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IDENTIFICATION OF ADENOSINE TRIPHOSPHATE IN HUMAN PLASMA AND THE CONCENTRATION IN THE VENOUS EFFLUENT OF FOREARM MUSCLES BEFORE, DURING AND AFTER SUSTAINED CONTRACTIONS

BY T. FORRESTER AND A. R. LIND

From the Institute of Physiology, University of Glasgow, and Indiana University Cardiopulmonary Laboratory, 6570 Aerospace Medical Research Laboratories, Environmental Medical Division, Wright–Patterson Air Force Base, Ohio, 45433, U.S.A.

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SUMMARY

1. When diluted human plasma is perfused through a frog heart, a marked augmentation of the heartbeat is produced which is very similar in action to that of low concentrations of adenosine triphosphate (ATP) on the heart.

2. It was established that the substance in the plasma responsible for the heart stimulation was ATP. The following tests were used: (*a*) the diluted plasma emitted light from firefly lantern extract characteristic of the light signal produced by a solution of ATP; (*b*) the stimulatory effect on the frog heart and luminescent effect upon the firefly extract were abolished by incubation of the plasma solution with the enzyme apyrase, which converts ATP to adenosine monophosphate (AMP); AMP does not stimulate the heart or cause light to be emitted from firefly extract; (*c*) the stimulatory substance in the plasma was eluted through a column of Sephadex G-25 in the same pattern as ATP; and (*d*) simultaneous assay of plasma solutions on frog heart and firefly extract produced the same quantitative result as that produced by a solution of ATP.

3. The amount of ATP in plasma from the venous blood of resting subjects ranged from 0·19 to 0·95 μg/ml. (mean 0·63 μg/ml., S.D. $\pm$ 0·25); up to half of the ATP detected could be attributed to blood platelet damage. Simultaneous arterial and venous samples of blood from four subjects at rest had mean concentrations of 0·19 μg/ml. (0·07–0·26 μg/ml.) and 0·70 μg/ml. (0·57–0·84 μg/ml.) respectively.

4. The concentration of ATP in the venous effluent from exercising forearm muscles was measured. The venous concentration consistently increased over the resting values in response to exercise while in one subject little change occurred in the arterial blood concentration during the exercise. It was concluded that the ATP was added to the blood in its passage through the muscle bed.

5. The origin of the ATP, including erythrocytes, blood platelets and active skeletal muscle, is discussed.

INTRODUCTION

In order to demonstrate the release of ATP from active frog skeletal muscle *in vitro*, techniques were developed which could identify and measure ATP in as low a concentration as 10^{-9} g/ml. (Forrester, 1966, 1967; Boyd & Forrester, 1968). In view of the proposal that ATP might be liberated into the blood stream from active skeletal muscle *in vivo* (Forrester, 1967), these very sensitive techniques were applied to peripheral human blood, despite the fact that human plasma contains enzymes capable of destroying ATP within a short period of time (Jørgensen, 1956; Ireland & Mills, 1964; Holmsen, Stormorken & Goote, 1965).

The first part of this paper describes the identification and assay of ATP in fresh human plasma obtained from the antecubital vein; the second part deals with the investigation of ATP in venous blood draining the forearm muscles before, during and after sustained forearm exercise. A preliminary communication of this work has been given to the Physiological Society (Forrester, 1969).

METHODS

Samples of venous blood were obtained from the antecubital vein using only a brief period of manual constriction around the upper arm in order to effect enough distension of the vein for a 'clean' venepuncture. In four subjects samples were obtained from a plastic cannula inserted retrogradely into one of the deep veins of the forearm; no occlusion of the upper arm was thus necessary to obtain samples from these subjects. In every case the subject was instructed to keep the forearm musculature as relaxed as possible just before and during the sampling. Venous blood was slowly drawn off into a plastic 'non-wettable' syringe and 4 ml. then transferred to a plastic tube containing 0·3% ethylenediamine-tetra-acetic acid (EDTA) and immediately put on ice for 5–10 min. After centrifugation at 1500 rev/min for 25 min at 4° C, the supernatant plasma was then diluted with either 0·9% pyrogen-free saline solution (w/v) or Locke solution of composition 154 mM-NaCl, 5·6 mM-KCl, 2·2 mM-$CaCl_2$ and 6·0 mM-$NaHCO_3$.

The methods of identification have been previously described by Boyd & Forrester (1968). The frog-heart perfusion system (Boyd & Eadie, 1961), molecular sieve chromatography (Boyd & Forrester, 1966) and apyrase tests were used without modification. In the firefly technique (see Strehler & McElroy, 1957) the same photomultiplier tube (14-stage E.M.I. 6262 with a conventional S11 photocathode) was

used as before, since the 'dark current' was virtually zero, permitting a large signal-to-noise ratio at high voltages.

Continuous perfusion of the frog heart with Locke solution was possible without alteration of performance (Forrester, 1967). The samples diluted with saline were assayed on firefly extract only. Solutions of ATP (disodium dihydrogen salt of adenosine-5′-triphosphoric acid, British Drug Houses) were made up in either pyrogen-free saline solution for assay on firefly extract or Locke solution for assay on frog heart.

Glassware was siliconized using 'Repelcote' (Hopkin and Williams, Ltd).

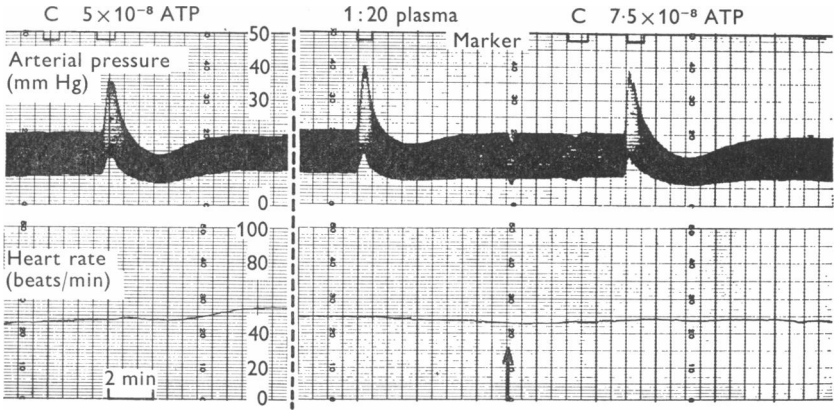

Fig. 1. Comparison of the action on a perfused frog heart of solutions of ATP and a diluted solution of fresh human plasma. Heart continuously perfused with Locke solution. C, control injection of Locke solution; vertical interrupted line, period of 25 min; ↑, fall in perfusion pressure during flushing of residual plasma solution from venous pressure capsule. Note the great similarity of action of both plasma and ATP solutions (g/ml.) on the blood pressure. There is little effect on the heart rate.

RESULTS

Pilot experiments showed that when suitably diluted fresh human plasma was perfused through a frog heart a pronounced stimulatory effect was produced. Figure 1 shows a comparison of the action of a diluted plasma solution and solutions of ATP on a frog heart. The effect of a 1:20 plasma solution has been 'bracketed' between the effects of ATP, 5×10^{-8} and $7{\cdot}5 \times 10^{-8}$ g/ml. There is clearly a great similarity of action between the test solution and the two solutions of ATP on the blood pressure. There is in all three cases an immediate positive inotropic effect on the myocardium, with little effect on the heart rate. Plasma from eight subjects was found to have the same qualitative action as ATP on the frog heart.

Since it is possible to discriminate the action of ATP from the actions of other nucleotide triphosphates on the frog heart (Boyd & Forrester, 1968), these preliminary results prompted the application of other tests for the identification of ATP.

Action of dilute plasma on firefly lantern extract. Extracts of firefly lanterns (*Photinus pyralis*) emit a slowly decaying flash of light when mixed with solutions of ATP, the time course of the signal decay being proportional to the concentration of ATP applied (McElroy, 1947). This reaction is reputed to be highly specific for ATP (Strehler & McElroy, 1957), although it was found in a previous investigation that the firefly extract

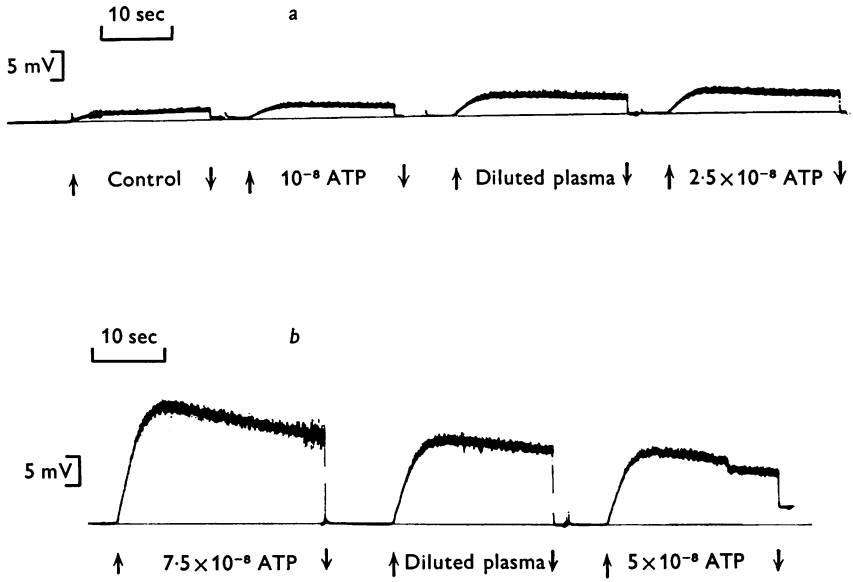

Fig. 2. Light emission from firefly extract in response to solutions of ATP (g/ml.) Upward deflexion indicates a light signal; ↑, voltage across photomultiplier tube switched on; ↓, voltage switched off. (*a*) Control, firefly extract + Locke solution; plasma sample, diluted 1:10 with Locke solution, has an effect almost equivalent to that of ATP $2{\cdot}5 \times 10^{-8}$ g/ml. (compare Fig. 5, last test). (*b*) A sample of plasma diluted 1:5 from another subject has its effect 'bracketed' between the effects of ATP 5×10^{-8} g/ml. and $7{\cdot}5 \times 10^{-8}$ g/ml. Note the similar time course of signal decay with plasma solutions and matching ATP solutions.

used (type FLE 50, Sigma Co.) also responds to low concentrations of other triphosphates (Boyd & Forrester, 1968). Samples of plasma from twenty-one subjects caused light to be emitted from firefly extract. In every case there was a light signal produced which could be matched in time course and intensity with that produced by small amounts of ATP. Figure 2 shows the effect of two plasma solutions, diluted 1:10 (*a*) and 1:5 (*b*) with saline, on extract of firefly. The light signals produced by the plasma solutions match those produced by the solutions of ATP.

Action of a specific enzyme on dilute plasma. The enzyme 'apyrase'

(adenosine-5′-diphosphatase, purified crystals, Sigma Co.) first converts ATP to ADP and 10 min later converts ADP to AMP. It has been demonstrated that the enzyme shows some specificity towards ATP (Krishnan, 1949), but it has been found in practice that it is not possible to distinguish ATP from ADP using apyrase in conjunction with the present methods (Boyd & Forrester, 1968). Figure 3 shows the effect of perfusing a dilute plasma solution, before and after incubation with apyrase, through a frog heart. Adenosine monophosphate does not stimulate the heart (Forrester, 1967). The stimulating effect of the plasma solution has been largely abolished after incubation and this is taken to indicate that the apyrase has had a dephosphorylating effect on the substance in the plasma which stimulates the frog heart.

Six plasma samples were tested in this way and in each case the positive inotropic effect was almost abolished by 10 min incubation with apyrase.

Column chromatography. In a molecular sieve chromatography procedure (Boyd & Forrester, 1966), it was established that the substance in the plasma which caused light to be emitted from firefly extract was eluted through a column of Sephadex G-25 in the same pattern as ATP. Undiluted plasma samples from five subjects were each tested in the following way. A 2 ml. plasma sample was mixed with blue dextran (a high molecular weight substance which acts as a visual indicator) and eluted through a column previously equilibrated with pyrogen-free saline solution. The column has the property of separating the protein and other molecules of large molecular weight from substances of smaller molecular weight. In general, the smaller the molecule, the more slowly it is eluted through the column. Consecutive fractions of eluate were collected from the column and then tested on firefly extract. It was found that the peak value of light emission from these fractions occurred at the same locus as that of ATP. Figure 4 shows the result of one such experiment. In this case a sample of plasma was obtained from a subject who was undergoing some mild forearm exercise; 2 ml. of the plasma sample was eluted through the column. Fractions of eluate were tested on firefly extract and the fraction which caused most light to be emitted was incubated with apyrase and then tested once again on the firefly. Figure 4(*a*) shows the pattern of light emission produced by the consecutive fractions. The hatched area shows the diminished light emission of that fraction after incubation with apyrase. Figure 4(*b*) shows the result of eluting a solution of ATP, 10^{-6} g/ml. through the same column 24 hr later. The greatest light signal is again produced by the fourth 2 ml. fraction.

It was not determined whether any destruction of ATP took place during its passage through the column, although this seems likely, since it was found on all five occasions when a solution of pure ATP was eluted

through a column after a sample of plasma had previously passed through it, that only a very small amount of ATP could be detected. Perhaps the column retains unknown substances from the plasma which have a dele-

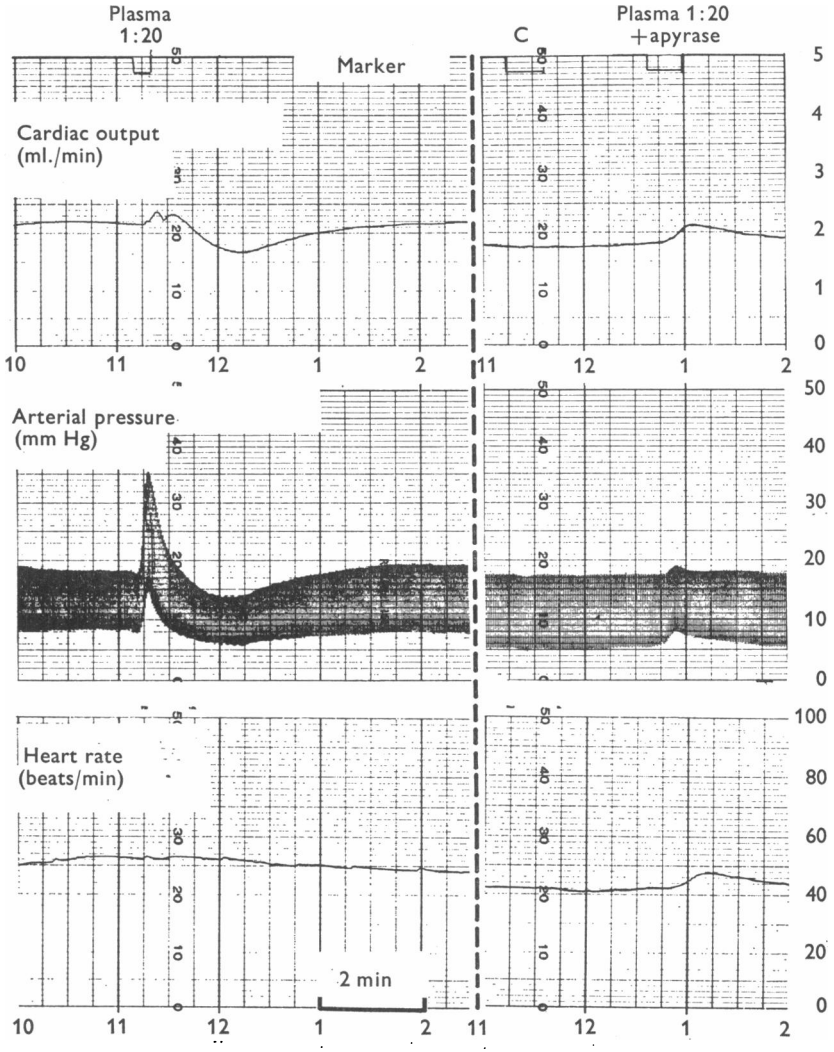

Fig. 3. The effect of a diluted plasma solution before and after incubation with apyrase on a perfused frog heart. Vertical interrupted line, interval of 20 min. The plasma sample, diluted 1:20, gives the usual marked positive inotropic effect; the cardiac output increased so rapidly at this point that the drop-counting mechanism failed. There is little effect on the heart rate. After incubation with apyrase the same solution has a much diminished inotropic effect. The increase in cardiac output has been caused by an increase in the heart rate.

terious effect on ATP. This phenomenon prevented accurate calculations of percentage regain of ATP from the column.

From these results—namely that the dilute plasma solution stimulates the frog heart in a manner similar to that of ATP, causes light to be emitted from firefly lantern extract, is eluted through a column of Sephadex G-25 in the same pattern as ATP and has its effect on both frog heart and firefly extract markedly reduced by incubation with apyrase—it was concluded that the substance in the plasma which stimulated the frog heart was ATP.

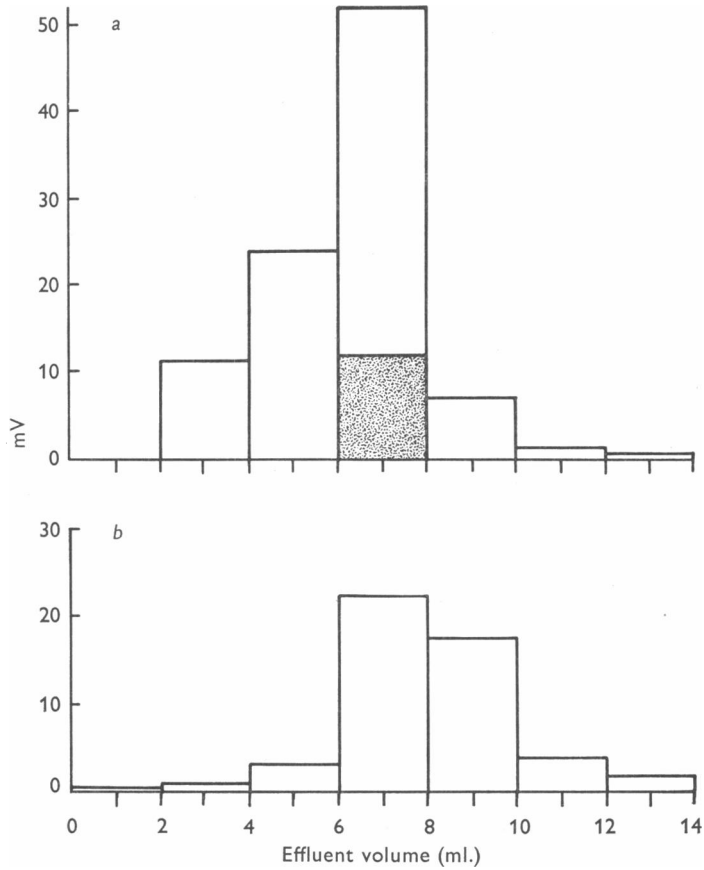

Fig. 4. Comparison of the behaviour of the substance in the plasma causing light emission from firefly extract and ATP in a column of Sephadex G-25. (*a*) Elution pattern from a plasma sample obtained from an exercising forearm. Peak light emission occurs in the fourth 2 ml. fraction. Hatched portion represents the light emitted from that fraction after incubation with apyrase. (*b*) Elution pattern obtained after passing a solution of ATP, 10^{-6} g/ml., through the same column 24 hr later. Again the peak light emission occurs in the fourth 2 ml. fraction.

Assay of plasma levels of ATP

An attempt to estimate the ATP in plasma was made, it having been established that small amounts of ATP could indeed exist in human plasma. Anticoagulated plasma samples from a total of thirteen subjects (9 ♂, 4 ♀) were assayed on firefly extract; three of those were assayed simultaneously on frog heart and firefly extract. The firefly procedure can distinguish between the actions of ATP and ADP but not between low concentrations

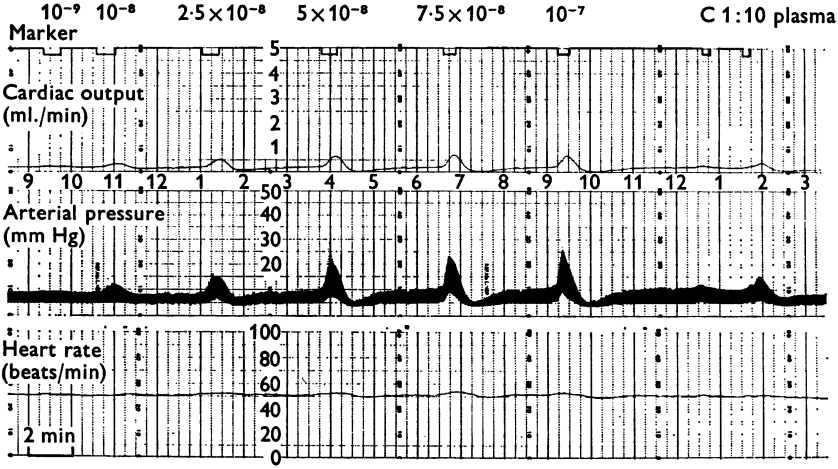

Fig. 5. Assay of a diluted plasma solution on a frog heart. From left to right, first six tests show the effects of graded concentrations of ATP in g/ml.; last test shows that the effect of a plasma solution diluted 1:10 is almost equivalent to that of ATP $2{\cdot}5 \times 10^{-8}$ g/ml. The same plasma solution was also assayed on firefly extract, with the same result (Fig. 2*a*).

of the various triphosphates; the frog-heart assay cannot distinguish between ATP and ADP but can distinguish between the actions of different triphosphates (Boyd & Forrester, 1968, figs. 5 and 8).

In each firefly assay a dose–response curve of light produced by the extract in response to ATP was obtained. The plasma samples were measured for light emission and an equivalent concentration of ATP assessed from the calibration curve (Fig. 6). In the three cases of simultaneous assay on the frog heart and firefly extract a dose–response curve of percentage output increase against concentration of ATP was derived on each heart and the test sample had its effect on cardiac output equated to a concentration of ATP. Figures 2(*a*) and 5 show the result of a parallel assay of a plasma solution. Figure 2(*a*) shows the effect of the solution, diluted 1:10, upon firefly extract. The effect of the solution is almost equivalent to that of a solution of ATP, $2{\cdot}5 \times 10^{-8}$ g/ml., giving the same

time course of signal decay. In Fig. 5 the initial tests on the heart show the gradually increasing effects of graduated concentrations of ATP on the cardiac output and blood pressure. The last test (on the right of the Figure) shows the effect of the same sample of diluted plasma; once again the effect lies between the effects of ATP, 10^{-8} g/ml. and $2{\cdot}5 \times 10^{-8}$ g/ml. The results obtained from the three parallel assays were not significantly different

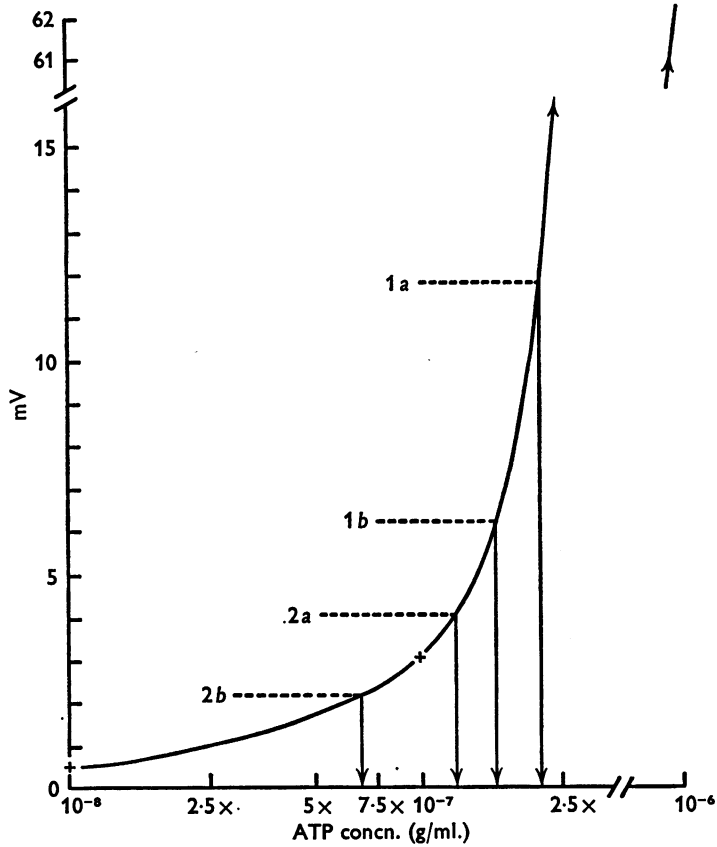

Fig. 6. Effect of time and 'non-wettable' glassware on ATP levels in plasma. Plasma sample from one subject divided into four parts, each diluted 1:5 with 0·9 % saline solution (w/v). Crosses, part of a dose–response relation of the action of ATP on firefly extract. Note interrupted axes in order to accommodate large light signal produced by ATP 10^{-6} g/ml. 1*a*, sample assayed immediately after centrifugation, using non-siliconized ware; assay result, 1·0 μg/ml. 1*b*, sample 1*a* assayed 10 min later; assay result, 0·85 μg/ml. 2*a*, sample assayed immediately after centrifugation, siliconized glassware used; assay result, 0·65 μg/ml. 2*b*, assay of sample 2*a* 10 min later; result, 0·35 μg/ml. Clearly in both 'wettable' and 'non-wettable' glassware the samples show a decline of ATP levels after 10 min. Siliconization also reduces the levels of ATP detected in human plasma.

from those obtained from the firefly extract alone, and so it was presumed that in every case the firefly extract gave an indication of the amount of ATP in the plasma solution and not of ADP or any of the other triphosphates.

The mean concentration of ATP measured in all samples of venous blood anticoagulated with EDTA was 0·63 μg/ml. (S.D. $\pm$ 0·25). The mean result for the four females tested was 0·64 μg/ml. (range 0·19–0·95 μg/ml.).

The effect of clotting on ATP levels in plasma. At an early stage in this work the blood samples were allowed to clot during the centrifugation period and the serum, gently expressed from the supernatant 'white' clot,

TABLE 1. Concentrations of ATP (μg/ml.) in samples of serum and plasma from four subjects. Last column shows the concentration of a 5 ml. solution of ATP, 0·5 μg/ml., after applying it to specimens of whole clot from each subject for a period of 1 min

Subject	Serum	Plasma	ATP, 0·5 μg/ml., + clot after 1 min
1	< 0·10*	0·23	0·34
2	< 0·10*	0·22	0·34
3	< 0·10*	0·15	0·35
4	—	0·10	0·50

* Samples of serum assessed on the lower part of calibration curve (see Fig. 6).

was then estimated for ATP. The time interval required to obtain serum samples was no different from that taken to obtain samples of plasma. From these early results there was an indication that the concentration of ATP in serum was lower than that in plasma, and so experiments were carried out to test whether the formation of a clot had any effect upon the levels of ATP in the plasma.

Blood samples were obtained by simple venepuncture from four subjects; each sample was divided into three parts. The first was allowed to clot during centrifugation, the second was routinely anticoagulated and the third portion was left to clot without centrifugation. The third samples had any residual supernatant blood decanted after a period of 20 min, leaving a clot behind in the tube. To this clot was added 5 ml. ATP, 0·5 μg/ml.; this mixture was left to stand for 1 min, after which time the ATP solution was decanted and assayed on firefly extract. The paired samples of serum and plasma were assayed on firefly extract immediately after centrifugation. Table 1 shows the results obtained for the concentrations of ATP in serum and plasma and the effect of applying a clot to ATP, 0·5 μg/ml., for 1 min. From these figures it is clear that, with the same samples and over the same period of time, the levels of ATP are markedly

reduced if the clotting process is allowed to take place. The application of an established clot to a known amount of ATP also diminishes its concentration within the space of 1 min. In three out of the four subjects the concentration was reduced, while in the fourth subject there was no change in the concentration. These experiments thus excluded the possibility that the clotting process might add to the amount of ATP detected in the plasma.

The effect of siliconized glassware on ATP levels in plasma. It is well known that for the collection and preservation of blood platelets the use of 'non-wettable' glassware is essential. Since blood platelets contain high concentrations of ATP (Born, 1958) it became essential to determine the effects on the plasma levels of ATP using glassware rendered non-wettable by siliconization. Samples of blood obtained from three subjects were divided into two portions, one group being handled in siliconized glassware, the other being centrifuged in non-siliconized tubes. In one subject the sample of blood was split into four portions, two for siliconized glassware and two for 'wettable' glassware. Figure 6 illustrates the result of an assay of these samples from the last subject on firefly extract. When the light emission was assessed on a dose–response curve, those samples handled in 'wettable' glassware (Fig. 6, 1*a*, 1*b*) had clearly a higher level of ATP than those which were contained in 'non-wettable' glassware (Fig. 6, 2*a*, 2*b*). In the case of the samples which were assayed immediately after centrifugation, sample 1*a* had a concentration of ATP, 1·0 μg/ml., whereas 2*a* had an ATP concentration of 0·65 μg/ml. Estimations 1*b* and 2*b* are the ATP concentrations of these samples measured 10 min later. It is to be noted that these assays were performed on the steepest part of the calibration curve, there being more than twice the light produced from sample 1*a* than from 2*a*.

The mean concentration of ATP in the samples where non-siliconized glassware was used was 0·64 μg/ml. (range 0·5–1·0 μg/ml.), virtually the same as the over-all assay figure; the mean concentration of ATP in samples contained in siliconized glassware was 0·41 μg/ml. (range 0·24–0·60 μg/ml.). Thus it seems that up to one-half of the ATP detected in the main series of assay results (mean 0·63 μg/ml.) is probably contributed by platelet damage when centrifuging was performed in tubes which had not been siliconized.

Rate of decline of ATP levels in plasma at room temperature. It was found that when a dilute solution of plasma was left at room temperature (18–22° C) for 1 hr after centrifugation, no ATP could be detected by the present methods. Obviously continuous ATPase activity could proceed in the plasma at room temperature.

An assay of two solutions of diluted plasma immediately following

centrifugation (1*a*, 2*a*) and then 10 min afterwards (1*b*, 2*b*) is illustrated in Fig. 6. After 10 min the ATP level in sample 1 declined from 1·0 to 0·85 μg/ml., while the level in sample 2 declined from 0·65 to 0·35 μg/ml. In another sample from the same subject there was a fall from 0·42 to 0·25 μg/ml., using siliconized glassware. An average fall in ATP concentration of 34% in the 10 min period 30–40 min after withdrawal of the blood from the vein would suggest that ATP can decay rapidly in diluted plasma, even at room temperature.

Release of ATP into the venous effluent of forearm muscles during and after sustained contractions

An assessment of the concentration of ATP in the venous effluent from forearms was made before, during and after voluntary sustained contractions of the forearm muscles.

Methods. The combination of three techniques was used for this study: (1) strain-gauge plethysmography (Whitney, 1953), (2) the firefly luminescence procedure, and (3) a method of accurately controlling static muscular contractions which allows measurement of blood flow during, as well as before and after, the muscular activity (Clarke, Hellon & Lind, 1958).

Five young, healthy men volunteered to act as subjects. The sustained contractions were performed on a simple hand-grip dynamometer (Clarke *et al.* 1958) on which the anatomical 'learning' responses were minimal. The maximal voluntary contraction (MVC) was determined for each subject; this was established on the day before the experiment to prevent the possibility that some residual influence of the MVC would persist and affect the venous concentration of ATP. The test contractions were chosen to be 10 and 20% of the MVC. The former provided a non-fatiguing contraction while the latter represented a tension which resulted in muscular fatigue.

The subjects lay on a bed, recumbent, throughout each experiment. At the outset a Teflon catheter was introduced into a vein in the antecubital fossa of the test forearm; the tip of the catheter was passed retrogressively to lie in a vessel draining the deep venous plexus in the muscle. Samples of venous blood were taken from the catheter mainly by allowing the blood to flow directly into centrifuge tubes containing EDTA. About 5 ml. blood was drawn for each sample. In a small number of cases, when the flow through the catheter was very low, samples were withdrawn into a syringe (non-wettable) and transferred at once to the centrifuge tubes. When samples were not being collected, the Teflon catheter was continually infused with pyrogen-free saline at a rate not exceeding 1 ml./min.

Forearm blood flows were measured by the Whitney (1953) mercury-in-rubber strain-gauge plethysmograph. To avoid any possibility of haematological trauma due to the inflation of plethysmographic cuffs, the blood flows were obtained on a separate occasion, under identical conditions. Changes in forearm blood flow in response to specific sustained contractions have been shown to be repeatable for individual subjects (Lind & McNicol, 1967).

Brachial arterial and venous blood samples were obtained from four subjects at rest and the ATP concentrations of these samples were measured.

Each subject made the test contractions of 10 and 20% MVC with his preferred hand; four of the five subjects were right-handed. The procedure was based on a 20

min cycle as follows: 2 min of resting control values, 4 min of the test contraction and 14 min of post-exercise examination. Venous blood samples were withdrawn once or twice during rest, three times during the contraction and four times during the post-exercise period. The samples were put on ice and centrifuged together at the end of each 20 min test. The first contraction for each subject was 10 % MVC; the subjects then underwent the same procedure at a tension of 20 % MVC. On one subject who performed only the 10 % MVC, blood samples were taken simultaneously from a Cournand needle introduced into the brachial artery of the arm to be exercised; after each sample the needle was flushed with a few ml. saline to keep the needle patent.

On two occasions solutions were diluted and frozen for a later parallel assay on firefly extract and on frog heart. The same quantitative result was produced, indicating that the firefly technique was measuring ATP and not ADP or any of the other nucleotides (Boyd & Forrester, 1968, figs. 5, 8*d*).

The concentration of ATP in each sample for each subject is shown in Fig. 7. Successive samples from resting forearms of the same subjects showed a remarkable consistency in the concentration of ATP. The samples taken during and after both contractions showed higher ATP concentrations than the resting values. In three out of the four subjects there was an increase in concentration throughout the 10 % MVC. At 20 % MVC, where, for technical reasons, results were available on only three out of four subjects, the last samples taken during exercise all had a lower concentration than the previous exercise sample.

A noteworthy feature of these results is the consistently different pattern of ATP concentration in the 10 % MVC compared to that of the 20 % MVC during the post-exercise period. After the 10 % MVC there was a marked peak of ATP concentration 2 min after tension was released; this was not the case at 20 % MVC, where the actual concentrations were lower, in general, than they were after the 10 % MVC. There was no obvious relationship between the venous concentration of ATP and the amount of blood flow. The forearm blood flow reaches a steady state at 10 % MVC, which is a contraction that does not result in fatigue, followed by a small post-exercise hyperaemia which was completed some 4 min after the contraction ended. At 20 % MVC, a tension which eventually results in fatigue, there was a continual rise of blood flow through the contraction to values higher than those obtained during the lower tension, followed by a clear post-exercise hyperaemia which persisted for 8 min or more. These findings are similar to those described before (Lind & McNicol, 1967).

It was clear that the concentration of ATP in the venous effluent was increased in association with sustained forearm exercise, but the actual amounts involved were difficult to assess. If ATP entered the blood stream at the site of the muscle bed, the concentration would of course be diluted by the concomitant vasodilatation during the exercise period. If the ATP has as its source a point proximal to the muscle bed, then the

increase in blood flow through the muscle would serve to increase the actual amounts of ATP appearing in the venous effluent per unit time. Thus it became essential to find whether ATP was present in the arterial blood passing to the forearm musculature.

Resting arterial and venous samples of blood were taken from four subjects. The mean arterial concentration of ATP was 0·19 μg/ml. (0·07–0·26 μg/ml.) and in venous blood of the same subjects it was 0·70 μg/ml. (0·57–0·84 μg/ml.), similar to the venous levels found previously (Forrester,

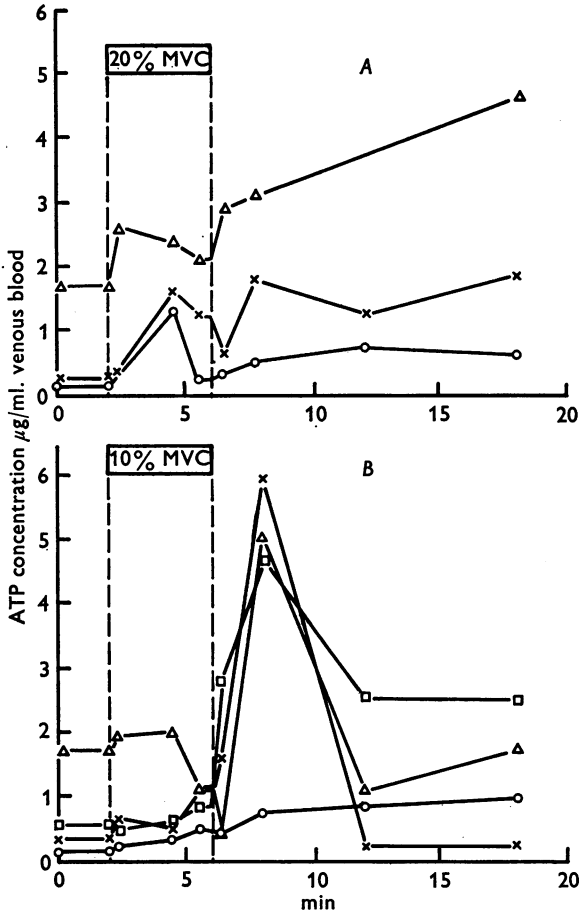

Fig. 7. The ATP concentration (μg/ml.) in the venous effluent before, during and after 20 % (*A*) and 10 % (*B*) maximum voluntary contraction. Different symbols represent the results of different subjects. The shaded bar indicates duration of the sustained forearm contraction. Note the slow rise in concentration of ATP during the post-contraction phase in the 20 % MVC (tension resulting eventually in fatigue) in contrast to the sharp rise and fall obtained in the non-fatiguing 10 % MVC.

1969). Thus it appeared that, under 'resting' conditions, blood coming from the forearm musculature contained more ATP than that travelling to it. One experiment was performed where simultaneous arterial and venous samples were taken from a subject before, during and after a 10% MVC. Figure 8(*a*) shows the concentrations of ATP obtained in the venous and arterial blood. Except for one sample, taken immediately after the start of the contraction, the concentration of ATP was clearly higher in

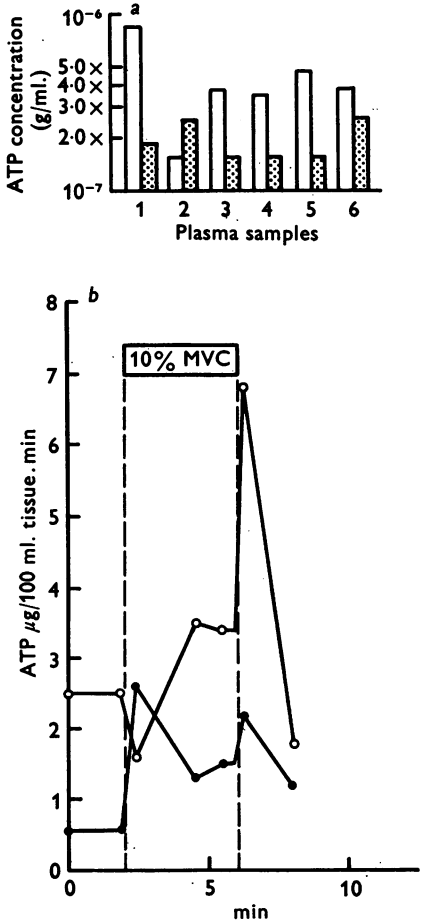

Fig. 8. Concentrations and amounts of ATP in arterial and venous blood of one subject before, during and after a 10% MVC: (*a*) shows a histogram of concentrations of arterial (▦) and venous (□) samples taken simultaneously; sample 1, resting value; samples 2, 3 and 4 taken at intervals during contraction; samples 5 and 6 obtained after the contraction. (*b*) shows comparison of total amounts of ATP in venous (○) and arterial (●) blood before, during and after the contraction.

the vein than in the artery, even during the period of vasodilatation. The blood flow through the forearm was measured at a 10% MVC and the actual amounts of ATP in arterial and venous blood before, during and after the contraction were expressed in μg/100 ml. tissue.min (Fig. 8*b*). Apart from the first venous sample during the work period, the amount of ATP in both arterial and venous blood increased during work and immediately after work. Two minutes after work the concentration of ATP had returned or nearly returned to normal resting levels. The blood flow during the contraction at this tension increased to a steady-state level approximately three times the resting value and then showed an increase to five times the resting value in the first 10 sec after work. It returned almost to the control value 2 min after the contraction ended.

DISCUSSION

Adenosine triphosphate has been identified in human arterial and venous blood and it has been shown that there is an increased concentration produced in the venous effluent from exercising forearm muscles. The main question arising from these results concerns the source of the ATP. Are the plasma levels of ATP in samples of blood obtained from resting subjects merely an index of damage to the formed elements of the blood?

The erythrocyte contains high concentrations of ATP (Bartlett, Savage, Hughes & Marlow, 1953). In the series of experiments where known amounts of ATP were applied to clotted whole blood there was inevitable mixing of free erythrocytes with the solution of ATP. Any liberation of ATP from these cells would almost certainly have been detected; in fact, the ATP concentration was reduced by this procedure (Table 1).

Blood platelets contain a higher concentration of ATP than other tissues, with the exception of the adrenal medulla and mammalian skeletal muscle (Born, 1958). Experiments in the present work indicate that platelet damage was contributing up to half of the total amount of ATP found in the plasma. Early observations by Zipf (1931) have indicated that whole blood, when exposed to mechanical disturbance, will increase its vasodilator property. Folkow (1952) has also shown that vasodilator agents are released even when slight handling of arterial blood takes place, and that the most important of these substances might be ATP released from the erythrocyte. It now seems likely from the present results that the increase in vasodilator property of shaken blood was due mainly to ATP released from damaged platelets, rather than from erythrocytes.

The increase in concentration of ATP in the venous effluent from exercising forearm muscles may prove to be significant. It is unlikely that muscles performing sustained contractions at the level of 10 and 20% of

the maximum voluntary contraction inflict any damage on the formed elements of the blood, although it is not known whether local effects of contracting muscle can render blood platelets more fragile, thus increasing the amount of ATP released from them. Abood, Koketsu & Miyamoto (1962) have shown *in vitro* that ATP is liberated from both nerve and muscle cells in association with depolarization. It may be that the increase in ATP concentration of blood coming from exercising forearm muscle represents some residual ATP produced by recent activity of muscle and nerve. If indeed ATP is released from active skeletal muscle *in vivo*, the question must be raised as to the part it may play in the hyperaemia occurring in muscles during and following contraction, since ATP is a powerful vasodilator agent.

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