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Systematic Variations in the Content of the Purine Nucleotides in the Steady-State Perfused Rat Heart

EVIDENCE FOR THE EXISTENCE OF CONTROLLED STORAGE AND RELEASE OF ADENINE NUCLEOTIDES

By DAVID J. BATES,* DAVID PERRETT† and JOHN MOWBRAY*
**Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, U.K., and †Department of Medicine, St. Bartholomew's Hospital, West Smithfield, London EC1A 7BE, U.K.*

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1. The contents of the major purine nucleotides in the isolated non-working perfused rat heart varied systematically during 80min of perfusion. In particular the amounts of ATP, ADP, GTP, cyclic AMP and cyclic GMP in the well-oxygenated myocardium showed changes ranging from 25 to 60% of the mean concentrations. The apparent periodicity was about 30min for some and about 60min for other nucleotides. 2. These data are in contrast with measurements of parameters reflecting heart performance, which remained constant over this period of perfusion. 3. The ATP/ADP ratio, the cyclic AMP content, the GTP content and the GTP/GDP ratio in the tissue bore a constant relationship to one another, and all showed the same temporal variation. 4. Increasing the energy demand on the heart by administration of bovine somatotropin (1 μg/ml) tended to damp the variations, and generally lower the content of all the nucleotides. 5. The total extractable adenine nucleotide pool also showed systematic temporal variations of as much as 1.3 μmol/g wet wt. of tissue within 10min. 6. These variations could not be accounted for as interconversion with adenosine, other purine nucleotides, nucleosides or purine-degradation products either in the tissue or in the perfusion medium. No evidence was found in this preparation of the purine nucleotide oscillations described by Lowenstein and his coworkers [see Tornheim & Lowenstein (1975) *J. Biol. Chem.* **250**, 6304–6314]. 7. Further, the pool size increases cannot be satisfactorily explained by either synthesis *de novo* or the breakdown of any purine macromolecular species in the cell. Thus it is suggested that an unsuspected substantial storage form of purine nucleotide may exist in heart.

We have previously reported that the contents of cyclic AMP and cyclic GMP in the perfused rat heart may vary widely and in an apparently systematic manner during perfusion (Mowbray *et al.*, 1975). Further, the cyclic nucleotide variation appears to be independent of external effectors. Administration of a purified bovine somatotropin (growth hormone) preparation to the hearts led to a rapid stimulation of the protein-synthesis rate and a lowered content of both cyclic nucleotides. However, neither the total content of either nucleotide, nor their ratio, correlated well with the protein-synthesis rate in control or somatotropin-treated hearts. It was concluded that the data were consistent with an intracellularly directed role for these nucleotides. Cyclic AMP has long been regarded as an intracellular mediator of β-adrenergic stimulation (Robison *et al.*, 1971; Posternak, 1974). There is also evidence that in perfused heart the cyclic GMP content is altered by cholinergic effectors, which decrease heart contractility (George *et al.*, 1970, 1973). Thus the observed variations in these cyclic nucleotides during perfusion appear to stand in contrast with the constant values given by several other parameters measured in this preparation. After a 10min wash-out perfusion, the heart is apparently in a steady state for up to 2h as judged by oxygen uptake, glucose utilization, nicotinamide nucleotide ratio, glycogen turnover, lactate turnover, alanine output (Mowbray, 1969; Mowbray & Ottaway, 1973*a*,*b*), protein-synthesis rate (Mowbray & Last, 1974; Mowbray *et al.*, 1975), beat rate and contraction amplitude (this study). On the other hand there may be no conflict if, as has been suggested, cellular feedback-regulation systems give rise to stable oscillations on which metabolic steady states rely (Hess *et al.*, 1969). Moreover, Tornheim & Lowenstein (1975) have observed oscillations in the concentrations of the adenine nucleotides and IMP in particle-free rat skeletal-muscle extracts, which appear to be related to glycolytic oscillations.

In seeking a driving force for the large changes in cyclic AMP and cyclic GMP previously observed (Mowbray *et al.*, 1975) and to test whether such oscillations in nucleotide content may be taking place in Langendorf perfused hearts, we have examined over 80min of perfusion the content of all the major purine nucleotides in the perfused non-working rat heart. A preliminary account of this work has been presented (Mowbray *et al.*, 1977).

Experimental

Materials

Cyclic [8-^{3}H]AMP was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Bovine somatotropin was purchased from Miles Laboratories, Kankakee, IL, U.S.A. Tris base, theophylline, xanthine oxidase (EC 1.2.3.2), nucleoside phosphorylase (EC 2.4.2.1) and adenosine deaminase (EC 3.5.4.4) were supplied by Sigma (London) Chemical Co., Poole, Dorset, U.K. Uricase (EC 1.7.3.3) and cyclic AMP were obtained from Boehringer Corp. (London), Lewes, East Sussex, U.K. Norit GSX charcoal was purchased from Hopkin and Williams, Chadwell Heath, Essex, U.K. All other reagents were of analytical quality.

Methods

Perfusion and extraction. Male Sprague–Dawley rats weighing 200–250g were deprived of food overnight. Hearts were removed under ether anaesthesia, weighed, wash-out preperfused for 10min and perfused with recirculation as described by Mowbray & Ottaway (1973*a*) under conditions in which variations in the tissue content of cyclic nucleotides had been previously observed (Mowbray *et al.*, 1975). The perfusion medium contained, in addition to 5.5mM-D-glucose, a mixture of L-amino acids found in rat plasma, but at four times normal concentrations (Mowbray & Last, 1974). Purified bovine somatotropin (see below) was added to the perfusate reservoir at 30min in some perfusions to give a final concentration of 1μg/ml (Mowbray *et al.*, 1975).

After the perfusion period, the hearts were clamped between liquid-nitrogen-cooled aluminium plates, and then homogenized at −10°C in 2ml of an aqueous solution of 10% (w/v) trichloroacetic acid containing 25% (v/v) methanol with an Ultra-Turrax homogenizer (Janke und Kunkel, Staufen i. B., W. Germany). The homogenizer was washed with another 2ml of the trichloroacetic acid/methanol. The homogenate was centrifuged at 9000*g* for 20min at 2°C, and the supernatant freed of trichloroacetic acid and methanol by extraction with water-saturated ether. It was adjusted to pH6–7 with Tris-base crystals and, together with samples of the perfusion medium, stored at −20°C until analysed.

High-performance liquid chromatography. The non-cyclic purine nucleotides in the heart extracts were determined by high-performance liquid chromatography as described by Perrett (1976).

Cyclic AMP assay. The heart extracts were freeze-dried and resuspended in 50mM-sodium acetate, pH4.2. Cyclic AMP was determined essentially by the saturation assay procedure of Tovey *et al.* (1974), by using a binding protein purified from frozen bovine skeletal muscle, except that theophylline and 2-mercaptoethanol were also included as in the assay of Brown *et al.* (1971). There was complete recovery in the assay of cyclic AMP standard added to the samples.

Cyclic GMP assay. The heart extracts were freeze-dried and resuspended in 50mM-Tris buffer containing 4mM-EDTA, adjusted to pH7.5 with HCl. Cyclic GMP was determined by using the radioimmunoassay kit supplied by The Radiochemical Centre. The assay is claimed to be 10^5 times less sensitive to cyclic AMP, and thus prior fractionation of the nucleotides was deemed not to be necessary. When tested with added cyclic AMP, the assay gave about 0.4pmol of apparent cyclic GMP per 100pmol of added cyclic AMP. Whether this implies that the assay is actually only 250 times less sensitive to cyclic AMP or that the added cyclic AMP contained 0.4% cyclic GMP is unknown. Even if the former were true it would only introduce an error of 5% in the cyclic GMP determinations at the maximum cyclic AMP/cyclic GMP ratio observed. The assay substrate in extracts was found to be removed by phosphodiesterase pretreatment, and the entire amount of standard cyclic GMP added to extracts was detected.

Measurement of purine-degradation products. The purines released into the perfusion medium by the heart were determined spectrophotometrically. Hypoxanthine (+xanthine), inosine and adenosine were assayed by sequential addition of xanthine oxidase, nucleoside phosphorylase and adenosine deaminase respectively, by using an Aminco dual-wavelength recording spectrophotometer to follow the increase in A_{293} corresponding to formation of uric acid, as described by Olsson (1970).

Uric acid was assayed by following the decrease in A_{293} after addition of uricase to a separate sample of perfusate, by using the method described by Scheibe *et al.* (1974).

Somatotropin purification. Bovine somatotropin was purified by ethanol precipitation and chromatography on Sephadex G-75 [Pharmacia (G.B.) Ltd., London W5 5SS, U.K.)] as previously described (Mowbray *et al.*, 1975).

Measurement of beat rate and contraction amplitude. The beat rate and amplitude of perfused hearts were monitored by connecting the apex of the heart to a Statham pressure transducer with cotton thread. The resulting signal was displayed on a chart recorder

with a fast response time (type R1 recorder, Devices Ltd., Welwyn Garden City, Herts., U.K.).

Results and Discussion

Content of the cyclic purine nucleotides in the perfused rat heart

The heart contents of cyclic AMP and cyclic GMP as a function of perfusion time are shown in Fig. 1. These findings are in agreement with the fluctuations in these nucleotides previously observed (Mowbray *et al.*, 1975). The apparent temporal positions of the maxima and minima tend to vary a little from one batch of experimental perfusions to another. Administration of bovine somatotropin to the hearts again decreased the fluctuations seen in the control perfused hearts.

It has been proposed that cyclic AMP may mediate the positive inotropic effect of β-adrenergic agonists on the heart, and this is broadly supported, with some exceptions, by the large amount of work which has sought a direct connection between increased cardiac contractility and intracellular cyclic AMP content (for review see Tsien, 1977). It might be expected, therefore, that the changes in cyclic AMP content seen in these hearts would be accompanied by detectable changes in mechanical activity. However, neither the amplitude of the heart beat nor the beat rate reflected the cyclic AMP fluctuations (Fig. 2).

In contrast with cyclic AMP, cyclic GMP in cardiac muscle has been proposed as a mediator of the negative inotropic effects of cholinergic agents (George *et al.*, 1970, 1973), and supporting evidence that cholinergic stimulation leads to an increased cyclic GMP content has been provided by other laboratories (Kuo *et al.*, 1972; Gardner & Allen, 1976). Further, the addition of cyclic GMP or its synthetic analogues has been found to mimic the effect of cholinergic agents on isolated cardiac cells (Krause *et al.*, 1972; Goshima, 1976; Ghanbari & McCarl, 1976) and cardiac tissue (Watanabe & Besch, 1975; Wilkerson *et al.*, 1976; Nawrath, 1976). However, the contractile performance of the hearts in the present study (Fig. 2) shows no correlation with changes in tissue cyclic GMP content (Fig. 1). It is possible that if both cyclic nucleotides changed together they might cancel out each other's effect on contractility (cf. Watanabe & Besch, 1975). It is not clear whether such an antagonism would require that the ratio of the overall cyclic nucleotide concentrations be proportional to indices of mechanical performance. Certainly this does not appear to hold in experiments reported here, since the cyclic nucleotide ratio (Fig. 3) shows significant changes between 0 and 30min ($P<0.01$) and between 60 and 80min ($P<0.05$).

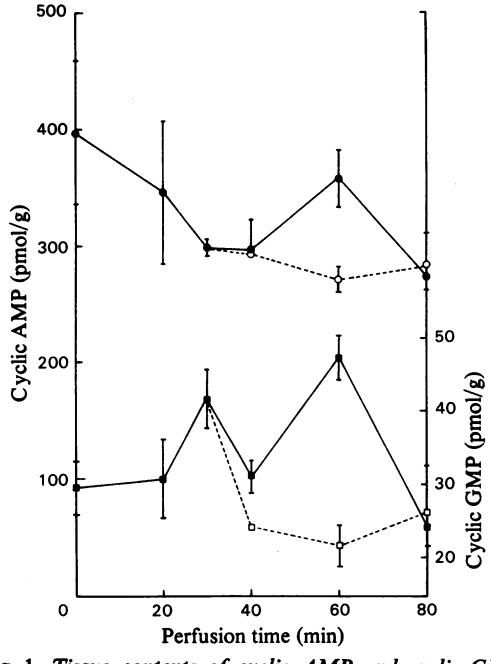

Fig. 1. *Tissue contents of cyclic AMP and cyclic GMP in the perfused heart*

Hearts were perfused in the absence (●, ■) or in the presence from 30min of somatotropin (1 μg/ml) (○, □). Further experimental details are given in the text. The cyclic AMP (●, ○) and cyclic GMP (■, □) values are shown as means±S.E.M. for at least four separate hearts (actual numbers are given in Table 1), except that at 40min in the somatotropin-treated series the values are from a single heart. The contents of cyclic AMP and cyclic GMP at 60min have been significantly lowered by somatotropin (*t* test: $P<0.0125$ and $P<0.0025$ respectively).

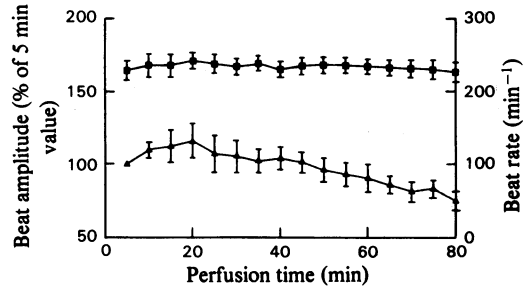

Fig. 2. *Mechanical performance of the perfused heart*

The Figure shows the beat rate (■) and beat amplitude (▲) of hearts perfused in the absence of somatotropin under the same conditions as in the experiment shown in Fig. 1. Further experimental details are given in the text. Each point represents the mean±S.E.M. for seven (■) or five (▲) hearts.

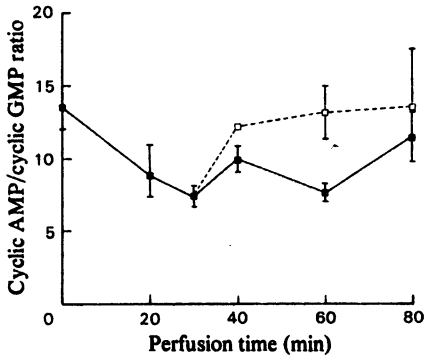

Fig. 3. *Calculated ratio of cyclic AMP/cyclic GMP*
The means (±S.E.M.) of the ratio of cyclic AMP/cyclic GMP in each heart are shown. The hearts (■, control; □, somatotropin-treated) were the same as those used in Fig. 1.

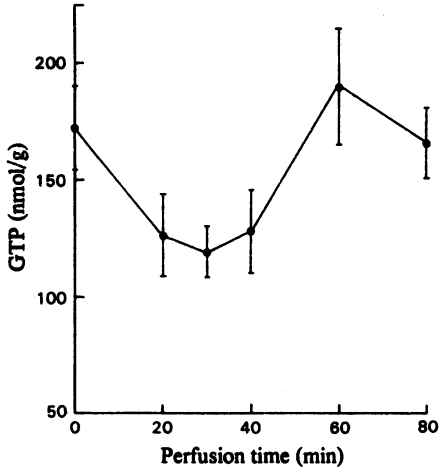

Fig. 4. *Tissue content of GTP in the perfused heart*
The Figure shows the GTP content of the hearts in the experiment shown in Fig. 1. Only the control hearts are given (●), since there was incomplete resolution in the high-performance liquid-chromatography system of GTP in the extracts from somatotropin-treated hearts. Each point represents the mean± S.E.M. for at least four separate hearts, except at 0 min and 30 min where the mean and spread are given, since the GTP content of only three hearts was adequately resolved.

Content of the non-cyclic purine nucleotides in the perfused rat heart

The contents of the major non-cyclic purine nucleotides were measured in the extracts of the same hearts in which the cyclic nucleotides had been found to vary. The contents of GTP (Fig. 4), ADP and ATP (Fig. 5) also varied markedly with perfusion time, and the GTP fluctuations, but not those of ADP or ATP, were remarkably similar in apparent periodicity to those observed for cyclic AMP. Administration of somatotropin to the hearts appeared to decrease the variations as it did the cyclic nucleotide variations. By contrast, the amounts of AMP, GDP and IMP remained relatively constant throughout the perfusion (Table 1).

The magnitude of the changes in the ADP and ATP content raises the question as to why they have not been previously observed. It has been generally found that the concentrations of the adenine nucleotides in the perfused heart remain constant despite their rapid turnover (Williamson, 1965, 1967; Neely *et al.*, 1970, 1972; Nayler & Seabra-Gomes, 1976; Nayler *et al.*, 1976). One possible explanation for this discrepancy may lie with the denaturation and extraction methods generally employed. The freeze-clamped tissue is usually ground up with or homogenized in $HClO_4$ or trichloroacetic acid at 0°C or at higher temperatures. These procedures have often been found to result in the redistribution of the phos-

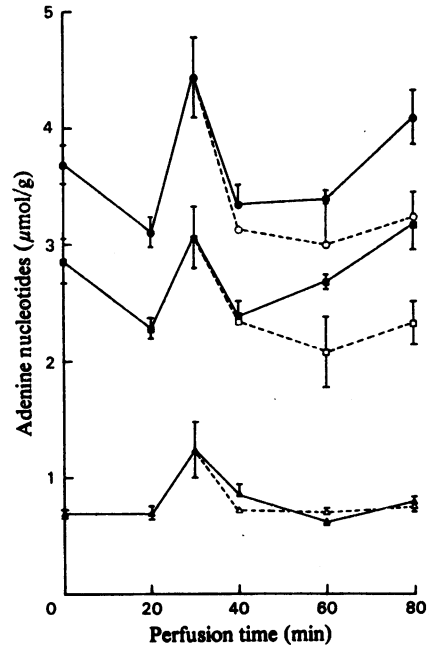

Fig. 5. *Tissue contents of ADP and ATP in the perfused heart*
The Figure shows the means±S.E.M. of the ADP content (▲) and ATP content (■) and the sum of the AMP, ADP and ATP contents (●) of the hearts in the experiment shown in Fig. 1. The somatotropin-treated hearts are depicted by the open symbols. The numbers of hearts are as given in Fig. 1.

Table 1. *Contents of AMP, IMP and GDP in the perfused heart*

The contents of AMP, IMP and GDP of the control hearts in the experiment shown in Fig. 1 are mostly given as mean values with S.E.M. Where incomplete resolution of the material from a large neighbouring peak made accurate measurement by high-performance liquid chromatography impossible, only values from three hearts are used. In these cases the data are shown as mean values with the spread in parentheses. In one case GDP was not resolved completely in any of the hearts (i.e. 30min).

Perfusion time (min)	No. of hearts	Content (nmol/g wet wt. of tissue) AMP	IMP	GDP
0	4	146±39	84±14	69 (42–95)
20	4	138±46	51±35	70 (65–74)
30	5	139±25	105±18	—
40	4	133 (95–175)	26 (20–30)	64 (37–80)
60	6	96±31	91±29	80±7
80	5	97±21	78 (60–100)	70±12

phate content of the adenine nucleotides, leading to low ATP/ADP and ATP/AMP ratios, low phosphocreatine and high P_i contents (Seraydarian *et al.*, 1961; Minard & Davis, 1962; Lowry *et al.*, 1964; Wijsman, 1976). Although some of these changes may be due to non-enzymic hydrolysis, certain enzymes, notably in this context adenylate kinase (Colowick & Kalchar, 1943; Williamson & Corkey, 1969) and creatine kinase (J. R. Griffiths, personal communication), are not destroyed by acid denaturation, although they are inactivated by acid/alcohol extraction at −10°C. If adenylate kinase and creatine kinase remain active in acid extracts, the values reported for adenine nucleotides in these conditions must be regarded as uncertain, especially when extraction is followed by enzymic assay involving incubation. In this study we have used trichloroacetic acid/methanol extraction at −10°C.

Other workers (Opie *et al.*, 1971) have observed significant changes in the adenine nucleotide content of perfused rat hearts which were induced to work harder, but only when they used a $HClO_4$/acetone/EDTA mixture as a denaturing agent: when $HClO_4$ was used alone no such variations were seen. This use of chelating agents in cold organic-solvent/aqueous mixtures has been found to prevent phosphate exchange in extracts (Seraydarian *et al.*, 1962; Lowry *et al.*, 1964).

The possibility that the observed variations in the purine nucleotides might be artifacts must be considered. Each measurement required the use of a single heart. Moreover the hearts used for each time point were perfused and extracted on different occasions. Despite this randomized sampling of the time course, the hearts behaved in a sufficiently synchronous manner that a significant difference between two points 10min apart could be established with only about four hearts per point. This suggests that inefficient chemical procedures during extraction cannot of themselves account for the data. Repeated extraction did not appreciably alter the yields of acid-soluble nucleotides. Also, the observed fluctuations in the content of the purine nucleotides could not have arisen from changes in the water content of the heart during perfusion (see Opie, 1965), since the data are standardized against the wet weight of the heart measured before perfusion. The protein content measured (Lowry *et al.*, 1951) after denaturation bore a constant relationship to wet weight measured in this way. The mean protein content (±S.E.M.) was 111±3mg of protein/g wet wt. of tissue.

Unlike the tissue contents of ATP and ADP, the ATP/ADP ratio (Fig. 6) did appear to vary in unison with the contents of GTP and cyclic AMP, and, on

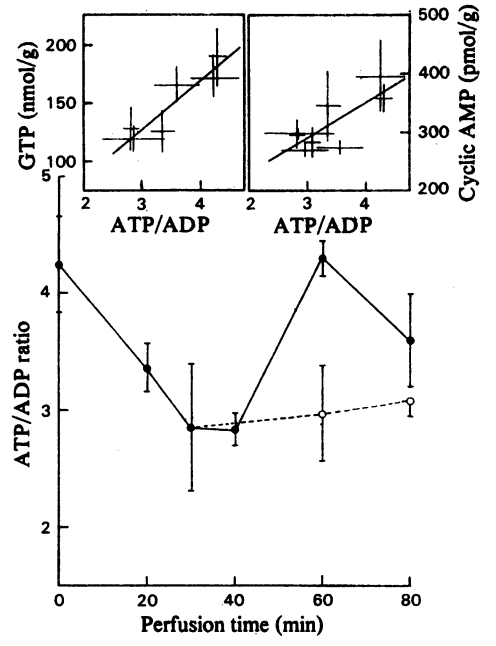

Fig. 6. *Calculated ratio of ATP/ADP in the perfused heart*

The Figure shows the mean values (±S.E.M.) of the ratio of ATP/ADP measured in each heart. The hearts (●, control; ○, somatotropin-treated) were those used in Fig. 5. The ratio at 60min was significantly lowered by somatotropin (*t* test: $P<0.01$). The insets show a significant correlation between the ATP/ADP ratio and both the cyclic AMP content ($r=0.838$; $P<0.01$) and the GTP content ($r=0.980$; $P<0.001$). The data are from Figs. 1, 4 and 7 and the bars represent S.E.M. of each value.

addition of somatotropin, the ratio showed little variation, much like the cyclic nucleotides. From 40min the cyclic GMP content of control perfused hearts would also appear to correlate with this ratio, but no clear relationship exists before this time.

The relationship between the ATP/ADP ratio and both the cyclic AMP content and the GTP content suggests a possible mechanism by which cyclic AMP may respond to changes in the ATP/ADP ratio. GTP has been found to be an activator *in vitro* of adenylate cyclase (EC 4.6.1.1) preparations from heart (Birnbaumer & Yang, 1974; Lefkowitz, 1974, 1975) and many other tissues (Birnbaumer & Yang, 1974; Bockaert *et al.*, 1972; Krishna & Harwood, 1972; Rodbell *et al.*, 1971; Wolff & Hope Cook, 1973). Further, GDP has been found to oppose the effect of GTP on adenylate cyclase (Birnbaumer & Yang, 1974; Harwood *et al.*, 1973). Since the GDP content of the hearts was unchanged throughout the perfusion (Table 1), the GTP/GDP ratio would follow the same pattern of variation as the GTP content (Fig. 4). Although the apparent GTP concentration in our hearts is much greater than required to activate fully broken-cell preparations of adenylate cyclase, it may be that the activity of adenylate cyclase in these hearts could be regulated by the GTP/GDP ratio, and the observed correlation between this and the cyclic AMP content would be consistent with such a mechanism.

Since the extramitochondrial GTP is probably synthesized in rat heart from GDP and extramitochondrial ATP by nucleoside diphosphate kinase (EC 2.7.4.6; Jacobus & Evans, 1977), a fall in the ATP/ADP ratio might well be followed by a fall in the GTP/GDP ratio, and ultimately by a decrease in the cyclic AMP content due to inhibition of adenylate cyclase. That the correlation is so good when the overall tissue concentrations are considered is all the more surprising, since the nucleotides may be unevenly compartmented within the cell.

The question remains as to how the fluctuations in the purine nucleotides arise. Such wide variations seem unlikely to be a property of this tissue *in vivo*, in which regulatory mechanisms maintain a much more constant concentration. However, these preparations are significantly different from the heart *in vivo* in at least two respects. First, the Langendorf perfused heart is not contracting against a circulatory load and thus presumably requires much less ATP for contraction. Second, *in vivo* the tissue is subjected to a barrage of extracellular regulatory effectors, both neural and humoral, and it may be significant that somatotropin (Figs. 1, 2, 5 and 6) and adrenaline (D. J. Bates, unpublished work) treatments tend to stabilize the fluctuations in the purine nucleotides. Thus while these variations might be generated by intracellular regulatory processes, the hearts are operating in conditions beyond the capacity of these systems to maintain constant concentrations of the nucleotides. Overcompensation in some part of the regulatory mechanism may then have led to the generation of an oscillatory process; the data are indeed compatible with oscillatory behaviour.

Variations in the total adenine nucleotide pool

The total extractable adenine nucleotide pool fell significantly during the first 20min of perfusion ($P < 0.05$; Fig. 5). This was almost entirely a result of a decrease in the amount of ATP (Fig. 5), but could not be explained as the operation of a purine nucleotide cycle (Tornheim & Lowenstein, 1975), since the heart content of IMP did not alter appreciably during this period (Table 1). Neither could an appreciable change in the total tissue nucleoside or free base content be detected (results not shown). However, sufficient purine nucleotide-degradation products were found to be released to the perfusion medium in this period to account for the tissue losses (Fig. 7). Small amounts of hypoxanthine, xanthine, inosine and adenosine were found, which is consistent with reports that these are released by the well-oxygenated myocardium (Rubio & Berne, 1969; Rubio *et al.*, 1972). In addition, uric acid was excreted into the medium in amounts commensurate with the known xanthine oxidase activity of rat

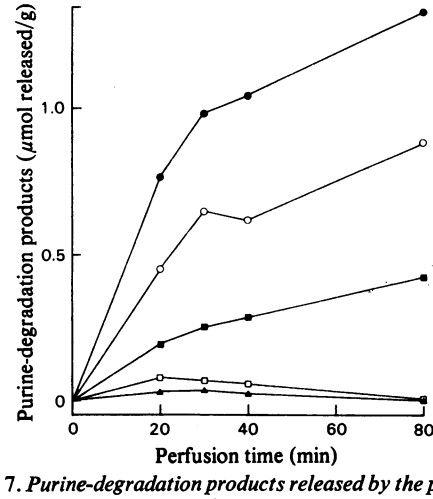

Fig. 7. *Purine-degradation products released by the perfused heart*

The Figure shows the purines or their degradation products released into the perfusion medium by the control perfused hearts in the experiment shown in Fig. 1. The data are expressed per g wet wt. of tissue and represent the mean values at each time point (▲, adenosine; □, inosine; ■, xanthine+hypoxanthine; ○, uric acid; ●, total purine-degradation products).

heart (Maguire *et al.*, 1972; Brunschede & Krooth, 1973).

Very surprisingly the average value found for the content of total adenine nucleotides at 30min increased by about 1.3μmol/g over the 20min value ($P<0.0125$; Fig. 5), representing an increase in pool size of around 40% in 10min. This change could not be due to reutilization of medium purine by the salvage pathways (Goldthwait, 1957; Kolassa *et al.*, 1970; Liu & Feinberg, 1971; Maguire *et al.*, 1972), since these compounds increased in amount during this period (Fig. 7). Moreover, the 30min value for the total adenine nucleotide content showed an increase over the zero-time value of around 0.7μmol/g. Neither could the increase be wholly attributed to conversion of other purine nucleotides in the heart, since their amount decreased by only 0.1μmol/g in this time (Table 1, Fig. 4).

It seems unlikely anyway that nucleotide production from the salvage pathways would contribute significantly to the observed increase. Although no data are available for rat heart, Liu & Feinberg (1971) have measured a maximum rate of nucleotide synthesis from adenosine in perfused rabbit heart of about 20nmol/h per g. The recovery *in vivo* of heart adenine nucleotide content after asphyxia in rabbits (i.e. salvage plus synthesis *de novo*) has been reported to be 1nmol/min per g (Isselhard *et al.*, 1970*a*), increasing to 10nmol/min per g during infusion with adenosine (Isselhard *et al.*, 1970*b*).

Synthesis of purine nucleotide *de novo* appears to be an equally unsatisfactory explanation. The rate in rat heart has been estimated from [1-^{14}C]glycine incorporation into adenine nucleotide and found to be 8.4nmol/h per g *in situ* and only 1.3nmol/h per g in perfusion (Zimmer *et al.*, 1973). The latter value can increase to 8.8nmol/h per g during post-ischaemic recovery (Gerlach & Zimmer, 1976), but this is still some 800 times less than the rate required by the observed change. Since the large changes in the amount of total adenine nucleotide in the acid-soluble fraction of the hearts cannot be explained by synthesis *de novo* or by purine interconversion, the conclusion appears to be that relatively large quantities of nucleotides can be reversibly sequestered in heart. Some adenine nucleotide binds to myofibrils. Heart has around 0.5μmol of actin and 0.14μmol of myosin per g, which are known to bind respectively 1 and 2mol of nucleotide per mol of protein (see, e.g., Wilkie, 1976). Thus these proteins could bind around 0.75μmol of nucleotide/g of heart, but this is non-covalent attachment and appears to be fully extracted by dilute acid (Seraydarian *et al.*, 1962). It also seems possible to ask whether other cellular processes such as nucleic acid turnover could substantially affect the purine nucleotide concentrations. Many mRNA molecules, for example, have polyadenylate tails. However, accepting the estimates for the proportion of cell RNA which is mRNA and assuming an average size for proteins and hence for message length, one can estimate the number of mol of messenger in heart. Then, assuming that each mol of messenger has 200mol of adenylate in its poly(A), this gives an upper limit (see Yannorell *et al.*, 1976) for the amount of adenine nucleotide that could be sequestered as poly(A). This turns out to be around 40nmol/g wet wt. of heart, i.e. 3% of the observed change in pool size. Turning to consider RNA itself, the size of the change seen would require the breakdown and resynthesis of about 125% of total heart RNA (1.45mg of RNA/g wet wt. of heart; C. M. Mackie, unpublished work), which is plainly not sensible. On the same basis about 275% of cell NAD and NADP (Burch *et al.*, 1963, 1967) would be required and can thus be excluded as a serious possibility. Trichloroacetic acid-insoluble extracts of rat liver have been found to contain mono(ADP-ribose) and poly(ADP-ribose) (Stone & Hilz, 1975; Stone *et al.*, 1976). If the heart also contained similar amounts of these compounds, their complete degradation would only account for an additional 22nmol/g.

Since none of the presently known sources of purine nucleotides in the heart could feasibly contribute to the rapid sequestration and release of adenine nucleotides observed here, we would suggest that an unsuspected substantial storage form of purine nucleotide may exist in the heart. Some evidence does exist for purine nucleotide store compounds. Diguanosine polyphosphates [e.g. guanosine(5')-tetraphospho(5')guanosine] have been found in considerable quantities as naturally occurring nucleotides in the brine shrimp (Finamore & Warner, 1963; Warner & Finamore, 1965) and in *Daphnia magnum* (Oikawa & Smith, 1966). A range of aquatic fungi also possess different, though as yet incompletely identified, polyphosphate compounds (Warner *et al.*, 1977; Goh & Lé John, 1977). The precise function of these nucleotides is not known, but it is possible that they serve as a stable energy reservoir, being converted into ATP with conservation of pyrophosphate bonds (Clegg *et al.*, 1967), and they appear to act as a purine store during embryogenesis in *Artemia salina*, when purine-synthetic pathways are absent (Warner & McClean, 1968).

The existence of a nucleotide store in the mammalian heart is supported by the observations that only 83% of the total [8-^{14}C]adenosine and 77% of the [8-^{14}C]inosine taken up by perfused rabbit heart appeared in the acid-soluble extract (Liu & Feinberg, 1971). The labelling of a nucleotide which is not extractable in acid provides a possible explanation for their low yields. Further investigations to ascertain the existence and nature of a purine nucleotide store in heart are required.

The GMP content of these hearts was negligibly low and thus, from Table 1 and Fig. 4, it is clear that

the tissue guanine nucleotide content is also changing with time. The values are significantly different between 40min and later times ($P < 0.001$). Although the proportional change in guanine nucleotide content was also around 40%, the variations were not in register with those of the adenine nucleotide content but rather followed the GTP time course.

It is pertinent to ask whether a controlled availability of adenine nucleotide seems a likely function in cellular regulation. Given the now widely held view that virtually all enzymes are substrate- and coenzyme-limited, the overall activity in cells is probably balanced between energy production and work (i.e. ATP synthesis and ATP utilization) by the effective adenine nucleotide concentration. Many cells, and certainly muscle tissue, are required *in vivo* to alter rapidly their basal activity by severalfold in response to demand. One possible mechanism for doing this would be to regulate adenine nucleotide availability. Interestingly, cell cultures have been observed to adjust their adenine nucleotide pool before changes in growth rate (Warren & Glinos, 1976): the total pool size declines by more than 50% in going from exponential growth to stationary phase and increases in a corresponding manner when growth is re-stimulated.

It seems clear that a complex interrelationship exists between the purine nucleotide availability, the adenine nucleotide ratio, the GTP content and/or the GTP/GDP ratio and the cyclic AMP content in steady-state rat hearts. If a rapidly controlled adenine nucleotide store does exist, it is not difficult to visualize in a general way how changes in net mobilization and sequestration could lead to alterations in adenine nucleotide ratio. It is tempting to speculate that cyclic AMP, which is involved in mobilizing glucose from storage, might also be involved in regulating the available adenine nucleotide pool size, and if cyclic AMP is indeed related in intact cells to the adenine nucleotide and guanine nucleotide ratios as suggested above, then the existence of such a loop of connections might well result in oscillatory behaviour under certain conditions. It is not possible to propose precise models of such a system without prior knowledge of the continuous variation and hence the precedence in the pattern shown by these parameters, although it does appear a suitable system where computer simulation might allow some quantitative evaluation of the relationships.

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