but there resulted no inhibitory action as exhibited by large amounts of ATP and ADP (fig. 6, 7). With extremely large amounts the action was marked and prolonged, but even with amounts ranging between $10^{-4}$ and $10^{-5}$ there was not seen so powerful an action as was noted with large amounts of ATP and ADP. Creatine and $Na_2HPO_4$, when given even in very large amounts, showed no action at all on the heart. $Na_4P_2O_7$ in amounts greater than $10^{-4}$ exhibited an inhibitory action. With small amounts of TTP ($10^{-5}$ to $10^{-6}$) there was seen no action on the amplitude. With amounts greater than $10^{-4}$ the amplitude was decreased and in some cases the heart beats became irregular. This phenomenon was seen in both the powerfully beating and weakened hearts. Next, investigations were made to find out if TTP in amounts of $10^{-4}$ and over, applied before or simultaneously, could remove the disturbance in function produced by various agents such as excess of $K^+$, $Ca^{++}$, $Ba^{++}$ and digitalis preparation on the isolated heart. The results showed that no such action was produced.

*Isolated cat's heart:* Depending on the individual specimen examined there were differences in the reactivity to the drug, but in general, the following

results were obtained. Adenosine in small doses of about $10^{-2}$ mg increased the coronary flow by more than 100 per cent. This increase lasted for only a short time, but slight enhancement of amplitude continued for some time in many cases. In the case of ATP and ADP, immediately after application there was seen a slight transitory inhibition followed by a slight or marked increase in amplitude and a short increase in coronary flow. PC and creatine did not increase the coronary circulation. PC in amounts of $10^{-2}$ mg and above showed slight increase of amplitude not accompanied by inhibitory action.

## DISCUSSION

Regarding the action of adenosine and related group of phosphoric acid compounds on the heart of warm blooded animals, since the report of Drury and Szent-Györgyi,[1] it has been generally confirmed that adenosine, A5MP and others possess the power of increasing the coronary flow. We succeeded too, in showing that excluding adenine, all members from adenosine to ATP, possessed in marked degree this action. However, regarding action on the amplitude Gillespie[8] found only an inhibitory action, while Drury[9] found A5MP to have the power of increasing the amplitude, which he considers to be due not merely to a secondary effect resulting from the increase in coronary flow. Green *et al.*[4] reported that ATP and ADP have a strong action of increasing the amplitude, but that A5MP shows a slight increase. Their results were confirmed by our findings. In the isolated heart of warm blooded animals, the influence of drugs tends to become complicated due to the changes occurring in the coronary circulation and to the sudden natural weakening. For this reason we used the heart of a cold blooded animal which does not possess coronary vessels, together with that of warm blooded. Lindner and Rigler,[3] Parnas and Ostern[10] and others found that ATP has a specific action of increasing the amplitude of the isolated forg's heart. Regarding A5MP, Parnas and Ostern,[10] and Flössner[11] found that there is seen a slight action resembling ATP. However, according to Gillespie[8] no such action is seen with ATP and A5MP. In our investigations the action of increasing the amplitude was seen specifically in ATP and ADP, and none such found in A5MP. Many have found the action of increasing the amplitude in A5MP is probably due to the presence of some amount of ATP or ADP intermixed in the drug employed. In recent years the biochemical action of ATP as an energy-rich compound has received attention, but a point of interest is that this substance also possesses a specific pharmacological action. It is clear that PC plays an important role as a pool for the energy-rich phosphate of ATP. It has been found that this substance possesses the power of increasing the amplitude of contraction of the isolated heart. This action is not so powerful as that seen in ATP and others, but it does not show a transitory inhibitory action and does not influence the coronary circulation. It is not known at present whether the action of increasing the amplitude possessed by ATP, ADP and PC is due to the passage of the substances through the membrane of the muscle cell and acts directly on the contractile mechanism of the muscle, or to some other mechanism. Regarding the action of A3MP on

the isolated heart, Parnas and Ostern[10] reported decrease in amplitude, while Flössner[11] reported an increase. Our experiments showed no enhancement. Regarding the inhibitory action accompanying the cardiac stimulation of ATP and ADP, in the cold blooded animal it is unrelated to the vagus nerve and is a direct one resulting from action on the heart muscle; and from the inhibitory powers of adenosine, it is probable that this action is related to this compound. However, the strong inhibitory action of ATP cannot be ascribed only to adenosine. Finally, regarding TTP, we were not able to find the strong cardiac stimulatory action on cold blooded animals reported by Plotka *et al.*[5] The isolated heart when observed for a long period of time, shows natural changes by conditions unrelated to the drug and TTP is a highly decomposable substance. Hence we consider as questionable the results of Plotka *et al.*

SUMMARY

The actions of ATP, PC, TTP and others related to this group of compounds were examined on the isolated hearts of the frog, toad and cat. ATP and ADP increased the amplitude of the heart beat of the frog and toad. These substances, besides increasing the amplitude, produced an increase in the coronary flow of the cat's heart. Adenosine, A5MP, ADP and ATP all increased the coronary circulation, but PC did not. PC showed an action of increasing the amplitude of contraction of the isolated heart, not accompanied by inhibitory action. TTP and A3MP showed only an inhibitory action on the isolated heart of cold blooded animals.

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## EXPLANATION OF FIGURES

FIG. 1. Effects of ATP 0.02 μg/ccm, ADP 0.05 μg/ccm and 0.02 μg/ccm on the amplitude of isolated toad's heart.

FIG. 2. Effect of ATP 0.5 μg/ccm on the amplitude of isolated toad's heart.

FIG. 3. Effect of ATP 10 μg/ccm on the amplitude of isolated toad's heart.

FIG. 4. Effects of ATP 0.03 μg/ccm and A5MP 6 μg/ccm on the amplitude of isolated toad's heart.

FIG. 5. Effect of adenosine 10 μg/ccm on the amplitude of isolated toad's heart.

FIG. 6. Effect of phosphocreatine 5 μg/ccm on the amplitude of isolated toad's heart.

FIG. 7. Effect of phosphocreatine 100 μg/ccm on the amplitude of isolated frog's heart.

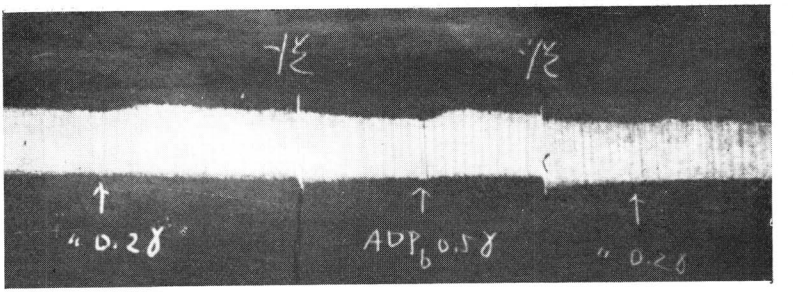


FIG. 1

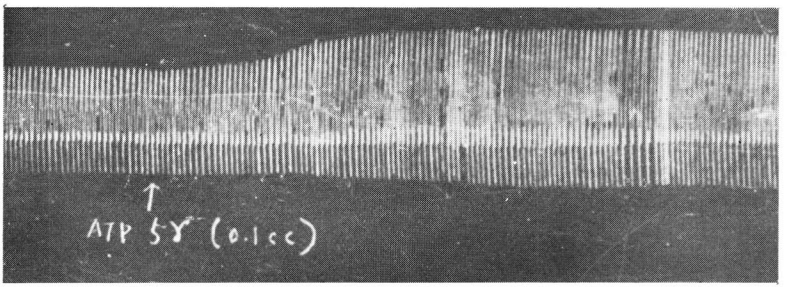


FIG. 2

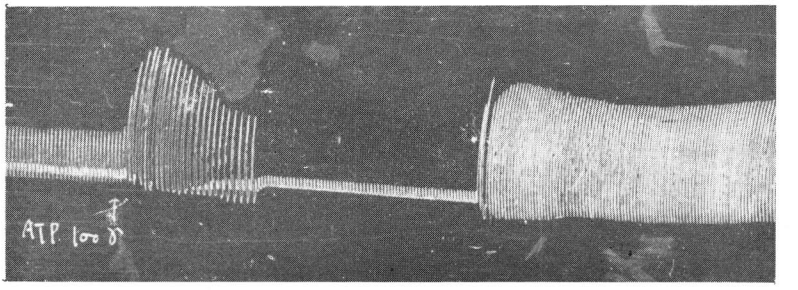


FIG. 3

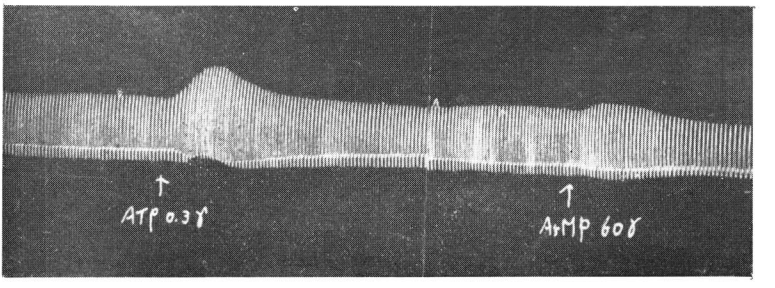


FIG. 4

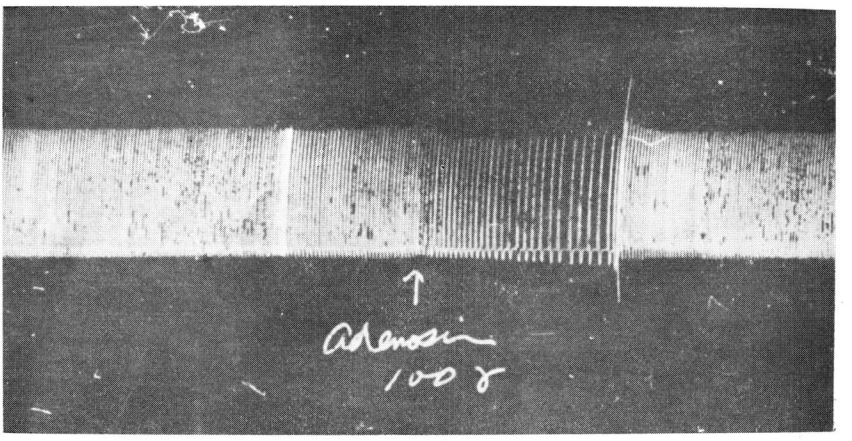


FIG. 5

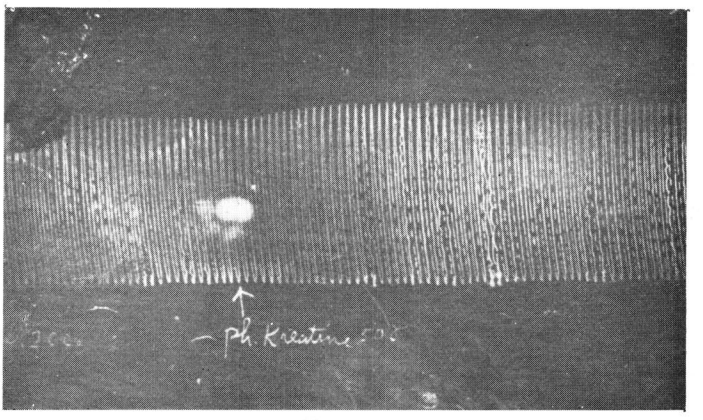


FIG. 6

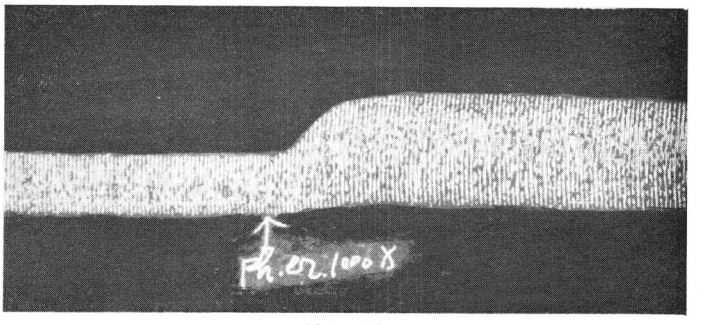


FIG. 7