

ENZYMOLOGICAL STUDIES ON THE CONTRACTION OF THE PREGNANT UTERINE MUSCULATURE

ON THE INFLUENCE OF ANORGANIC IONS ON ACTOMYOSIN-ATPase
OF THE HUMAN PREGNANT UTERUS

BY

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In the previous reports^{1,2)} of the authors the influences of anorganic ions and placental substances of the rat and woman on actomyosin ATPase of the rat were investigated. As for the ions, Ca, Mg and Mn activated, and Fe and Cu inhibited the actomyosin ATPase of the rat's pregnant uterus. In the present paper the actomyosin ATPase was prepared from the human pregnant uterus and examined under action of anorganic ions.

EXPERIMENTAL METHOD AND MATERIAL

Substrate: Adenosine triphosphate sodium salt (Wako pure chemical) was used.

Buffer: Tris (hydroxy-methyl) amino-methane-HCl buffer (pH 7.0) was used.

Enzyme solution: Normal muscular tissues were resected out of a human uterus in the fifth month of pregnancy complicated by cervical cancer, and used for preparing actomyosin ATPase according to the procedure described in the previous reports¹⁾.

Composition of experimental solution was as follows,

enzyme solution	1.0 ml
tris-buffer	4.0 ml
6 M/10 KCl	1.0 ml
added substance	1.0 ml
distilled water	1.0 ml

After keeping at 37°C for one hour 1.0 ml of M/400 ATP was added into the solution, which was incubated for 15 minutes.

Determination: The solution above was deproteinized by mixing with the same volume of 10% trichloro-acetic acid. Then, liberated anorganic phosphoric acid was colorimetrically determined by Youngburg & Youngburg method.

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RESULTS

Influence of Ca⁺⁺ and Mg⁺⁺: At terminal concentrations of M/1000 CaCl₂ and M/1000 MgCl₂ the activity of AM-ATPase of the human pregnant uterus was accelerated as shown in Table 1.

Table 1. Influence of Ca⁺⁺ and Mg⁺⁺ on AM-ATPase of the human pregnant uterus

control	0.250*
CaCl ₂	0.308*
MgCl ₂	0.343*

* extinction coefficient

Influence of Cu⁺⁺ and Fe⁺⁺:—At terminal concentration of M/1000 CuSO₄ and M/1000, M/10000 FeCl₃ the enzyme activity was observed (Table 2).

Both Cu⁺⁺ and Fe⁺⁺ inhibited the enzyme activity at terminal concentration of M/1000, but FeCl was without effect at concentration of M/10000.

Table 2. Influence of Cu⁺⁺ and Fe⁺⁺ on AM-ATPase of the human pregnant uterus

control	0.250
M/1000 CuSO ₄	0.130
M/1000 FeCl ₃	0.142
M/10000 FeCl ₃	0.243

Influence of CN⁺⁺:—As shown in Table 3, NaCN inhibited the enzyme activity at terminal concentration of M/100, and exerted no influence at concentration of M/1000.

Table 3. Influence of CN⁺⁺ on AM-ATPase of the human pregnant uterus

control	0.250
M/100 NaCN	0.165
M/1000 NaCN	0.233

Influence of NaF:—As Table 4 shows, NaF inhibited the enzyme activity at terminal concentration of M/100, but it was without effect at M/1000.

Table 4. Influence of NaF on AM-ATPase of the human pregnant uterus

control	0.250
M/100 NaF	0.169
M/1000 NaF	0.247

Influence of KI:—KI had no effect on the enzyme activity even at higher concentration as seen in Table 5.

Influence of AgCl:—AgCl had also nothing to do with the enzyme

activity under conditions described in Table 6.

Table 5. Influence of KI on AM-ATPase of the human pregnant uterus

control	0.25
M/100 KI	0.243
M/1000 KI	0.260

Table 6. Influence of AgCl on AM-ATPase of the human pregnant uterus

control	0.25
M/100 AgCl	0.260
M/1000 AgCl	0.262

DISCUSSION

With raw enzyme of actomyosin ATPase prepared out of the rat's pregnant uterus the authors obtained various interesting results in the previous reports. It is quite important whether the biological and biochemical characters of the enzyme could be completely different from each other according to the species. However, as confirmed in the present experiments, the attitude of actomyosin ATPase for anorganic ions was the same when the enzyme was prepared from the human pregnant uterus instead of the rat's uterus in gestation. Of course such facts have to be confirmed further after purification of the raw enzyme. Never-the-less studies on factors dealing with the enzyme activity of the pregnant uterus seem to be interesting for understanding the onset of labor in animals as well as in women.

SUMMARY

1. Out of a human pregnant uterus raw enzyme of actomyosin ATPase was prepared.
2. Ca^{++} and Mg^{++} activated, and Cu^{++} and Fe^{++} inhibited the enzyme.
3. NaF, NaCN, KI and AgCl were without influence on the enzyme even at the concentration of M/1000.
4. As far as the influence of anorganic ions was concerned, the enzyme of actomyosin ATPase of the pregnant uterus of women was similar to that of the rat.

REFERENCES

- 1) Fujii, K., Kato, K. and Hirakawa, K. Bull. Tokyo Med. and Dent. Univ. 8: 55, 1961.
- 2) Fujii, K., Kato, K. and Hirakawa, K. Bull. Tokyo Med. and Dent. Univ. 8: 61, 1961.