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Isolation of Epithelial Like Cells from the Rabbit Myometrium: the Distribution of Creatine Kinase and Plasminogen Activator

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토끼 자궁근층에서 분리한 상피세포의 배양 분리 : 크레아틴 카이네이스와 플래스민 활성인자의 분포

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抄錄 : 가토 자궁 근층에서 분리한 상피세포를 10% 송아지혈청, 3mM glutamine이 함유한 Basal Eagle 배지에서 배양한 결과 세포 성장 시간은 53시간이 소요되었고, estrogen에 insulin을 첨가했을 때는 40시간으로 감소하였다.

Creatine kinase 활성 단위는 단백질 mg당 0.019 unit이었다. 활성의 30%가 이온상태가 높은 완충액에서 추출되었고, plasminogen 활성 인자는 세포 백만당 140 CTA unit였다.

Introduction

Although myometrium is characterized by the predominance of smooth muscle cells, the tissue is made up of heterogeneous components. Nearly 40% of the myometrial volume is known to be occupied by nonmuscle constituents such as fibroblasts, histocytes, macrophages, collagen and elastin[10,20,24].

In initiating a smooth muscle cell culture, it is customary to treat the tissue with collagenase or elastase, trypsin, viokase, EDTA or with any of these combinations plus mechanical agitation[12,19]. The true muscular nature of the growing cells thus isolated is evidenced by spontaneous contractions at least at the initial stage of culture. To name a few examples, contracting cells have been isolated from vas deferens of guinea pig[5], chicken gizzard[7], rabbit mesenteric arterioles[33] and rabbit thoracic aorta[23].

Although dispersed cells from a muscular tissue may yield immediately a culture of predominantly muscle cells[28], a combined usage of selective serial passage and cloning is essential to obtain a putative smooth muscle cell line[19]. This implies that nonmuscle cells present in the muscular tissues must be deleted. Thus the fate of the non-muscle cells found in cultures of muscular tissues remains obsecure.

In this paper we report the isolation from myometrium of the population of cell which have the

epithelioid morphology. In an attempt to assess the functional significance of these cells in myometrium, we have grown the culture and studied the distribution and some properties of enzymes, creatine kinase(CK) and plasminogen activator(PA) during 16 serial passages.

Materials and Methods

Longitudinal muscle including perimetrium was stripped from the uterus of a young adult rabbit(4 kilograms, New Zealand) bathed in oxygenated Ringer's solution containing 100 units/ml each of penicilin and streptomycin and 5mM glucose. The minced pieces of myometrium were trypsinized in 10 volumes of 0.05% trypsin, 0.2% EDTA and 5mM glucose in phosphate buffered saline, pH 7.4. The cells were agitated by magnetic stirrer at 37°C for five min. and the supernatant was discarded. The cells liberated during the next 10 min. were collected and plated at an initial cell density of 1×10^4 cells/cm^2, in waymouth medium, 3 mM glutamine, 3% porcine serum, 100units/ml of penicillin and streptomycin and 2.5μg/ml fungizone. Cells were grown in an atmosphere of 95% CO_2 and 5% air at 37°C and were fed with the same medium twice a week for the next two weeks fellowed by two more weeks of feeding with the Basal Eagle's Medium with glutamine containing 10% chick embryo extract and 10% fetal calf serum. Confluency was observed by this time. Subcultured cells were found to thrive in various media(MEM, Dulbecco-Vogt, waymouth etc.) containing different sera(horse, porcine, newborn calf), and in the presence or absence of chick embryo extracts. The cells could survive at the pH extremes of 6.5 to 8.0 for at least a day. Normal growth was observed in the absence of glutamine at least for one month. The entire experiments were conducted over the period of 6 months. During this period the cells underwent the total of 16 to 18 subcultures.

Preparation of cells for creatine kinase activity: Three types of isolation procedure were used to determine the distribution of CK activity within the cell. First, the enzyme soluble in 0.25M sucrose was isolated. This was accomplished by homogenizing the cells in 0.5ml of the wash solution and centrifuging at 60,000×g for 10 min[20].

Secondly, the amounts of CK in mitochondria, microsome and cytosol were determined. For this purpose roughly 100 to 300mg(30 to 100 million cells) were scraped off the plates and washed three times in 20 volumes of 0.25M sucrose, 0.001 M EDTA in 0.1 mM 2-mercaptoethanol. Cells were homogenized in 15 volumes of the wash solution in a loose fittine Potter-Elvejem homogenizer(20 strokes with cooling). Differential centrifugation was conducted at 700×g. for 10 min(nuclear and cell debris), 12,000 ×g for 30 min. for the isolation of mitochondrial fraction and 100,000×g for 1 hour for microsomal fraction[7].

Thirdly, CK activities extractable in solutions of low and high ionic strength were determined. For this purpose, approximately 100 million cells were washed twice with 0.15 M NaCl and 0.02 M phosphate buffer, centrifuged and frozen overnight. Thawed cells were suspended in 1.3 ml of low ionic strength buffer containing 0.05 M KCl in 0.01 M imidazole, pH 7.0 and 0.01mM 2-mercaptoethanol, and the cells were disintegrated by homogenizing in cold(3×10 strokes) in Potter Elvejem homogenizer. The residue was rehomogenized in 1.3ml of 0.6 M KCl, 0.0 M imidazole, pH 7.0 and 0.1 mM 2-mercaptoethanol. The homogenate was gently stirred for 30 min. at 0°C before separating the insoluble residue by centrifugation at 10,000×g for 20 min. The pellet was resuspended in 1 ml of the high ionic strength buffer and extracted overnight with gentle stirring. The CK activity was determined separately in the two extracts.

Creatine kinase and creatine kinase isoenzyme assay: Creatine kinase activities were determined at 25°C using calbiochem CPK Max-Pack kit[3] which is based on the method of Oliver[25] and Rosalki[27]. Approximately 10μl portions of 10 times diluted cellular supernatant was added to 1ml of reaction mixture containing 1.2mM ADP, 3.6mM AMP, 1,700mIU glucose 6-phosphate dehydrogenase, 4mM glucose, 1,700mIU hexokinase, 8.4mM $MgCl_2$, 1.8 mM NADP, 0.05 M Pipes buffer, pH 6.5 and 0.02 M creatine phosphate. The activity was expressed

as μ moles NADPH produced per min. per mg protein by measuring absorbancy at 340nm[13].

CK isoenzymes were separated on the cellulose polyacetate strip membrane in a Beckman microzone chamber. Cellular extract in the amount of 0.25 μl containing approximately 1 milli unit of CK was applied to the membrane and was electrophoresed for 45 min. at 250 V in 0.025 M Tris-0.19 M glycine buffer, pH 8.6 in 0.1 mM 2-mercaptoethanol. Strips were stained for CK activity by layering the cellulose acetate strips over the reaction gel[36] of the following composition: 5mg/ml agar noble; 100 mM triethanolamine-HCl buffer, pH 7.2; 5mM glucose; 5mM $MgCl_2$; 5mM AMP; 0.8mM ADP; 1 mM NADP; 4mM creatine phosphate; 0.5 mg/ml nitroblue tetrazolium; 0.0025 mg/ml phenazine methosulfate, 4 unit/ml hexokinase; 2 unit/ml glucose 6-phosphate dehydrogenase. strips layered over the reaction gels were incubated at 37°C until good fomazan color developed. Immunodiffusion plate of 4×9cm was suitable to solidify 10 ml of reaction gel mixture[26].

Plasminogen activator assay: Since serum contains inhibitors of PA, it was necessary to delete serum from the culture medium prior to the enzyme assay. When the cells were grown to confluency, which occurred on the 6th or 7th day after subculture, serum in the culture fluid was replaced by 1 % bovine serum albumin(sigma). The confluent cells were rinsed twice with serum free culture medium. Medium was changed each day for 4 consecutive days and both extra and intra cellular PA activities were measured using fibrin-agar plates[32] based on the method of Astrup & Mullertz[1]. This method estimates the content of plasmin as well as of plasminogen activators. By heating the fibrin plates for 35 min. at 85°C, the plasminogen in the fibrin plates is destroyed and the substrate reacts with plasmin only.

For the measurement of intracellular PA activity, $2^1/_2$ million cells from a 21 cm^2 flask were scraped off and washed three times with phosphate buffered saline and were centrifuged at 700×g for 10 min. The packed cells were sonicated in a total volume of 0.5ml of saline solution and were centrifuged at 12,000×g for 30 min. Au aliquot of 10 μl supernatant was applied to 2.3mm hole in a fibrin-agar plate which had been preincubated at 85°C for 35 min. The plates were prepared by clotting 0.2% fibrinogen in 1% agar, 50 mM sodium phosphate buffer, pH 7.0, 0.15 M NaCl, 0.1% NaN_3 with a 5 NIH units of bovine thrombin. The plates were incubated at 37°C for 18 hourse. The area of lysis was calculated and activities were expressed as CTA units per million cells. CTA units were calculated from the graph(log mm^2 lysis vs log absolute CTA units) which was standardized against Serano Pharmaceutical Urokinase, 92,000 CTA units per mg protein. Proteins were estimated by the method of Lowry *et. al*[21].

Myosin detection by Immunodiffusion: The cellular extracts were tested immunologically for the presence of myosin using goat antiserum against bovine uterus smooth muscle myosin. Column purified smooth-muscle myosin was isolated according to the method of Wachsberger and Pepe[37]. Approximately 4 mg of purified myosin in 2 ml complete Freund's adjuvant was injected. A two-days post bleeding was performed to generate control goat plasma for the immunological experiments. Biweekly booster injections of 1 mg myosin in incomplete adjuvant were given. The gamma globulin antiserum fraction was then obtained by a triple ammonium sulfate precipitation of 33%[22]. Immunodiffusion was performed in a 1.0% agar gel matrix suspended in 0.05M sodium phosphate buffer, pH 7.0, containing 0.15 M sodium chloride and 0.1% sodium azide.

Effects of insulin, estrogen and cis-hydroxy proline on cell growth: To study the effects of hormones, the cells were fed with culture medium containing either insulin or insulin plus estradiol. Some of the cells were also fed with cis-hydroxyproline which was reported to selectively suppress the growth of fibroblasts.

Insulin, estradiol and cis-hydroxyproline dissolved in minimum amount of 95% ethanol were spread on petri dishes and were allowed to dry under the uv lit hood. The dried powder was first absorbed in newborn calf serum which was then added to the growth medium(hormone enriched medium contained

20 μg insulin/ml and 0.1 μg estradiol/ml). The concentration of cis-hydroxyproline was 100 μg/ml.

Cells were counted on Levy hemocytometer. Cells grown in normal and insulin enriched medium were fixed in Bouin's fluid after 7 days in culture and stained with Harris hematoxylin and aqueous eosin.

Behavior of the cells under electric stimulation was studied by exciting the cells under AC field of 10 volt/cm by immersing microelectrodes right above cells in the culture fluid. Excitation was also attempted by bringing the electrode in contact with the cells.

Antibiotics and antimycotics containing penicillin, streptomycin and fungizone were obtained from Grand Island Biological Co., Grand Island, New York; Waymouth medium, porcine serum and chick embryo extract were obtained from Flow Laboratories, Rockville, Maryland, U.S.A.; trypsin was obtained from Worthington Biochemical, Freehold, New Jersey, U.S.A.; fibrinogen, thrombin, insulin, cishydroxyproline(Hydroxy-D-Proline-Allo) and β-estradiol were obtained from Sigma Co., St. Louis, Missouri, U.S.A.; Freund's adjuvant was obtained from Cappel Laboratories, Downington, PA, U.S.A.

Results

Cells liberated from the myometrium upon treatment with trypsin and EDTA during 5 to 15 minutes were of mixed nature. Observation of cells under the phase contrast microscope immediately after trypsinization revealed the presence of short crescent shaped cells, elongated bipolar cells and polygon shaped cells with fuzzy outlines. within a week in culture some of the attached cells could be seen to have multiplied in several parts of the culture flasks. The multiplying colonies were either of short crescent shape or polygonal shape. At the end of four weeks the confluent cells were mainly of polygonal epithelioid type.

Cells attached to the flask at the onset of subculture are of various shapes(Fig. 1). Some of the spindle-shaped cells are spiked at polar ends and contain halos around the plasma membrane near the nuclei. The length of the bipolar cells is 120μ and the diameter around the nucleus is approximately

Fig. 1. Cells attached to the culture flask at the onset of subculture. Cells are bipolar spindle shaped. Some are polygon shaped, or stellate(700 ×).

Fig. 2. Cells in log phase of growth. Cells with cytoplasmic processes are seen in noncontiguous area(700 ×).

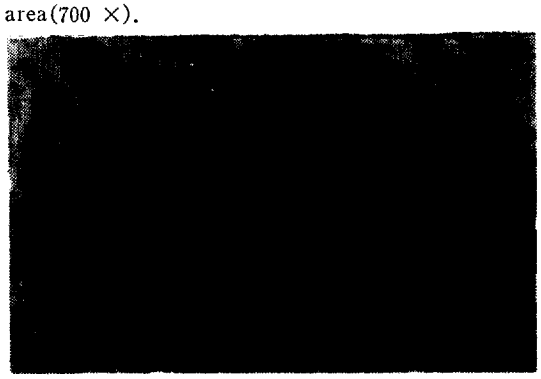

Fig. 3. Confluent cells at day 7. Cells in general are of rounded appearance. Spindle shaped cells are no longer seen(700 ×).

5μ. Figure 2. represents cells in log phase of growth. The cells that are contiguous show epithelioid morphology and are characterized by flat, round nuclei with one or two nucleoli. Figure 3. shows the confluent cells at day 7. The spindle-shaped cells are no longer seen.

The isolated uterine cells had a doubling time of

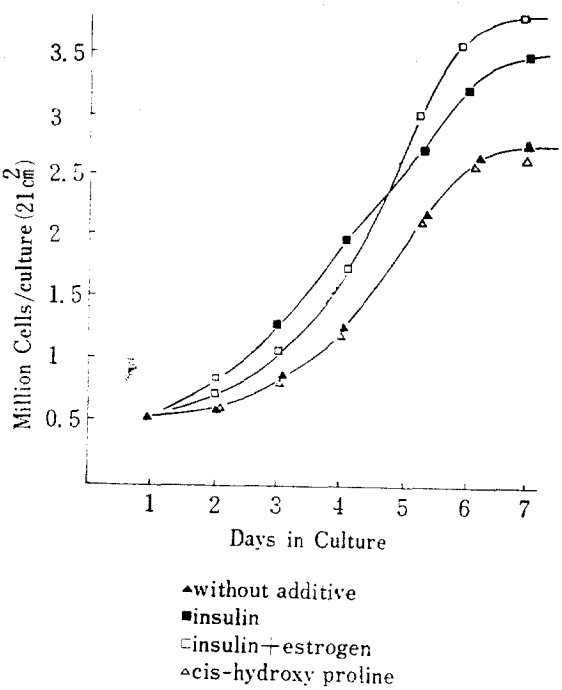

Fig. 4. Growth curve of the cells in culture in the presence of different additives.

53 hours, when grown in the presence of 10% fetal calf serum. Insulin enhanced the growth rate and this was most pronounced on day 3(Fig. 4). The growth rate was highest in the presence of insulin plus estradiol and this estrogen-insulin effect was highest on day 5. Cis-hydroxyproline in the amount of 100μg/ml had no effect on the growth rate.

The isolated cells exhibited both CK activity(Table 1) and PA activity(Table 2). The CK activity of 0.019 unit/mg protein was obtained when the rabbit uterus cell line was allowed to grow to confluency. A culture of fetal calf left ventricle in a stationary phase was determined to be 0.0117 unit/mg protein whereas the same culture in mid-log phase of the growth cycle, the CK activity was 0.022 unit per mg protein.

The isoenzyme distribution on the cellulose polyacetate strip revealed that the cell line contained mainly BB form of CK and a trace amount of MM form(Table 2).

When the cells were fractionated, 96% of the total activity was found to be in cytosol fraction, 3.3% in the mitochondria and the remaining 0.8% in the microsome(Table 3).

Table 1. Creatine kinase activities of the rabbit uterus and fetal bovine left ventricle in culture

Cells in culture	A_{340}/min-mg protein	μmole NADPH/min-mg protein
Rabbit uterus cell line in stationary phase	0.12	.019
Fetal calf left ventricle in culture in stationary phase	0.73	.117
Fetal calf left ventricle in culture in mid-log phase	0.14	.022

Table 2. Creatine kinase isoenzyme distribution of the rabbit uterine cell line and tissues of other sources

	MM	MB	BB
Rabbit uterine cells in culture	+		+++
Dog heart	+++		
Dog brain			+++
Beef heart	++	+	
Bovine non-pregnant uterus myometrium	+		+++
Bovine uterus endometrium			+++
Bovine uterus cotyledon			+++
Fetal calf thoracic aorta in culture			+++
Fetal calf left ventricle in culture	+++	+	

+ indicates trace amount;
+++ indicates great amount

Table 3. Intracellular distribution of creatine kinase

Cell fractions	unit per million cells	% total activity
Mitochondria	0.0027	3.3
Microsome	0.0006	0.7
Cytosol	0.079	96

When the spindle-shaped cells were isolated by cloning, they were again grown to epithelial shape upon reaching confluency. The differential plating was also attempted by separating the cells which settled down in the first 40 min. from those which remained afloat. Four successive serial passages

Table 4. Plasminogen activator of rabbit uterine cell line(RU-4) Remaining Quiescent in 1% bovine serum albumin

Number of days in BSA	Fxtracellular CTA units per million cells	Intracellular CTA units per million cells
1	70	140
2	60	500
3	40	450
4	70	400

thus carried out did not significantly increase the number of spindle-shaped cells. The differences in the CK activities between the cells that settled to the culture dish quickly and those that did not are illustrated in Fig. 5. No dramatic difference was achieved during the three serial passages.

In Table 5 are summarized in greater detail the results that are illustrated in Fig. 5. Creatine kinase extractable in low and high lonic strength buffers during serial passage was recorded. A significant amount of creatine kinase was found in the high ionic strength buffer which is customarily used to extract myosin from muscle system[14,34]. The average value for the relative activities of CK in the low ionic strength was 12.6 unit per mg protein as opposed to 5.7 unit per mg protein extractable by the high ionic strength. The average amount of protein isolated from 12 million cells in low ionic strength was 0.78mg and in high hionic strength

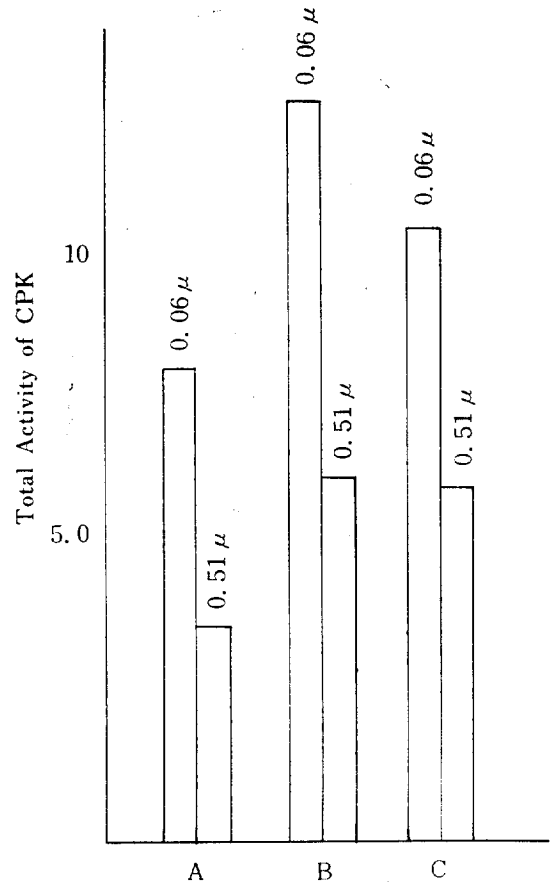

Fig. 5. Creatine kinase activities extractable in low and then in high ionic strength salt solution.
A : Cells were allowed to settle down to the bottom of culture dish.
B : Cells which floated in the first passage.
C : Cells which floated in the 1st passage but settled in the 2nd.

Table 5 CK Activities of the cells in serial passage extracted from the low and then high ionic strength solution

Description of the cell	Extracted in 0.06μ			Extracted in 0.51μ		
	CPK activities $\triangle A_{340}$ per min.	mg protein	CPK activities $\triangle A_{340}$ per min. protein per min.	CPK activities $\triangle A_{340}$ per min.	mg protein	CPK activities $\triangle A_{340}$ per min per mg protein per min
Cells settled in the 1st serial passage	8.06	0.65	12	3.6	0.52	7.0
Cells floated in the 2nd serial passage	12.6	0.78	15	5.2	0.52	5.2
Cells settled in the 2nd serial passage	10.4	0.91	11	5.0	0.4	5.0
Average		.78	12.6		.48	5.7

See Figure 3 for detailed experimental procedure.

Tabie 6. Stability of CK after storage at 0°C in the solution of high ionic strength

Description of cells or tissue		CPK A_{340} per min.				
	protein in mg	0 min.	6 hours	24 hours	48 hours	6 days
RU-4 cells settled in the 1st serial passage	0.052	0.036	0.026		0	
RU-4 colls floated in the 2nd serial passage	0.052	0.052	0.054		0	
RU-4 cells settled in the 2nd serial passage	0.04	0.05	0.015		0	
Bovine gravid uterus myometrium in low ionic 0.05 M.	0.02	0.200				0.120

0.48mg.

The cells which settled in the second serial passage contained a total of 1.3mg protein of which 0.52mg was found to be extracted only by the solution of high ionic strength. The cells which settled rapidly at the bottom of the culture flask contained 1.2mg protein of which 0.4mg was extractable only by high ionic strength solution. Relative specific activity of CK was 15 per mg protein for the floating cells in the second serial passage which was extracted by the low ionic strength solution. The activity for the protein extractable only with high ionic strength was 5.1 per mg protein.

Studies were also conducted to measure the relative stability of CK in cell culture. Proteins from the cells extracted in low and high ionic strength salt solutions were kept at 0°C. Table 6 shows CK activity at time 0, 6, 24, 48 hrs. No decrease in the activity was detected at 6 hrs. after homogenization. The activity was nearly completely gone in the high ionic strength extract by 48 hrs. The activity of the fresh homogenates of bovine uterine tissue(although there was a species difference) on the other hand retained nearly 60% of the initial activity after 6 days at 4°C.

Extracellular production of plasminogen activator remained relatively constant(Table 4) at approximately 50 units per million cells over 4 days after the serum content of the culture medium was replaced by bovine serum albumin. The greater portion of activity was found intracellularly amounting to 140 CTA per million cells on day 1. The activity increased 3-fold the next day and decreased slightly on day 4 from 500 CTA to 400 CTA/million cells.

Immunoprecipitin band was formed when the cellular extract on Ouchterlony double diffusion plate was allowed to react with anti-goat serum against bovine uterine smooth muscle myosin.

It was also noted that the cells which were allowed to grow to confluency and were left without being subcultured for two weeks deposited amorphous materials. These materials were resistant to trypsin. No contraction was observed when the cells were stimulated under the electrical field.

Discussion

The rabit uterine cells which we have isolated by trypsinization of young rabbit myometrium can be grown in culture. At the onset of subculture the cell shapes are varied and therefore it is not possible to ascertain whether they are muscular(smooth muscle), fibroblastoid or epithelioid. Upon approaching confluency, however, nearly the entire population of the cells assumed epithelial shape. Similar variability in shapes of lone cells in culture under sparse plating conditions has been described of fibroblasts[8]. Thus phenotypic characteristics seemed to be clearly defined only after reaching confluency.

It is apparent that cells of epithelial type are liberated preferentially when the cells are dispersed from the original tissue by trypsin and EDTA. Since muscle cells in culture do not proliferate as rapidly, the population of the epithelial cell type might have selectively increased during repeated subculture. whether or not the muscle cells were totally eliminated during the six month's serial pa-

ssage remains uncertain.

The rounded appearance of our cells in culture stained in hematoxylin and eosin and the lack of contractility under electrical stimulation are evidence that these cells are neither fibroblasts nor smooth muscle cells. The electrical field of 10 volts/cm which we applied to the cells is above the threshold voltage under which excitation is known to be induced in muscle cells even in the absence of added calcium[6].

Two enzymes, CK and PA, were chosen to study phenotype markers of these cells in the myometrium. CK acts as an energy transducer for specilized cell functions like muscular and nervous activity. Plasminogen activator is a highly specific protease whose level is higher in the uterus than any other tissue measured[1]. PA is known to be elborated by epithelial cells in the kidney[22]. The source of the enzyme in the uterus is not established.

The extent of CK activity in the cell line is comparable with activities reported in such non muscle-cells as fresh rat liver and rat kidney tissue. This value is five to six fold lower than the values reported for the rat myogenic cell line in stationary phase[19].

Most of CK is localized in the cytosol. However, next to cytosol mitochondrial fraction contained the highest enzyme activity. The fact that more creatine kinase is found in mitochondria than in microsome suggests that synthetic functions of the cells was not at its peak when the cells attained confluency. It also suggests that energy transduction from oxidation is taking place at high levels.

This distribution pattern makes an interesting comparison with the values obtained from the gravid and non-gravid bovine uteri. In the non-gravid myometrium of a cow, 97% of the total CK was found in cytosol and 3% in the microsome, whereas with the gravid tissue about 10 to 12% of the sucrose soluble activity was found in the microsomes and the rest in the cytosol[22]. Actomyosin in this medium, which sediments with nuclei and other insoluble fragments, is also found to contain 1 to 2% of tightly bound CK, which could be separated only by gel permeation chromatography on agarose.

The role of CK in tissues may be dependent upon the enzyme location within the cell. CK of the mitochondria is known to play a unique role in fascilitating the transport of ATP molecules produced by oxidative phosphorylation[30,17]. This enzyme, unlike soluble cytosolic CK, is known to be bound to the outer portion of the inner mitochondrial membrane.

CK which was extractable only in high ionic strength extractant may be associated with myosin. Myosin isolated from uterine[16] and other smooth muscles has been shown to bind a protein kinase which can only be removed by chromatography. However, the presence in our cell line of a cross reactive protein against myosin antibody is not indicative of the presence of muscle cells[9], since myosin as well as muscle type of CK are also found in a number of non-muscle cells in small quantities. Coexistence of both MM and BB within non-muscle cells has been observed in cells of ascites of rat and mouse tumors as well as in normal mammary glands, aorta and lung of the rat and liver, kidney and lung of the mouse.

The process of serial passage is often used to separate muscle cells from fibroblasts, since trypsinized fibroblasts are known to settle down rapidly to the bottom of the culture flasks. According to the data in the second serial passage, it appears as though the floating cells possess more CK activities than the rapidly settling cells. It is also noted that proportionately greater amount of proteins are extractable by the high ionic strength solution than in the case of rapidly settling cells.

Our cells in culture contains an appreciable level of plasminogen activator and continue to excrete the enzyme into the culture medium. Our results do not exclude the possibility that other types of cells of the uterus might also be responsible for the high levels of uterine PA. However, it has been reported that enzyme with fibrynolytic activity is exclusively contributed by the endothelial cells, even in tissues with the highest activity such as human myometrium[34]. If this is indeed the case, our cell line may have risen from the endothelium embedded intimately with the myometrium at an

interface. Or it is also possible that PA production is not necessarily confined to endothelium.

The fact that cells proliferated rapidly in the presence of cis-hydroxyproline which is a known depressor of fibroblasts, may be characteristic of epithelial cell line. The concentration of cis-hydroxyproline(100μg/ml) is known to be deterimental even to the normal growth of skeletal muscle cell culture. In view of the fact that uterus undergoes many diversified functions, the use of enzyme markers may be meaningless in defining the state of differentiation. There may be cells capable of producting unusually wide spectrum of enzymes. If this is the case, our problems is not to determine the presence of certain enzymes, but to ascertain how much of a certain enzyme is found in what area at what time[31,34],

There has been a controversy over how much of extracellular matrix or muscle cells grow in greater extent during dregnancy. Since muscle cells as well as fibroblasts can form collagen, the increase in the muscle cell volnme may be concomitant with the increase in extra cellular matrix. The need to increase the amount of extracellular matrix may depend upon the size of fetus which a particular species must accomodate.

Our cultured cells from the rabbit myometrium show response to insulin and estrogen. The growth response to estrogen is evidenced even in the culture medium containing 10% calf serum. More rigorous analysis of estrogen effect will be necessary, however, to substantiate the unequivocal response of these cells to estrogen. Furthermore, it will be necessary to grow the cells in more defined medium by depleting the calf serum, the composition of which one is not completely sure of.

We do not exclude the possibility that the cells we grew in culture are a contaminant from perimetrium or endometrium. But if these cells indeed arise from myometrium, the presence of such cells in the myometrium should have functional significance in being able to produce extracellular matrix to increase the uterine size during pregnancy. These cells may be involved in the postpartum involution of myometrium by producing plasminogen activator. Nonspecific proteases in the serum are known to be activated by PA. The cells we have isolated may also have hitherto unknown primary effects related to hormone sensitivities crucial to tlje onset of labor.

We cannot overemphasize the importance of different cell types in accessing the degree of hormonal effects, as the responses are not only organ specific but also cell specific[18].

Summary

Cells with an epithelioid morphology were isolated from the rabbit myometrium and were grown in culture. The cells had a doubling time of 53 hours when grown in the presence of 10% fetal calf serum in Basal Eagle's medium with 3mM glutamine. In the presence of estrogen plus insulin, doubling time was reduced to 40 hours. Creatine kinase activity upon reaching confluency was determined to be 0.019 unit per mg protein. Approximately 30% of the activity was extractable only in high ionic strength buffer. Cells also contained plasminogen activator with a specific activity of 140 CTA units per million cells. Creatine kinase was mainly BB form. The cells contained a cross reactive protein against bovine smooth muscle uterine anti-myosin.

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