Some Properties of the Actomyosin-like Protein of the Uterus

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Comparatively little work has been done on the protein extractable from uterus muscle under conditions similar to those used for extracting actomyosin from skeletal muscle. Csapo (1949, 1950*a*, *b*) has used 0·5M-KCl extracts of rat, rabbit and human uterus and, by comparing the viscosity before and after addition of adenosine triphosphate (ATP) with the viscosity of skeletal-muscle extracts similarly treated, has concluded that uterus muscle contains actomyosin but less than that in skeletal muscle. Csapo (1949, 1950*a*, *b*) has correlated this actomyosin content with functional activity in different states of the uterus. Schwalm, Cretius & Lange (1955) have studied variations in the viscosity response to ATP of extracts made with M-KCl from human uteri in different states.

With extracts considered, from their similar specific viscosity after ATP addition, to contain similar amounts of myosin, Csapo (1949) found that the adenosine triphosphatase (ATPase) activity was less in those from the uterus than from skeletal muscle. He also made purified preparations by dilution precipitation of the protein soluble in 0·5M-KCl; the ATPase of such preparations, like skeletal-muscle actomyosin, was activated more by Ca^{2+} than by Mg^{2+} ions. In all this work ATPase activity was measured by a viscosimetric method, and from the results only approximate values can be deduced for the enzymic activity in terms of inorganic P liberated/mg. of actomyosin N. Westgren (1950) has also prepared protein from human, cow and rabbit myometrium, following the method of Szent-Györgyi (1951) for preparation of myosin B. He found the ATPase of the once-precipitated material to be activated by either Mg^{2+} or Ca^{2+} ions, rather more by the latter; he gives a pH–activity curve with several peaks, the most important being about 7·3 and 8·5, and suggests that more than one ATPase might be present. Since no protein concentrations are reported, it is not possible to compare the ATPase activity with the values of other workers.

In connexion with work which we are doing on the proteins of the uterus, it seemed desirable to make a more detailed and quantitative study of the ATPase activity of uterus actomyosin. Csapo and Westgren seem to have assumed that the protein they were dealing with from smooth muscle was the same as the actomyosin of striated muscle. Crepax (1952), on the other hand, studying the electrophoretic behaviour of 0·5M-KCl extracts of uterus muscle, considers the proteins present to be different. Snellman & Tenow (1954) also believe the contractile element of the uterus to have a different protein composition from that of skeletal muscle. In the present paper the term actomyosin will be used, for convenience, for the protein prepared from the uterus by the methods ordinarily used for the preparation of myosin B from skeletal muscle; this is done without any intention of deciding at the present stage the question of the identity of the uterus protein.

Singher & Millman (1947), using rat uterus, reported that homogenates of the whole organ showed three times as great ATPase activity as homogenates of skeletal muscle. In the present work it has been found that only a small part of this activity can be due to actomyosin; the greater part is due to cell particles which can be centrifuged down at 40000 to 50000 *g*. In such homogenates the particles are derived from the endometrium as well as the myometrium and, as will be shown below, the ATPase activity of cell particles from rat uterus is much higher than that of particles from pig or rabbit myometrium. Nevertheless, in the smooth-muscle cells of the myometrium the particulate fraction (8–9% of the total N) is likely to be quantitatively more important than in many skeletal muscles—certainly than in rabbit skeletal muscle ordinarily used for actomyosin preparation (Chappell & Perry, 1953). In preparing uterus actomyosin we have therefore taken care to remove the particulate fraction from the extract before precipitation of the actomyosin by dilution. A study of the characteristics of actomyosin and of cell-particle ATPase from the whole uterus or the myometrium alone shows that the two enzymes can be clearly distinguished.

A few experiments were made on the viscosity of the actomyosin before and after ATP addition, to make certain that our preparations gave the expected changes.

A preliminary report of this work has been published (Needham & Cawkwell, 1955).

EXPERIMENTAL

Materials

Homogenate of rat uterus for the study of ATPase distribution. This was made by grinding rat uteri (all selected at the stage of late oestrus) with 10 vol. of 1·25 M-KCl, containing 0·08 % ATP (sodium salt) and buffered with 0·03 M-$NaHCO_3$ at pH 7·9. In order to facilitate the disintegration, the cooled tissue was allowed slowly to freeze solid in air at −15°, and the frozen material was ground with the medium and about 0·5 g. of acid-washed sand/g. All subsequent work was done at about 4°. After measured samples had been removed, the homogenate was centrifuged at 600 *g*, and extracted twice more, each time with 5 vol. The combined supernatants were centrifuged at 600 *g* to remove any remaining debris, and then at 41 000 *g* for 1 hr. to sediment the cell particles. All values given for *g* are average values. Uniform suspensions in 0·5 M-KCl were made of the particle pellet and of the residue.

Preparation of actomyosin. This was made by a method based on those described by Bailey (1942), Szent-Györgyi (1951), Portzehl, Schramm & Weber (1950) and Hasselbach & Schneider (1950–51) for the preparation of myosin B. Pregnant uterus was used in all cases; pig material for preparation I was taken after 40 days' pregnancy; for preparations II and III after 30 days. The rabbit and rat material was taken very near term. Glass-distilled water was used for all solutions and for the precipitation by dilution.

The sections of pig uterus to be used were cooled and brought to the laboratory as quickly as possible. All further work was carried out in a cold room at 4°. After being slit open, the uterus was scraped free from endometrium and the myometrium was peeled away as much as possible from the outer fibrous layer. Histological examination showed that the material used was nearly all myometrium. This was minced and a weighed amount of about 50 g. was homogenized in a Waring Blendor with 3 vol. of 0·5 M-KCl containing 0·08 % ATP (sodium salt) and 0·05 M-KH_2PO_4; the latter had been brought to pH 7·5 by addition of N-KOH. The homogenization was for six periods of 30 sec., with 1 min. intervals. This stage was reached about 1·5 hr. after the death of the animal.

The homogenate was stirred slowly for some hours, then left at 0° overnight. Next day the extremely viscous suspension was further diluted with 1 vol. of extractant and then centrifuged at 3000 *g*. The supernatant was filtered with gentle suction through a layer of paper pulp (1–2 cm.) previously saturated with extractant. The filtrate was centrifuged in the Spinco ultracentrifuge, model L. Table 1 shows the effect of filtering and of duration and speed of centrifuging on the protein-N content of the dilution precipitates and of the particle material spun down. In later work, in order to hasten the preparation, the filtration was omitted and centrifuging in the Spinco centrifuge for 1 hr. at 105 000 *g* was used as a routine.

The supernatant was first diluted about fivefold with water at 0° and the pH was adjusted, by means of 0·5 N acetic acid, to 6·5–6·7, with the glass electrode. The dilution was then continued to 14-fold. A precipitate appeared which quickly flocculated. The preparation was allowed to stand at 0° overnight, then the clear fluid was decanted and the suspension centrifuged. The precipitate was dissolved in about 40 ml. of 0·6 M-KCl, any traces of undissolved matter being spun off. The precipitation by dilution to 0·04 M-KCl was repeated once or twice more, care being taken that the pH remained about 6·6. Finally, the preparation was stored at 0° as a solution in 0·5 M-KCl containing about 1 mg. of actomyosin N/ml. The pH was brought to 7·0 by means of a few drops of 0·6 M glyoxaline buffer.

With the uteri from rabbit and rat the initial stages could be carried out more quickly. With the rat the whole uterus was used after washing and blotting.

The cell-particle suspension. This was made by adding a few drops of cold 0·5 M-KCl to the pellet in the centrifuge tube and working up gently with a glass rod to a uniform suspension; more solution was gradually added up to the required volume.

Special chemicals. ATP was used as the sodium salt supplied by the Sigma Chemical Co.

Glyoxaline (from L. Light and Co., Ltd.) was sometimes coloured faintly yellow. On these occasions it was recrystallized first from benzene, then from ethanol.

2:4-Dinitrophenol (DNP) was recrystallized first from ethanol, then from water–ethanol (50 %, v/v).

$MgCl_2$ and $CaCl_2$ solutions were made from A.R. reagents and standardized by titration with silver nitrate.

Table 1. *Effect of various treatments on the amount of cell-particle preparation and actomyosin precipitate*

Pig preparation I was used. Filtration was through paper pulp as described in the text; the unfiltered extract had a preliminary centrifuging at 600 *g* for 20 min. to remove cell debris. Centrifuging in the Spinco centrifuge was for 1 hr. at 41 000 *g*; or for 1 hr. at 41 000 *g*, followed by 1 hr. at 105 000 *g*.

	Period of centrifuging (hr.)	Protein N derived from 1 g. wet wt. of myometrium (mg.)
Whole extract, unfiltered	—	10·3
Whole extract, filtered	—	9·7
Actomyosin pptd. from filtered extract	—	1·30
Sediment from unfiltered extract	1	0·83
	2	1·72
Sediment from filtered extract	1	0·40
	2	1·00
Actomyosin pptd. from filtered, centrifuged extract	1	1·48
	2	0·88

Methods of estimation

Inorganic P was estimated by the method of Fiske & Subbarow (1925) or, for quantities below 20 μg., by the method of Weil-Malherbe & Green (1951). Protein N was estimated by the micro-Kjeldahl method of Chibnall, Rees & Williams (1943).

Ribonucleic acid (RNA) was estimated by a modification of the method of Schmidt & Thannhauser (1945). Phospholipids were first removed by the method of P. D. Mitchell and J. M. Moyle (personal communication); the mixture was extracted three times with an equal volume of ice-cold *n*-butanol, being allowed to stand each time in the centrifuge tube at 0° for 20 min. with frequent stirring; the layers were

separated by centrifuging, and after removal of the last butanol layer the aqueous phase was treated with cold trichloroacetic acid to a concentration of 5% to remove acid-soluble P; the precipitate was washed twice with 5% trichloroacetic acid, the temperature being kept about 0°. After separation of the deoxyribo- and ribo-nucleic acids, the ribonucleic acid P was estimated by Allen's (1940) method for total P, or if the amounts were below 20 μg., by a modification of the method of Weil-Malherbe & Green (1951). In this case, incineration was done in the presence of 0·3 ml. of conc. H_2SO_4 and 0·2 ml. of 6N perchloric acid (Hanes & Isherwood, 1949); any pyrophosphate formed was hydrolysed by diluting to about 3 ml. and heating for some minutes at 100°. No further acid was added, ammonium molybdate solution in water taking the place of the usual acid molybdate; shaking with *iso*butanol for 5 min. was necessary for complete extraction of the phosphomolybdate under these conditions.

Measurements of enzyme activity

Measurement of ATPase activity. The reaction mixture (final volume 2 ml.) contained unless otherwise stated 0·002M ATP; 0·066M glyoxaline buffer, pH 7·0; and 0·01M-$CaCl_2$. KCl was always present and the concentration is given for the different experiments. The reaction was started by addition of 0·2 ml. containing 0·07–0·2 mg. of actomyosin N, or 0·1 mg. or less of particle N. At these concentrations the rate of reaction was found to be proportional to the amount of enzyme present. The reaction time was 5 min. and the temperature 28°. It was ascertained that the rate remained constant during this period. The reaction was stopped by addition of 1 ml. of trichloroacetic acid (15%, w/v).

Testing of lecithinase activity. Purified α-toxin (from *Clostridium welchii*) dissolved in equal parts (v/v) of 1% NaCl and glycerol was obtained from the Wellcome Research Laboratories through the kindness of Mrs Irene Batty. The solution contained 100 Lf units/ml. (van Heyningen, 1948) or, very approximately, 1·7 Keilley–Meyerhof units (Keilley & Meyerhof, 1950). The preparation was shown to be highly active by the turbidimetric method of van Heyningen (1941), with 1·6 Lf units, 0·8 ml. of egg-yolk solution, 0·04M glyoxaline buffer and 0·001M-$CaCl_2$ in 1·6 ml. At 25° maximum turbidity appeared in 15 min. Addition of particle suspension (about 0·18 mg. of N) had no effect on the development of turbidity.

Viscosity measurements

An Ostwald viscosimeter was used, about 8 ml. in capacity, with a flow-time of 88 sec. for water at 18°.

RESULTS

ATPase activity. For the preliminary experiments with homogenates of rat uterus the results given in Table 2 are typical. They show that about 60% of the ATPase activity is due to the particulate material. Moreover, most of the activity of the residue is probably due also to cell particles not

Table 2. *Distribution of ATPase activity in rat-uterus homogenate*

The preparation of the fractions and the reaction mixture was as described in the text. The KCl concn. was adjusted to 0·1M. Incubation was for 3 min. at 25°. The measured samples of homogenate or tissue fraction added to the reaction medium contained 0·08–0·13 mg. of N.

	P liberated/g. wet wt. of uterus (mg.)
Homogenate	2·83
Particulate fraction	1·72
Supernatant	0·42
Residue	0·84

Table 3. *Effect of* KCl *concentration on ATPase activity of actomyosin and cell-particle preparations from the uterus*

The conditions of incubation and the composition of the reaction mixture were as described in the text.

		Inorganic P liberated/mg. of N/min. (μmoles)	
		With 0·1M-KCl	With 0·6M-KCl
Pig I	Actomyosin, once pptd.*	0·38	0·68
Pig II	Actomyosin, once pptd.*	0·24	0·48
	Actomyosin, twice pptd.*	0·28	0·56
	Particle preparation	0·56	0·49
Pig III	Actomyosin, twice pptd.	0·34	0·63
	Actomyosin, twice pptd.†	0·094	0·49
	Particle preparation†	0·60	0·48
Rat	Actomyosin, twice pptd.	0·56	0·52
	Particle preparation	7·5	5·4
Rabbit	Actomyosin, three-times pptd.	0·19	0·70
	Particle preparation	1·10	1·10

* Actomyosin pptd. from extracts before high-speed centrifuging. In the other cases centrifuging was for 1 hr. at 105000 *g*.

† 0·001M-$CaCl_2$ used.

removed in the extraction; even after seven extractions it was found that the residue still contained about 20% of the total ribonucleic acid. The ATPase activity of the supernatant containing the actomyosin was only about 15% of the whole.

The ATPase activity of all the uterus-actomyosin preparations tested (Tables 3 and 4) is very low compared with the activity of actomyosin from skeletal muscle under similar conditions. Thus Greville & Needham (1955) give values of about 6 μmoles of P liberated/mg. of N/min. at 25° in 0·1 M-KCl, pH 7·0. The ATPase activity of the cell-particle preparations was higher, especially in the rat; these very high values have been found in many experiments with rat preparations. Raising the KCl concentration to 0·4 M or above about doubles the ATPase activity of the actomyosin preparations (with the exception of the rat preparation) whilst having only a slight inhibitory effect on the cell-particle preparations. With the uterus actomyosin from the rat, several KCl concentrations between 0·025 M and 0·6 M were tried without affecting the ATPase activity.

Another clear distinction between the actomyosin and the particulate ATPase is to be found in the effect of Ca^{2+} and Mg^{2+} ions. In every case Ca^{2+} ions activate actomyosin ATPase much more strongly than Mg^{2+} ions, whilst sometimes the one ion, sometimes the other, is slightly the better activator of cell particles (Table 5).

2:4-Dinitrophenol ($3–5 \times 10^{-3}$ M) also, in the presence of 0·6 M-KCl, raises the actomyosin-ATPase rate considerably, without much affecting the particle ATPase (Table 6). The behaviour of the rat material is again anomalous in that actomyosin activation is also obtained at low KCl concentrations, and this will be discussed later.

The effect of pH upon the activity in 0·6 M-KCl of uterus actomyosin from the rabbit is shown in Fig. 1. Three buffers, 0·06 M citrate, 0·035 M glyoxaline and 0·05 M aminotrishydroxymethylmethane (tris) were used, overlapping to cover the pH

Table 4. *Effect of* KCl *concentration on the ATPase activity of uterus actomyosin*

The composition of the reaction mixture was as described in the text. The actomyosin (pig II) was precipitated twice from extract which had been centrifuged for 1 hr. at 105000 *g*.

Concn. of KCl (M)	Inorganic P liberated/mg. of N/min. (μmole)
0·1	0·16
0·2	0·17
0·4	0·50
0·6	0·51
0·8	0·50
1·0	0·48

Table 5. *Effect of* Ca^{2+} *and* Mg^{2+} *ions on ATPase activity of actomyosin and particle preparations from the uterus*

The conditions of incubation and the composition of the reaction mixture were as described in the text. The actomyosin preparations were all made from extracts which had been centrifuged for 1 hr. at 105000 *g*; they were precipitated twice, except for the rabbit preparation, which was precipitated three times.

			Inorganic P liberated/mg. of N/min. (μmoles)	
		Activator	With 0·1 M-KCl	With 0·6 M-KCl
Pig II	Actomyosin	0·01 M-$CaCl_2$	0·16	0·58
		0·002 M-$MgCl_2$	0·10	0·09
	Particle prep.	0·01 M-$CaCl_2$	0·56	0·49
		0·002 M-$MgCl_2$	0·68	0·63
Pig III	Actomyosin	0·001 M-$CaCl_2$	—	0·67
		0·01 M-$CaCl_2$	—	0·76
		0·001 M-$MgCl_2$	—	0·069
		0·01 M-$MgCl_2$	—	0·069
	Particle prep.	0·001 M-$CaCl_2$	0·30	0·25
		0·001 M-$MgCl_2$	0·30	0·30
Rat	Actomyosin	0·01 M-$CaCl_2$	0·46	—
		0·002 M-$MgCl_2$	0·037	—
	Particle prep.	0·01 M-$CaCl_2$	7·5	—
		0·002 M-$MgCl_2$	5·6	—
Rabbit	Actomyosin	0·01 M-$CaCl_2$	0·19	—
		0·001 M-$MgCl_2$	0·019	—
	Particle prep.	0·01 M-$CaCl_2$	1·10	1·10
		0·001 M-$MgCl_2$	1·55	1·30

Table 6. *Effect of 2:4-dinitrophenol on ATPase activity of actomyosin and particle preparations from the uterus*

The conditions of incubation and the composition of the reaction mixture in the control were the same as described in the text. The actomyosin and particle preparations were the same as those used in Table 5.

		Concn. of DNP (mM)	Inorganic P liberated/mg. of N/min. (μmoles) With 0·1 M-KCl	With 0·6 M-KCl
Pig II	Actomyosin	—	—	0·36
		3·5	—	0·52
	Particle prep.	—	—	0·56
		3·5	—	0·53
Pig III	Actomyosin	—	0·34	0·76
		3·5	0·28	1·22
		—*	—	0·069
		3·5*	—	0·12
	Particle prep.	—	0·54	—
		3·5	0·54	—
		4	0·24	—
Rat	Actomyosin	—	0·51	0·38
		3·5	0·87	0·87
	Particle prep.	—	—	0·54
		3·5	—	0·54
Rabbit	Actomyosin	—	0·19	0·63
		3	0·20	1·47
		5	—	1·88
	Particle prep.	—	—	0·74
		3	—	0·90
		5	—	1·01

* 0·001 M-$MgCl_2$ was used as activator.

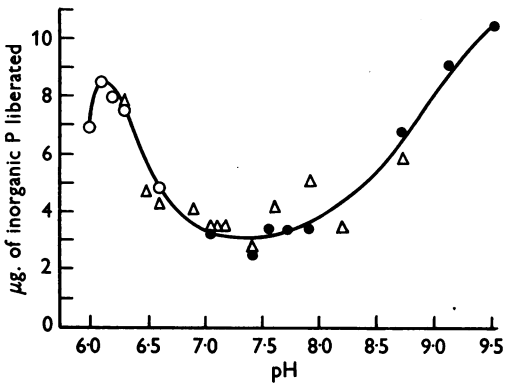

Fig. 1. pH–Activity curve for uterus actomyosin. The preparation was from the rabbit and had been three times precipitated; 0·1 mg. of actomyosin N was used, in a final volume of 2 ml. The reaction medium contained 0·002 M ATP (sodium salt), 0·01 M-$CaCl_2$ and 0·6 M-KCl. The buffers used were 0·05 M tris (●); 0·035 M glyoxaline (△); 0·06 M citrate (○). Reaction time, 3 min. at 25°.

Table 7. *Ribonucleic acid content of actomyosin and particle preparations from the uterus*

The actomyosin and particle preparations were the same as those used in Table 5. The ATPase activities used are those obtained in the usual conditions (see text) with 0·6 M-KCl.

		RNA P/mg. of N (μg.)	Inorganic P liberated/μg. of RNA P/min. (μmole)
Pig II	Actomyosin	11·1	0·047
	Particle prep.	51·0	0·011
Pig III	Actomyosin	11·5	0·055
	Particle prep.	60·0	0·010

range 6·0–9·5. There was usually good agreement between the rates with different buffers at any given pH.

In some experiments the ribonucleic acid content of the particle preparations was estimated and compared with that of the actomyosin preparations. It is not certain that all the RNA content of

the actomyosin was due to contamination by particles, but a maximum value for possible contamination is obtained. Calculation of the ATPase activity/μg. of RNA P in each case showed that the activity of the actomyosin preparations was 4–5 times that which could be due to particulate matter carried down (Table 7).

Myokinase. Myokinase was found to be absent from the actomyosin tested, pig preparation III (Fig. 2). The liberation of inorganic P ceased after half the easily hydrolysable P had been split off, in spite of the addition of more enzyme at 30 min. With the cell-particle preparation, on the other hand (also from pig III and washed once with 0·5 M-KCl), the liberation of inorganic P continued steadily well beyond the half-way point. This indicates either that myokinase was present as well as ATPase, or that the particle preparation contained an apyrase capable of splitting off two phosphate groups from ATP.

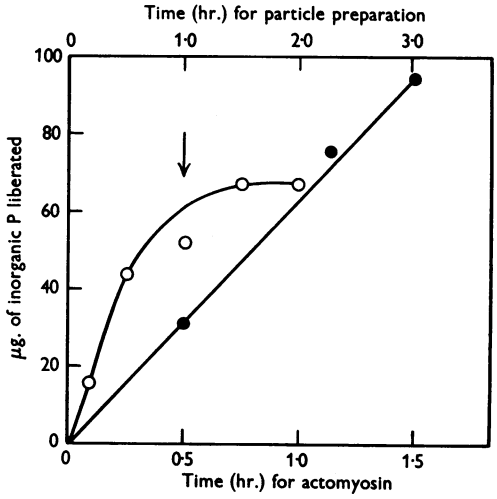

Fig. 2. Testing of actomyosin and washed-particle preparation for myokinase. The medium (final volume 2 ml.) contained 0·001 M ATP (sodium salt); 0·01 M-$CaCl_2$; 0·6 M-KCl and 0·066 M glyoxaline buffer. At the arrow a second addition of actomyosin solution was made. Temp. 28°. ○, Actomyosin; ●, particle preparation.

Effect of lecithinase. In the early stages of this work, before the activating effect of high KCl concentration and of DNP on the actomyosin ATPase was known, it was thought that it might be possible to confirm the activity of actomyosin ATPase from the rat, as distinct from the very active particle ATPase, by the susceptibility of the latter to lecithinase (Keilley & Meyerhof, 1948, 1950). It was, however, not found possible to cause more than 10–20 % inhibition of the ATPase of the particle preparation, although the conditions, described in the Experimental section as optimum for egg-yolk flocculation, were varied by increasing the lecithinase content, by increasing the time of action to 1 hr. and by substituting histidine buffer (as used in the experiments of Keilley & Meyerhof) for the glyoxaline buffer. Thus a typical particle preparation (0·8 mg. of N) lost about 20 % of its ATPase activity on incubation for 1 hr. with 4 Lf units of lecithinase in 1·6 ml. final volume; the control, similarly treated but without lecithinase, lost 9 %. The percentage effect on ATPase activity was the same whether Ca^{2+} or Mg^{2+} ions were used as activator in testing the ATPase.

Viscosity changes. Pig preparation I was tested at three stages for viscosity changes on ATP addition, and the results are shown in Table 8. The values for specific viscosity and for the fall caused by ATP are somewhat greater than those recorded by Snellman & Tenow (1954) for pregnant uterus, but considerably smaller than those given by Csapo (1949). The value for Z (see Table 8) can

Table 8. *Changes in viscosity of uterus actomyosin on ATP addition*

The actomyosin was from pig I, and was dissolved in 0·6 M-KCl containing 0·05 M-KH_2PO_4 brought to pH 7·0 with N-KOH. Soln. A contained actomyosin pptd. from filtered extract before high-speed centrifuging. Soln. B contained actomyosin pptd. from the extract after centrifuging for 1 hr. at 41 000 *g*. Soln. C contained actomyosin pptd. from extract after centrifuging for 1 hr. at 41 000 *g*, followed by 1 hr. at 105 000 *g*. A portion (8 ml.) of solution was used in each case, the outflow time for the buffer being 85 sec. The volume of 0·1 M ATP added was 0·1 ml. Temp. 18°.

	Specific viscosity			Protein concn. (%)	Z_η* before ATP	ATP sensitivity (%)*
	Before ATP	After ATP	Returning to			
Soln. A	0·67	0·38	0·67	0·45	0·11	57
Soln. B	0·74	0·38	0·74	0·45	0·13	64
Soln. C	0·37	0·24	0·37	0·26	0·078	40

* $Z_\eta = \dfrac{2{\cdot}3 \times \log \eta_{rel.}}{\text{concn. (g./l.)}}$. ATP sensitivity (%) $= \dfrac{\log \eta_{rel.} - \log \eta_{rel.\ ATP}}{\log \eta_{rel.\ ATP}} \times 100$,

where $\eta_{rel.}$ and $\eta_{rel.\ ATP}$ are the relative viscosities before and after addition of ATP (Portzehl *et al.* 1950).

be compared with that of about 0·3 given by Portzehl *et al.* (1950) for actomyosin from skeletal muscle under similar conditions; these authors give values up to 165 for the percentage ATP sensitivity of actomyosin from skeletal muscle.

DISCUSSION

Evidence has been presented that the ATPase activity of actomyosin preparations from the uterus is due to the actomyosin itself and not to contaminant particles, and also that a specific ATPase is concerned.

The behaviour of the uterus-actomyosin ATPase shows some resemblances to that of striated-muscle actomyosin. The viscosity changes on ATP addition are of course similar, though on a smaller scale. If we assume that the uterus actomyosin in 0·6 M-KCl is readily dissociated into actin and a myosin-like protein, then this myosin-like protein, in the same way as the myosin of skeletal muscle, is activated by DNP (Greville & Needham, 1955; Chappell & Perry, 1955), and its pH–activity curve is very similar (Mommaerts & Green, 1954). There are, however, some differences in behaviour; thus, skeletal-muscle actomyosin is known to show, under the conditions similar to those used in this work, decreasing ATPase activity with increasing KCl concentration (Mommaerts & Green, 1954; Greville & Needham, 1955). This is taken to mean that at pH 7·0 the ATPase activity of the undissociated actomyosin is greater than that of myosin. With uterus actomyosin the increased activity with high KCl concentrations would indicate that the myosin-like protein has greater ATPase activity than the actomyosin. Uterus actomyosin from the rat is anomalous here, being neither activated nor much inhibited by high KCl concentrations, and being activated by DNP at low KCl concentrations as well as at high. This might indicate that this actomyosin is dissociated with particular ease.

In this work we found that actomyosin ATPase keeps its activity for at least 2–3 weeks. The loss of actomyosin shown in Table 1 which occurred on prolonged high-speed centrifuging was also noticed with actomyosin from skeletal muscle. Crepax (1952) comments on the instability of the uterus proteins soluble in solutions of high ionic strength and contrasts this behaviour with that of the proteins from skeletal muscle; but it is not made clear wherein the instability consists.

The amount of protein obtained as actomyosin from the myometrium by the method described was very small, only 1·0–1·5 mg. of N/g. wet wt. after two precipitations. No quantitative figures seem available for the yield from skeletal muscle after comparable treatment. This yield from the myometrium was certainly not quantitative. Experiments with whole uterus from the pregnant rat by Robinson's (1952) method for complete extraction and precipitation with ethanol (15%, v/v) gave values of 3·5–4·5 mg. of actomyosin N/g. wet wt.; these are to be compared with a content of about 18 mg. of actomyosin N/g. wet wt. for skeletal muscle (Hasselbach & Schneider, 1950–51).

The cell particles after the treatment of the tissue as described for actomyosin extraction are no doubt in a damaged condition, and it is to be expected that their ATPase activity would be fully unmasked (cf. Keilley & Keilley, 1951). The lack of activation by DNP is in agreement with this. With mitochondria from pigeon-breast muscle showing no latent ATPase activity, Chappell & Perry (1953) found consistently greater activation by Mg^{2+} ions than by Ca^{2+} ions, especially at concentrations of 0·01 M and below. With the cell particles from the uterus as used in this work, the superiority of Mg^{2+} ion activation was neither so consistent nor so great.

The ATPase activity of the particles from rat uterus is exceptionally high. Chappell & Perry (1953) found about 1·5 μmoles of P liberated/mg. of N/min. for unmasked mitochondria from pigeon-breast muscle; and Lardy & Wellman (1953) found 2·5 μmoles for mitochondria from rat liver in the presence of DNP. The resistance of the particulate ATPase to lecithinase attack was unexpected, since the material from rat muscle found by Keilley & Meyerhof (1948) to contain the lecithinase-sensitive enzyme was centrifuged down between 10000 and 18000 *g* and was considered by them to be microsomal in origin; these particles, like those in our work, must have been damaged by their previous treatment. The different behaviour of the uterus particles might depend on the difference in tissue or on differences in previous treatment. This matter is being investigated further.

SUMMARY

1. An actomyosin-like protein prepared from pig and rabbit myometrium and from whole uterus of the rat showed specific adenosine-triphosphatase activity. In 0·1 M-KCl at pH 7·0 this activity was 3–10% of that of skeletal-muscle actomyosin under similar conditions. Like the latter, it was activated by Ca^{2+} ions much more than by Mg^{2+} ions. A pH–activity curve is given.

2. With the protein from pig and rabbit myometrium, the activity was about twice as high in 0·4–0·8 M-KCl as in 0·1 M-KCl. This behaviour contrasts with that of skeletal-muscle actomyosin, which is inhibited by raising the KCl concentration. In the presence of high KCl concentration there was activation by $3–5 \times 10^{-3}$ M 2:4-dinitrophenol, as with skeletal-muscle actomyosin.

3. The actomyosin-like protein from rat uterus showed some anomalies, being neither activated nor inhibited by changing the KCl concentration, and being activated by 2:4-dinitrophenol at low as well as high KCl concentrations.

4. In the course of the actomyosin preparation, a cell-particle fraction was separated; its adenosinetriphosphatase activity showed very different characteristics from those of the actomyosin.

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Metabolism of Calcium, Phosphorus and Nitrogen in Hypervitaminosis A in Young Rats

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The production of toxic effects in human beings as well as in experimental animals by the administration of large doses of pure vitamin A is well recognized clinically (Josephs, 1944; Knudson & Rothman, 1953). Among the experimental animals, rats appear to be specially sensitive to these toxic effects. Skeletal fractures and haemorrhages are among the important features in this malady (Herbst, Pevcek & Elvehjem, 1944; Moore & Wang, 1945; Pevcek, Herbst & Elvehjem, 1945). Although the skeletal changes that lead to these fractures have been known for some time, the mechanism remains obscure. Further, since previous workers' preparations of vitamin A contained large amounts of vitamin D, it is not known with certainty whether the recorded effects are due to vitamin A or to vitamin D. Histological studies by Wolbach (1947) suggested that accelerated epiphyseal cartilage sequences and remodelling processes are responsible for the production of fractures, and that these are mediated independently of the pituitary (Wolbach & Maddock, 1952). Fell & Mellanby (1950), in their investigations of tissue cultures of bone, have shown that vitamin A acts directly. In an attempt to elucidate the mechanism of action of pure vitamin A, free from vitamin D, on bones, the effects of hypervitaminosis A on the chemical composition of bones