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HYPOXANTHINE NUCLEOTIDES AND MUSCULAR CONTRACTION

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SUMMARY

1. Experiments have been devised to test the suggestion of a number of investigators that the interconversion of adenine and hypoxanthine nucleotides may provide the energy for muscular contraction.

2. Several methods for the assay of very small amounts of these nucleotides in muscle extracts have been examined. These involve spectrophotometry, radioactivity, electrophoresis and chromatography. They have been applied to a study of the production of the hypoxanthine nucleotides during single contractions of the frog rectus-abdominis muscles at 0°.

3. Small amounts of the hypoxanthine di- and trinucleotides were detected in resting muscles but there were no significant net changes in either the adenine or hypoxanthine nucleotides during single contractions.

INTRODUCTION

LOHMANN in 1934[1] demonstrated that enzymically active, dialyzed extracts of muscle were incapable of hydrolysing phosphorylcreatine except in the presence of added adenine nucleotides. This and further work[2] led to the conclusion that in living muscle, the hydrolysis of ATP during contraction to form ADP must necessarily precede in time the breakdown of phosphorylcreatine. Thus ATP was proposed to be the immediate source of energy for muscular contraction by way of the LOHMANN reaction:

$$\text{ATP} \longrightarrow \text{ADP} + \text{P}_i$$

$$\text{ADP} + \text{phosphorylcreatine} \longrightarrow \text{ATP} + \text{creatine}$$

Belief in this mechanism has been greatly strengthened by the striking effects of the nucleotides on muscle-model systems[3] and by the absence of an alternate pathway of phosphorylcreatine utilization in muscle tissue[4,5].

A number of workers have carried out analytical experiments with living muscles in an attempt to test the operation of the LOHMANN reaction *in* [illegible]ly work by LUNDSGAARD[6,7] on iodoacetate-treated muscles demonstrated a correlation between

Abbreviations: 3-PGA, 3-phosphoglyceric acid; PCr, phosphorylcreatine.

phosphorylcreatine hydrolysis and the work done in muscles. There were no significant changes in ATP content ("pyrophosphat") until rigor developed. MUNCH-PETERSEN[8], CALDWELL[9], FLECKENSTEIN, JANKE, DAVIES AND KREBS[10], MOMMAERTS[11,12], CHANCE AND CONNELLY[13], CARLSON AND SIGER[14] and HOHORST, REIM AND BARTELS[15] all found that during single or small numbers of contractions the small or statistically insignificant changes observed in the concentrations of the adenine nucleotides were insufficient to account for the work done by the muscles. Even prolonged series of contractions failed to alter the ATP concentration until the muscle approached a state of rigor at which time a rapid breakdown of ATP occurs[6,7]. These efforts followed an initial report by MOMMAERTS AND RUPP[16] in which they described their finding of a decrease in the ATP content in contracted muscles by an amount sufficient to account for the work done.

LANGE[17-19] on the other hand found that tetanic contractions (up to 10 sec in duration) brought about a decrease in the ATP concentration of 0.25 μmole/g muscle with a concomitant increase of 0.1–0.4 μmole/g in the content of inosine monophosphate (hypoxanthine mononucleotide, IMP).

WAJZER, WEBER, LERIQUE AND NEKHOROCHEFF[20] reported finding changes in the ultraviolet absorption of living muscle during contraction, which they interpreted as a qualitative demonstration of a "reversible dephosphorylation–deamination of ATP during a single twitch". In later analytical experiments WAJZER, NEKHOROCHEFF AND DONDON[21] found a decrease of 1.5 μmoles/g in the total adenine nucleotides and an increase of 0.5 μmole in the hypoxanthine nucleotides. In view of these contradictory findings, a series of experiments have been undertaken to investigate the role of hypoxanthine nucleotides in the process of muscular contractions.

METHODS

Basic experimental procedure

Female *Rana pipiens* stored at 0–5° in tap water were used unless otherwise stated. The frogs were killed by severing the spine and the spinal cord was destroyed to eliminate spontaneous twitches during the dissection. The rectus abdominis muscles were dissected free with the xiphisternal and pubic attachments intact and separated along the midline. They were placed in an open petri dish of physiological bicarbonate saline solution[22] and allowed to rest for at least 1 h at room temperature. The muscles were then singly transferred to an isotonic lever which traced on a kymograph, adjusted to their rest length *in vivo* and cooled to 0° by immersion for 4 or 5 min in bicarbonate–saline solution kept at 0°. This solution was then quickly removed and the muscle was rapidly frozen in place[23] by surrounding it with a container of dichlorodifluoromethane (−160°) or chlorotrifluoromethane (−185°) either at once (control muscle) or after electrical stimulation (12 pulses/sec for 1–2 sec) to nearly maximal shortening (experimental muscle). During isotonic shortening the experimental muscle performed about 125 g × cm work/g muscle.

The frozen muscle was cut from its attachments and shattered in a stainless steel percussion mortar[23]. The pellet obtained was transferred to a stainless steel centrifuge tube (International No. 298) where 0.25 ml of frozen 0.25 M $HClO_4$ was ground into the pellet with a close-fitting stainless steel ball attached to a handle. These procedures were all carried out at −196°. The paired samples were then simultaneously thawed

by adding 1–4 ml of cold 0.25 M $HClO_4$, extracted for 3 min at 0° and centrifuged for 2 min (International high-speed attachment No. 2550). The supernatant extract was recovered and rapidly neutralized at 0° on a pH meter (Beckman Model H 2) with KOH, frozen and stored at −35°. When required for analysis the extract was thawed and the $KClO_4$ precipitate was removed by centrifugation at 0°.

Spectrophotometric method

The ratio of hypoxanthine nucleotides to adenine nucleotides was determined on samples of the extracts by differential spectrophotometry according to the method described by SZENTKIRALYI[24].

^{32}P-labelling of isolated muscles

After the usual 1-h rest at room temperature, the isolated muscles were transferred to equal portions (2.7 ml) of bicarbonate–saline solution containing 0.1 mM [^{32}P]P_i (100–200 μC per muscle). Each muscle was removed from the ^{32}P-solution 60–90 min later, suspended at the kymograph and electrically stimulated 3 times for 3 sec each time, to do a total of about 400 g × cm external work/g muscle. The purpose of this procedure was to cause a turnover of all of the compounds involved in muscular contraction and to ensure a closer approach to uniform specific activity among the phosphorus compounds. After re-equilibration in the radioactive solution for another 60–90 min, the muscles were taken through the remainder of the procedure as described above.

^{32}P-labelling in vivo

A frog which had been in hibernation for about 4 months was given 300 μC of [^{32}P]P_i by intraperitoneal injection from the dorsal side. It was kept in the cold room (0–4°) in about 50 ml of tap water for 3 days and then transferred to a 25°-room for 1 day to allow more a rapid turnover of the ^{32}P. The frog was cooled overnight at 0–4° and then killed. The muscles were quickly dissected out, allowed to recover in phosphate-free bicarbonate–saline solution at 25° for about 30 min and then taken through the usual contracted *versus* control experimental procedure.

Separation and measurement of nucleotides

Since the β- and γ-P-atoms of all of the nucleotides have approximately the same specific activity and the α-P-atom has negligible activity (see later), the measurement of the total radioactivity associated with each nucleotide in a portion of the extract becomes a measure of the total amount of that nucleotide present. This is the basis of the calculations used to measure the concentrations of the nucleotides in the ^{32}P experiments.

Samples of the nucleotides were added to the neutralized extracts to act as carriers, and the nucleotides in these extracts were then separated from the other ^{32}P-labelled compounds by adsorption onto and elution from small charcoal columns by a modification of the method described by THRELFALL[25], or by elution from paper after chromatographic separation with Solvent 1 (absolute ethanol – 1 M ammonium acetate (pH 3.8) – 0.1 M sodium EDTA (pH 8.2) (75:29:1, v/v)), descending for 25 h at 23°. This solvent was found to separate P_i, PCr, hexose monophosphates, Fru-1,6-P_2 and the glyceric acid phosphates from the nucleoside di- and tri-phosphates (Fig. 1);

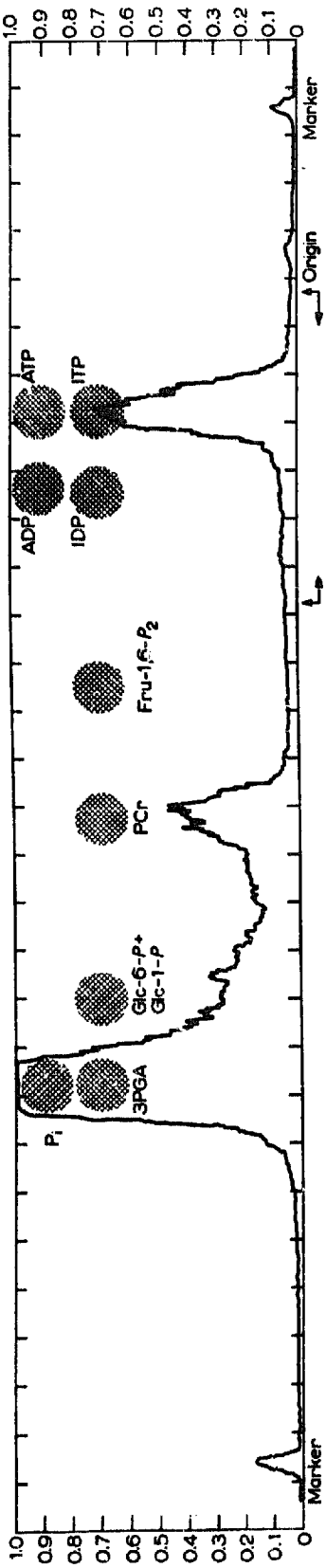

Fig. 1. Distribution of ^{32}P on a chromatogram of a radioactive muscle extract. The separation system was Solvent 1. The hatched areas indicate the location of carrier compounds and the area between the arrows indicates the portion taken for elution. Full-scale deflection of the scanner was 10 000 counts/min.

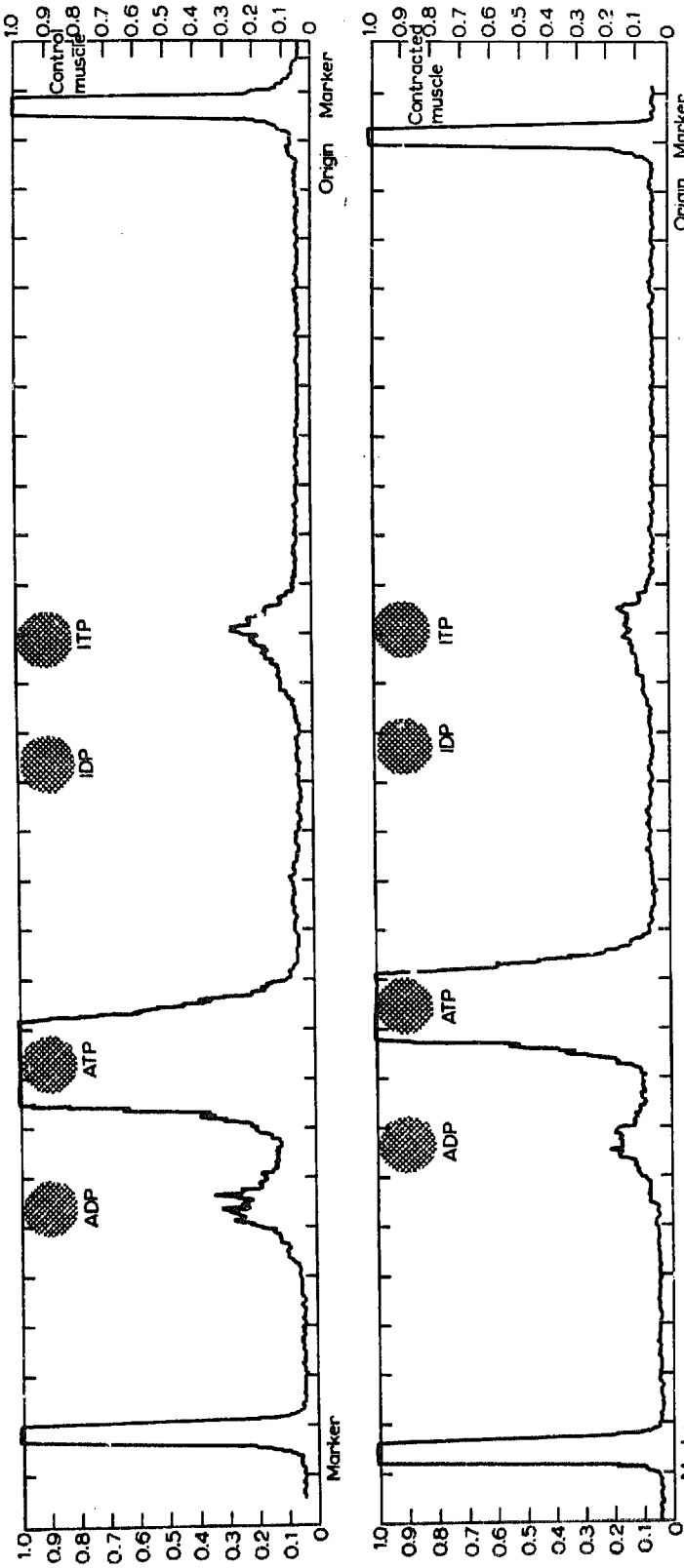

Fig. 2. Distribution of ^{32}P on a chromatogram of the eluted region of Fig. 1 or of the eluates from the charcoal columns. The hatched areas indicate the location of the carrier nucleotides. The separation system was Solvent 2. Full-scale deflection was 1000 counts/min. The quantitative data obtained from these and similar chromatograms are given in Table IV. N.B. The amounts of radioactive material applied to the chromatograms were not made equal since only the relative amounts of activity in the various components were used in the calculations.

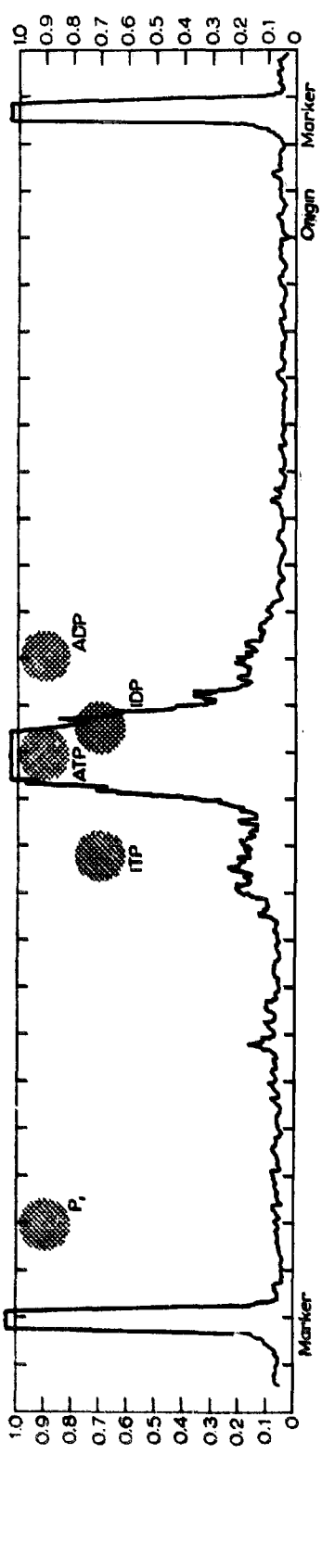

Fig. 3. Distribution of ^{32}P on a paper-electrophoresis strip of the radioactive compounds in the eluted area of Fig. 1. The buffer used was pyridine – glacial acetic acid – water (100:10:890, v/v). Note the virtual absence of ^{32}P in the Pi region. Full-scale deflection of the scanner was 1000 counts/min.

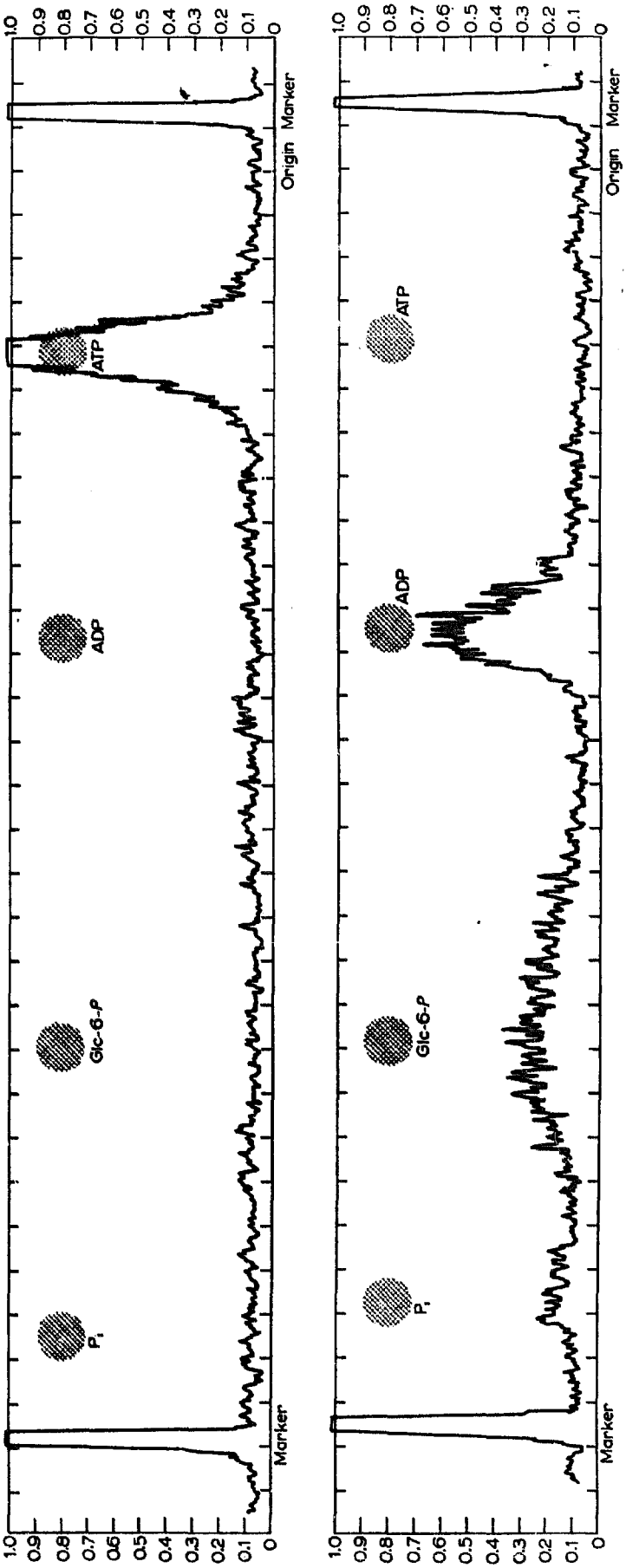

Fig. 4. The upper tracing describes the distribution of ^{32}P on a paper chromatogram of material isolated from the ATP region of Fig. 2. The separation system used was Solvent 1. The lower tracing shows the separation of the products obtained on treatment of the isolated [^{32}P]ATP with glucose and hexokinase. Full-scale deflection of the scanner was 300 counts/min.

the EDTA greatly increases the resolution obtained. Final separation of ATP, ADP, ITP and IDP was obtained by paper chromatography in an isobutyric acid solvent system (Fig. 2), Solvent 2, (isobutyric acid–1 M NH_4OH–0.1 M sodium EDTA (pH 8.2) (100:60:1.6, v/v)) descending for 35 h at 23°. The nucleotides were located with a Mineralight SL-2537 ultraviolet lamp and the radioactivity associated with each nucleotide was determined by means of a strip scanner equipped with an automatic integrating device[26].

Since P_i runs near ATP in Solvent 2 any contamination or breakdown during the elution, drying, etc. would be measured as ATP. To check this possibility, a portion of the nucleotides eluted from the Solvent 1 chromatograms was examined by paper electrophoresis (EC Apparatus Co., Swarthmore, Pa.) (pyridine – glacial acetic acid – water (100:10:890) (pH 6.0) at 24 V/cm, for 4.5 h at 0°). The freedom from contamination by $^{32}P_i$ and other labelled phosphorus compounds is shown in Fig. 3.

In order to measure the relative specific activities of the β- and γ-P-atoms of ATP, [^{32}P]ATP was isolated from paper chromatograms after separation in Solvent 1 then Solvent 2 and the isolated material was reacted with excess glucose in the presence of hexokinase. The reaction volume was 0.2 ml, with the following reagent concentrations: 25 mM glucose, 10 mM Tris (pH 8), 5 mM $MgCl_2$, 0.05 mM EDTA and 100 μg crystalline yeast hexokinase (Boehringer & Soehne, Mannheim, W. Germany) for 10 min at 37°. Sufficient enzyme was used to turnover at least 0.2 μmole of ATP/min. The products of the reaction were then separated in Solvent 2 (see Fig. 4).

Because of the stoichiometry of the reaction and the negligible radioactivity found in the α-P atom (see later), the ratio of the radioactivity of the ADP to that of Glc-6-*P* is a measure of the ratio of the β- to γ-P-atoms of [^{32}P]ATP.

RESULTS

Spectrophotometry

When compared with the previous work of SZENTKIRALYI[24], LANGE[17–19], WAJZER *et al.*[20–21] surprisingly large values were obtained by the SZENTKIRALYI method for the hypoxanthine nucleotide content of both contracted and control muscles. As shown in Tables I and III, these values range from 23 to 42 % of the total nucleotides.

Since the expected values could not be obtained with standard solutions of "pure"

TABLE I

SPECTROPHOTOMETRIC ASSAY: RECOVERY OF NUCLEOTIDES ADDED TO A MUSCLE EXTRACT

Muscle number and additions	*Total nucleotide concentration (Measured at 248 mμ)*	*Inosine nucleotides as percentage of total nucleotides measured at:*					*Recovery (%)*
		255 mμ	*260 mμ*	*265 mμ*	*Obs. mean*	*Expected mean*	
40 A-B	$1.99 \cdot 10^{-2}$ mM	30.7	32.7	25.2	29.5	—	—
40 A-B + $0.20 \cdot 10^{-2}$ μmole* IMP/ml	$2.19 \cdot 10^{-2}$ mM	37.8	39.3	31.0	36.0	35.9	102
40 A-B + $0.84 \cdot 10^{-2}$ μmole* IMP/ml	$2.83 \cdot 10^{-2}$ mM	49.1	49.1	54.4	50.9	50.5	102
40 A-B + $0.83 \cdot 10^{-2}$ μmole* AMP/ml	$2.82 \cdot 10^{-2}$ mM	74.1	74.5	83.7	77.4	79.2	96

* The amount of nucleotide added is determined by the change in absorbancy at 248 mμ, following SZENTKIRALYI's equation[24].

IMP and AMP (Pabst Laboratories) or with known mixtures of these standards (Table II) the methods and data given by SZENTKIRALYI were examined. The spectral constants which she used were described as those of "pure adenosine and inosine taken from KALCKAR's paper" (see ref. 27). However, these data were not specifically reported in that paper and were apparently obtained graphically from the spectral curves for AMP and IMP by KALCKAR[27]. The critical point of SZENTKIRALYI's method using differential spectrophotometry is the wavelength of the isosbestic point. She reported this to be 248 mμ. Both by carefully replotting the data presented in the Pabst Circular OR-17 and by the spectral curves we have run on Pabst AMP and IMP with a calibrated Beckman DU, we find the isosbestic point to be 250 mμ by both methods (range −0.1, +0.3 mμ). Since this region is on a very steep portion of the AMP curve, small errors in wavelength cause large errors in the molar ab-

TABLE II

SPECTROPHOTOMETRIC ASSAY OF MIXTURES OF STANDARD NUCLEOTIDE SOLUTIONS

	Total nucleotide concentration (measured at 248 mμ)	*Adenosine nucleotides as percentage of Total nucleotides measured at:*				
		255 mμ	*260 mμ*	*265 mμ*	*Obs. mean*	*Expected mean*
Standard AMP	$1.94 \cdot 10^{-2}$ mM	117.5	112.4	116.5	115.5	100
Standard IMP	$2.23 \cdot 10^{-2}$ mM	8.5	1.3	−1.3	2.8	0
78.9% AMP 21.1% IMP	$2.46 \cdot 10^{-2}$ mM	105.7	101.3	105.4	104.1	78.9
4.3% AMP 95.7% IMP	$2.33 \cdot 10^{-2}$ mM	15.2	8.6	5.2	9.7	4.3

sorbancy coefficient which is used to determine the total nucleotide values. Thus an error of 2 mμ (*e.g.* using 248 instead of 250 mμ) in the isosbestic point could result in a 16% error in the determination of the total nucleotide content of a solution. The effect of this error is demonstrated in Table II. It is pertinent to note here that many Beckman spectrophotometers as used are significantly more precise than accurate[38]. Another error of this method when applied to biological extracts is the presence of ultraviolet-absorbing material other than adenine and hypoxanthine nucleotides. Also, FLECKENSTEIN[39] has reported that the frog rectus abdominis muscle during caffiene contracture at 20° produces a considerable amount of IMP. This does not occur at 0°. No IMP is visible on the ultraviolet photographs of their chromatograms. This means that the tissue concentration of IMP must be less than about 0.2 μmole/g in this muscle.

On the basis of all of these considerations we believe that the apparent high values for the inosine nucleotide content obtained by SZENTKIRALYI's spectrophotometric method are erroneously high and do not constitute an accurate measurement of the real concentration in this tissue.

However, the ability of the method to detect small changes in the tissue concentrations of adenine and hypoxanthine nucleotides is not affected by the above criticisms and is shown in Table I. Satisfactory recovery of both AMP and IMP when added to muscle extracts was obtained by this method of differential spectrophotometry.

It has been demonstrated that an isotonic contraction of 125 g × cm work/g muscle brings about an increase in the tissue content of inorganic phosphate of about 0.5 μmole/g muscle[23,30,31]. This is in agreement with the amount of breakdown of a "high-energy" phosphate compound releasing about 10000 cal of free energy/gram-mole calculated to be equivalent under physiologic conditions to the work done by the muscle assuming 50 % efficient energy transduction[32].

TABLE III

SPECTROPHOTOMETRIC ASSAY OF NUCLEOTIDES IN MUSCLE EXTRACTS

Muscle number	*Contracted or control*	*Total nucleotide concentration (Measured at 248 mμ)*	*Inosine nucleotides as percentage of total nucleotides measured at:*				*Change on contraction*
			255 mμ	*260 mμ*	*265 mμ*	*Mean*	
40 A-B	Control	$1.99 \cdot 10^{-2}$ mM	30.7	32.7	25.2	29.5	—
41 A	Contracted	$1.29 \cdot 10^{-2}$ mM	27.2	28.7	18.6	24.8	+1.5 %
B	Control	$1.56 \cdot 10^{-2}$ mM	27.0	25.0	18.0	23.3	
42 A	Control	$1.46 \cdot 10^{-2}$ mM	32.2	33.6	21.3	29.0	+2.9 %
B	Control	$1.58 \cdot 10^{-2}$ mM	33.6	35.5	26.6	31.9	
43 A	Control	$1.48 \cdot 10^{-2}$ mM	44.6	46.0	35.8	42.1	−3.6%
B	Contracted	$1.42 \cdot 10^{-2}$ mM	40.2	42.3	33.1	38.5	

Based on the total nucleotide content of 3.5 μmole/g in the rectus abdominis (of *Rana esculenta*)[10], the values of the hypoxanthine nucleotides observed (23–42 %, Table III) would correspond to 0.8–1.5 μmoles/g muscle. The changes observed in the two contracted *versus* control muscle pairs were small and similar to that found in the control *versus* control pair. A change in the hypoxanthine nucleotide content of 0.5 μmole/g would bring about an increase in the ratio of the hypoxanthine nucleotides to the total nucleotides of 14 % (from 1.09/3.5 to 1.59/3.5 *i.e.* 31–45 %). On the basis of the recovery experiments a change of this magnitude would have been detected readily, but did not occur.

^{32}P *Experiments in vivo*

A more direct demonstration of the presence and concentration changes of the nucleoside monophosphates could be made if sufficient ^{32}P could be incorporated into them. Incubations *in vitro* of the isolated muscles for as long as 4 h produced labelled ATP which, however, contained only 1 or 2 % of its radioactivity in the α-P-atom although the β- and the γ-P-atoms of ATP and the P-atom of PCr were nearly equal in specific activity.

Since the α-P-atom is very stable metabolically we attempted to incorporate ^{32}P here by means *in vivo* as described in METHODS. The labelled compounds of the muscle extracts were separated electrophoretically, chromatographically and with small charcoal columns as described. The IMP and AMP regions were devoid of sufficient activity for further work. Furthermore, when the isolated [^{32}P]ATP was hydrolysed for 7 min at 100° with 1 N HCl, over 95 % of the radioactivity was found to be associated with inorganic phosphate. Thus the direct measurement of the nucleoside monophosphates by equilibration *in vivo* with [^{32}P]P_i was not feasible even after 4 days.

Ratio of the ^{32}P-labelled nucleotides: ATP, ADP, ITP and IDP

^{32}P was introduced into these metabolically active phosphorus compounds of the rectus abdominis muscles of the frog by the methods described *in vitro* or *in vivo*. The muscles were then taken through the usual experimental procedure and the labelled nucleotides were separated as shown in Figs. 1 and 2.

The results of this experiment with six contracted *versus* control muscle pairs and 1 control *versus* control pair are given in Table IV. The concentrations given are calculated from a total nucleotide value of 3.5 μmoles/g which has been reported for the rectus abdominis[10]. In these experiments the amount of radioactivity in the α-P-atom is negligible and the β-P-atom has 88.1 % of the specific activity of the γ-P-atom. This measurement was made by isolating [^{32}P]ATP from paper chromatograms and reacting the isolated [^{32}P]ATP with glucose in the presence of hexokinase, as described in METHODS. Because of the stoichiometry of the reaction, the ratio of the activity of ADP to Glc-6-*P* (plus a small amount of P_i) gives the ratio of β/γ P-atoms of ATP. Consequently the concentrations of ADP and IDP are related to ATP by multiplying the percentage radioactivity found in the ADP and IDP areas by the factor 2.14. ITP is related directly to ATP.

These experiments specifically demonstrate the very low tissue concentrations of ITP and IDP (0.22 and 0.07 μmole/g respectively). They further show a lack of

TABLE IV

NUCLEOTIDE CONTENT OF MUSCLES AS DETERMINED BY THE DISTRIBUTION OF ^{32}P

Values are expressed as percentage of total nucleotide radioactivity in individual nucleotide areas on the paper chromatograms and as μmoles/g muscle (method of calculation described in the text).

Muscle number	State	ATP		ADP		ITP		IDP	
		(%)	(μmoles per g)	(%)	(μmoles per g)	(%)	(μmoles per g)	(%)	(μmoles per g)
68 E	Control	81.7	2.57	8.73	0.59	8.55	0.27	0.97	0.07
F	Contracted	80.2	2.54	8.47	0.57	10.4	0.33	0.95	0.06
69 A	Control	85.6	2.76	7.10	0.49	5.88	0.19	0.91	0.06
B	Contracted	85.9	2.77	6.65	0.46	6.54	0.21	0.93	0.06
69 C	Contracted	84.6	2.71	6.59	0.45	7.02	0.22	1.78	0.12
D	Control	85.8	2.81	6.04	0.42	7.37	0.24	0.70	0.05
69 E	Control	81.2	2.59	7.38	0.50	10.3	0.33	1.23	0.08
F	Control	80.8	2.56	7.41	0.50	11.0	0.35	1.24	0.08
70 A	Control	88.5	2.80	6.3	0.43	5.2	0.16	1.4	0.10
B	Contracted	86.4	2.66	8.2	0.54	5.3	0.16	1.9	0.13
71 A	Control	86.8	2.83	6.2	0.43	7.0	0.23	—	—
B	Contracted	83.8	2.74	6.2	0.44	10.0	0.33	—	—
Average values									
	Control		2.75		0.47		0.22		0.07
	Contracted		2.69		0.49		0.25		0.09
	Change on contraction		−0.06		+0.02		+0.03		+0.02
	(±S.E.M.)		±0.021		±0.022		±0.018		±0.020

a net change of ATP, ADP, ITP and IDP by an amount sufficient to provide the energy for contraction. These data also exclude the possibility of changes in AMP and IMP since the production of these compounds should be reflected in a corresponding decrease in the quantity of the diphosphates and triphosphates, and this was found not to occur.

DISCUSSION

LANGE[17-19] studied the effect of contraction by electrical stimulus or pharmacological agents on the nucleotide contents of the frog *rectus abdominis*. The experiments were apparently carried out at room temperature and then, after a delay of several seconds in transfer, the muscles were extracted in a blendor with 60 % ethanol. The nucleotides were separated chromatographically and electrophoretically on paper and assayed spectrophotometrically and by phosphorus analysis. This author found contraction to bring about a decrease in the tissue concentration of ATP by 0.25 μmole/g muscle and an increase in the IMP content of 0.1–0.4 μmole/g thus suggesting a simultaneous dephosphorylation–deamination of ATP during muscular contraction.

However, the contractions were of prolonged duration (up to 10 sec) and there is no reason to believe that the techniques which were employed were rapid enough to detect changes associated with only the primary event in muscular contractions.

WAJZER *et al.*[21] reported finding a decrease of 1 5 μmoles/g in the adenine nucleotides when one of the paired muscles (rectus internus major of the frog) was electrically stimulated to do a single contraction at 0°. The muscles were then immediately denatured and extracted by rapid blending in perchloric acid at 0°. The purines were isolated and measured by the method of PARNAS[33].

The measurements by the method of differential spectrophotometry which are described in the present paper suffer from an error in the method which prevents the estimation of absolute quantities or the real ratios of adenine and hypoxanthine nucleotides in the extracts. The technique is, however, very sensitive to changes in the ratio of these nucleotides and the data obtained clearly establish that the large changes in both adenine and hypoxanthine nucleotides reported by LANGE[17-19] and by WAJZER *et al.*[21] certainly did not occur during contraction under the conditions which we employed. The large changes these authors describe are also in conflict with the lack of sufficient net changes of ATP found by numerous investigators and may be due to the blending procedures which were used to denature the muscle since damaged muscle has been shown to deaminate the adenine nucleotides very rapidly[24,33].

The experiments with the ^{32}P-labelled nucleotides demonstrate the very low tissue concentration of ITP and IDP. Changes in their concentration by the amount expected if ITP or IDP were the primary energy source for contraction (0.5 μmole/g) would been readily detected but certainly did not occur.

It is, of course, possible that the hypoxanthine (or adenine) nucleotides may function as intermediates in the process of energy transduction during muscle contraction, but the lack of a quantitative change during contraction clearly bars their operation as the net energy source in these experiments.

Early work by LUNDSGAARD[6,7] and more recent experiments by CARLSON AND SIGER[14] and by MOMMAERTS, SERAYDARIAN AND MARECHAL[34] have shown that PCr functions as the net energy source during multiple contractions. Recent work in our

laboratory[23,30,31,35–38] has shown that the breakdown of PCr to form P_i and creatine is also the net energy source for single contractions of working muscle. This observation has been confirmed independently by MOMMAERTS[39–41]. However, these experiments did not resolve the question of whether PCr is the immediate energy source for contraction or whether it merely regenerates ATP (or some other compound) which is the real immediate energy source for contraction.

Recent reports by CAIN AND DAVIES[42], CAIN, INFANTE AND DAVIES[43] and INFANTE AND DAVIES[44] describe experiments in which muscles were treated with FDNB which is an inhibitor of ATP–creatine phosphoryltransferase[45]. These muscles whilst liberating P_i during contraction did not break down PCr. Under these conditions, it was possible to demonstrate a net breakdown of ATP during both single full contractions and single twitches of working muscles.

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