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故 朴相允博士를 追慕함

韓國動物學會會長　　**李　　澤　　俊**

本學會의 會長職을 歷任하였고 多年間 本學會 理事로 계셨던 朴相允 博士께서 지난 2月 8日 突然히 幽明을 달리하셨읍니다. 故 朴相允博士의 靈前에 우리나라의 큰 人材를 잃은 슬픈 마음으로 故人의 冥福을 빌 뿐입니다.

故 朴相允교수께서는 祖國光復 직후 서울大學校 文理科大學 生物學科를 卒業하시어, 春川農大·釜山大學校·高麗大學校를 차례로 在職하시다가 成均館大學校 교수로 오늘날까지 在職하시면서 三十餘年間 교육과 연구에 전념하신 生物學者이셨읍니다.

이 긴 세월동안 오로지 動物生理學을 專攻하시면서, 수 많은 新進氣銳의 學者들을 養成하셨고, 또 수 많은 論文과 著書들을 펴 내셨읍니다. 그의 訓導下에서 자란 젊은 學者들은 지금 이나라 各 大學에서 그를 이어 動物生理學을 硏究하고 있으며, 그의 論文과 著書들은 수 많은 後進들의 學問的 길잡이가 되고 있읍니다.

朴博士는 硏究生活의 初期부터 動物生理學가운데서도 특히 比較生理學에 커다란 關心과 硏究의 焦點을 두고 있었읍니다. 이러한 그의 硏究關心의 産物이 1961年에 發刊된 그의 代表作「動物生理學」이었읍니다. 1960年이라면 우리나라에 專門書籍이라는 것은 그야말로 不毛地帶였던 時期입니다만, 당시 弘志社의 採算을 無視한 決斷으로 「生物學叢書」가 10卷가까이 出版될 때, 35歲의 若冠으로 動物生理學의 執筆을 맡아 그의 流麗한 文章으로 우리나라 최초의 動物生理學專門書籍을 펴낸 것입니다. 이 책은 그 後 約 10年동안 動物生理學의 唯一한 책이었고 全國 各 大學에서 다투어 敎材로 사용되었읍니다.

朴博士는 比較生理學을 이렇듯 初期부터 關心을 가지고 硏究하면서 進化論의 槪念이 담기지 않은 比較生理學은 意味가 없으며 또 進化의 개념이 含蓄되어 있지 않는 生物學은 아마추어 博物學에 지나지 않는다고도 입버릇처럼 말해왔읍니다. 따라서 그의 實驗室에서 나온 수 많은 珠玉같은 論文들에는 한결같이 系統學的 考察이 담겨져 있었던 것입니다. 이러한 初期의 그의 論文가운데 代表的인 것이 1964年 동물학회지에 發表된「척추동물 뇌조직의 에너지代謝에 관한 系統學的 比較硏究」로 그는 이 論文으로 1965年에 서울大學校에서 理學博士 學位를 수여받았읍니다.

이와같이 博士는 우리나라 최초의 比較生理學者이셨고, 또 生理學을 進化論에 密着시킨 進化論者이셨으며, 또 動物機能에 進化論的 考察을 가하면서 生化學的 方法論을 일찍이 導入한 先驅者이셨읍니다.

朴博士의 進化에 대한 깊은 關心과 理解는 1980年에 펴낸 그의 著書「進化」(電波科學社 刊)에도 잘 나타나 있읍니다. 이 책은 古典 生物學的 進化의 개념에 아울러 生化學的 進化의 모습을 簡潔하게 다루었고, 末尾에는 그의 該博한 漢文知識을 驅使하여 進化論이 開化期의 우리나라에 미친 영향까지도 다루고 있는 것입니다.

博士는 回甲을 바라보는 최근까지도 硏究生活에 고삐를 늦추지 않아, 近年에는 各種 同位酵素의 生化學的 分析을 역시 系統學的 觀點에서 다루어 오시면서 수 많은 新進 博士도

輩出하셨고, 이들은 또한 現在 國內 여러 大學에서 그의 思想을 이어 活躍하고 있읍니다.

그의 學問的 關心은 이와 같이 주로 比較生理學的 面에서 본 進化에 있었으나 그의 多才多能은 수많은 隨筆, 論說文 등 이루 헤아릴 수 없는 글들을 남겨 놓고 있읍니다. 多年間 高等動物의 腦의 比較生理學 및 比較生化學을 연구하셨던 그는 1978年에는 Halacy의 「Man and Memory」를 「記憶의 秘密」이라는 題目으로 번역하신 적도 있읍니다.

朴博士는 大學敎育 및 硏究에 情熱을 쏟으시는 한편 韓國動物學會, 韓國生化學會, 韓國科學史學會의 育成에도 남달리 活躍을 하시었읍니다. 特히 韓國動物學會 會長職在任時에는 어려운 財政難을 克服하면서 學會誌 發刊에 勞苦를 아끼지 않으셨고 運營의 所任을 다 하시어 오늘의 韓國動物學會의 기틀을 마련해 주셨읍니다.

한편 朴博士는 우리나라 生物學界에서는 보기드문 名文章家이어서 그의 著述이나 論文은 學問的 知識뿐만 아니라 文章技法까지도 後學들에게 가르치고 있읍니다.

이제 우리는 博士님의 모습을 찾아볼 길 없겠으나 남겨놓으신 많은 業績은 길이 빛날것이며 후배들이 이어 받아 動物學分野의 發展에 貢獻할것을 믿어 疑心치 않읍니다.

끝으로 故 朴博士께서 그동안 쌓아 놓으신 功德에 찬사를 드리고 博士님의 靈前에 冥福을 비는 바입니다.

Effects of Catecholamine on the Fusion of Chick Embryo Myoblasts *in vitro*

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鷄胚筋原細胞의 融合에 미치는 카테콜아민의 影響

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요 약

근원세포가 분화하는 과정에서 신경전달물질의 영향을 연구하기 위하여 배양한 근세포에 dopamine과 epinephrine을 처리하여 근원세포의 융합과 세포내 cAMP의 농도에 미치는 효과를 조사하였다.

Dopamine $(3 \times 10^{-5}M)$과 epinephrine $(3 \times 10^{-5}M)$을 세포배양후 34시간에 처리했을때 근원세포의 융합이 크게 억제되었으며, 특히 dopamine의 효과가 epinephrine보다 현저하게 나타났다. 한편, 세포내 cAMP농도는 dopamine과 epinephrine을 처리해도 거의 변화가 없었다.

근원세포의 분화에 cAMP가 관계하는지를 조사하기 위해 dbcAMP, PGE₁ 및 aspirin을 처리하였는데, dbcAMP $(1 \times 10^{-4}M)$는 근원세포의 융합을 억제한 반면, PGE₁ $(3 \times 10^{-6}M)$은 오히려 융합을 촉진하였고, PG 합성효소의 억제물질인 aspirin은 융합 억제효과를 보였다.

Dopamine과 epinephrine이 근원세포의 융합과정에 작용하는 가능성있는 기작에 대해서 고찰하였다.

INTRODUCTION

Myoblast differentiation involves the fusion of cell membranes to form postmitotic multinucleate myotubes and the concomitant elaboration of muscle specific proteins such as creatine phosphokinase (Turner *et al.*, 1974), acetylcholine receptor (Paterson & Prives, 1973), and contractile proteins (Devlin & Emerson, 1978). Myoblasts, cultured *in vitro*,

proliferate, then synchronously fuse to form myotubes. The synchronous differentiation of myoblasts *in vitro* seems to be due to the change of the extracellular environment. However, no mechanism by which myoblasts commit *in vivo* or *in vitro* has been known.

Kalderon & Gilula (1979) have proposed a model for myoblast fusion based on ultra-structural analysis. They suggest that the cytoplasmic particle-depleted vesicles initiate the generation of particle-depleted membrane domaines, both being essential components in the fusion process. This model is quite similar to that of exocytosis of secretory vesicles in other cells (Miller & Nelson, 1977; Lawson *et al.*, 1977). In connection with this view, exocytosis of secretory vesicles in various kinds of cells was shown to be affected by neurotransmitter (Birge, 1970; Bower *et al.*, 1974; Miller & Nelson, 1977; Dunlap & Fischbach, 1978; Horn & McAffe, 1980).

In addition, there are some evidence that the differentiation of myoblast, as well as myotube, is affected by innervation. Bonner (1978) found that chick myoblast populations were changed by *in vivo* manipulation of the nervous system and Filogamo *et al* (1978) observed the contacts between myogenic cells of somites and primitive axons growing from the neural tube as early as 35 hr of incubation in chick embryos. Although the mechanism of interaction between neurons and myogenic cells has not been elucidated, these evidence suggest that myoblast differentiation may be affected by neurotransmitter. Because the protocol of cell culture eliminates neurons from cultured myoblasts, it may give some insight into the nature of synchronous differentiation of myoblast *in vitro* to study the effect of neurotransmitter on cultured myoblasts.

The present investigation was undertaken to study the effect of dopamine and epinephrine as neurotransmitter on myoblast differentiation *in vitro*, and demonstrates that dopamine and epinephrine inhibit myoblast fusion and this effect is not mediated by the increase in intracellular cAMP pool.

MATERIALS AND METHODS

Cell Culture

Cultures were prepared by the method of O'Neill & Stockdale (1972) with minor modifications. Pectoral muscle was dissected out from 12-day old chick embryos. After removing connective tissues, the muscle was minced and incubated for 10 min at 37°C in 0.05% trypsin with gentle agitation. The supernatant was discarded and the tissue clumps were rinsed with RPMI 1640 and reincubated for 10 min at 37°C in 0.125% trypsin with gentle agitation. The supernatant was centrifuged to collect cells. The cell pellet was resuspended in complete medium (RPMI 1640 supplemented with 10% horse serum, 4% chick embryo extract, and 1% antibiotics; designated 8104 medium) and filtered through four layers of lens paper. The cell suspension was preplated on collagen-coated dishes for 10 min to remove fibroblasts. Cells were then plated at 2×10^5 cells/35 mm plastic culture

dish for chemical treatment or 2.4×10^6 cells/90 mm culture dish for cAMP assay. The cells were grown for the first 24 hr in 8104 medium and the medium was changed at 24 hr and at 2-day interval thereafter to fresh medium (RPMI 1640 supplemented with 10% horse serum, 2% chick embryo extract, and 1% antibiotics).

Dopamine, epinephrine, dibutyryl cyclic AMP (dbcAMP) and prostaglandin E_1 (PGE_1) were dissolved in PBS and used at a final concentration of $3 \times 10^{-5}M$, $3 \times 10^{-5}M$, 1×10^{-4} M, and $3 \times 10^{-6}M$, respectively. Aspirin was dissolved in 50% ethanol in PBS and used at a final concentration of $1 \times 10^{-3}M$. The chemicals were added at 34 hr after plating.

Measurement of Cell Fusion

Cell fusion was determined by direct microscopic examination of ethanol-fixed and Giemsa-stained cultures at a magnification of 400X. It was considered as fused cell only if there was clear cytoplasmic continuity and at least three nuclei present in each myotube. Randomly chosen 20 fields were counted for each culture.

Cyclic Adenosine 3′, 5′-Monophosphate (cAMP) Assay

Dishes were washed twice with 0.85% NaCl. After the addition of 0.2 ml of 50 mM sodium acetate buffer, pH 5.2, preheated to 90°C, cells were rapidly scraped off with a rubber policeman. The cell suspension was then incubated at 90°C water bath for 60 min and subjected to vigorous vortexing for 2 min. After standing at 5°C for 30 min, samples were stored at −70°C and assayed within 1 week.

Immediately prior to assay, the samples were thawed, and cellular debris was removed by Eppendorf centrifugation at 5°C for 5 min. Before the centrifugation, an aliquot of sample was taken to measure the DNA content. The supernatants were analyzed for cAMP using Amersham's cAMP assay kit. The assay procedure is based on the competition between unlabelled cAMP and [³H]-AMP for cAMP-binding proteins.

DNA Estimation

The DNA content was estimated by the method of Rissane & Robins (1958) with minor modifications. The samples were incubated at 60°C for 45 min with 0.1 ml of 40% (w/v) 3,5-diaminobenzoic acid that had been decolorized with charcoal. The reaction was stopped by the addition of 2 ml of 1 N HCl, and fluorescence was then measured (excitation 405 nm; emission 520 nm). Calf thymus DNA was used as a standard.

Affinity Chromatography on Lysine-agarose

Agarose (Sepharose 4B) was activated with cyanogen bromide (CNBr) by the method of Cuatrecasas *et al.* (1968). One hundred ml of settled agarose was suspended in 100 ml of distilled water. Two hundreds ml of 5% CNBr was added to the agarose suspension and the pH was adjusted to and maintained at 11 by titration with 4 M NaOH. The reaction was allowed to proceed at room temperature for 9 min and stopped by washing with 1.9 l of cold 0.1 M sodium carbonate buffer, pH 8.9 on a Büchner funnel. Fifty ml of 40% (w/v) L-lysine monohydrochloride, pH 8.9 was then added at room temperature to 150 ml suspension containing 100 ml of activated agarose. The slurry was gently stirred

at 5°C for 24 hr.

Chromatography was performed according to Hatzfeld *et al* (1982). Three hundreds ml of horse serum was centrifuged at $19,000 \times g$ for 20 min and loaded onto a lysine-agarose column (20×1.5 cm, flow rate of 20 ml/hr, 4°C) previously equilibriated with 0.1 M phosphate buffer, pH 7.4. Embryo extract was chromatographed in the same way as horse serum after incubation at 37°C for 1 hr with deoxyribonuclease (20 ug/ml), ribonuclease (10 ug/ml), and hyaluronidase (5 ug/ml).

RESULTS

The Effect of Catecholamine on Myoblast Fusion

To observe the effect of catecholamine on myoblast fusion, 3×10^{-5}M dopamine or 3×10^{-5}M epinephrine was added to the cultures at 34 and at 72 hr after plating.

Dopamine or epinephrine markedly inhibited myoblast fusion, and dopamine was more potent than epinephrine (Fig. 1). The myoblast in the control medium initiated fusion at 48 hr and nearly completed at about 72 hr, whereas the myoblast added with dopamine or epinephrine kept proliferating up to 72 hr and only after 72 hr they started fusing (Fig. 3).

Cell proliferation was hardly affected by the treatment of catecholamine (data not shown). Furthermore, the catecholamine-treated myoblast advanced fusion in spite of the addition of catecholamine at 72 hr. These results indicate that the inhibitory effect of catecholamine seems not due to toxic effects.

The Effect of dbcAMP or PGE_1 on Myoblast Fusion

Though cAMP seems to be involved in myoblast differentiation, many results are contradictory one another. dbcAMP, when added at a concentration of 1×10^{-4}M,
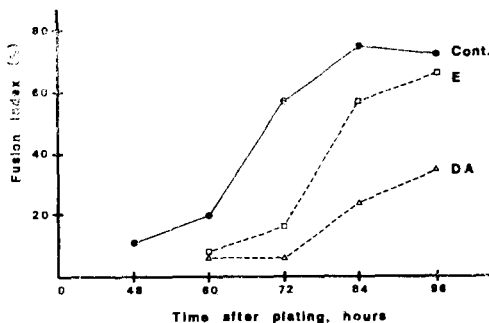

Fig. 1. Effect of catecholamine on myoblast fusion. ●—●, control; △—△, 3×10^{-5}M dopamine; □—□, 3×10^{-5}M epinephrine. The chemicals were added at 34 and 72 hr after cell plating.
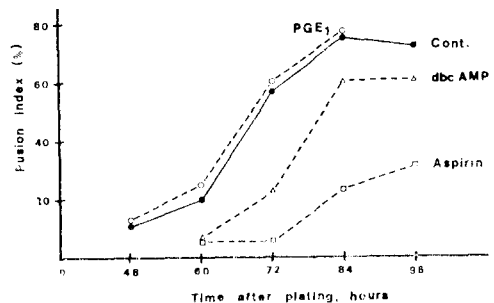
Fig. 2. Effect of dbcAMP, PGE_1, and aspirin on myoblast fusion. ●—●, control; △—△ 1×10^{-4}M dbcAMP; ○—○, 3×10^{-6}M PGE_1; □—□, 1×10^{-3}M aspirin. The chemicals were added at 34 hr after cell plating.
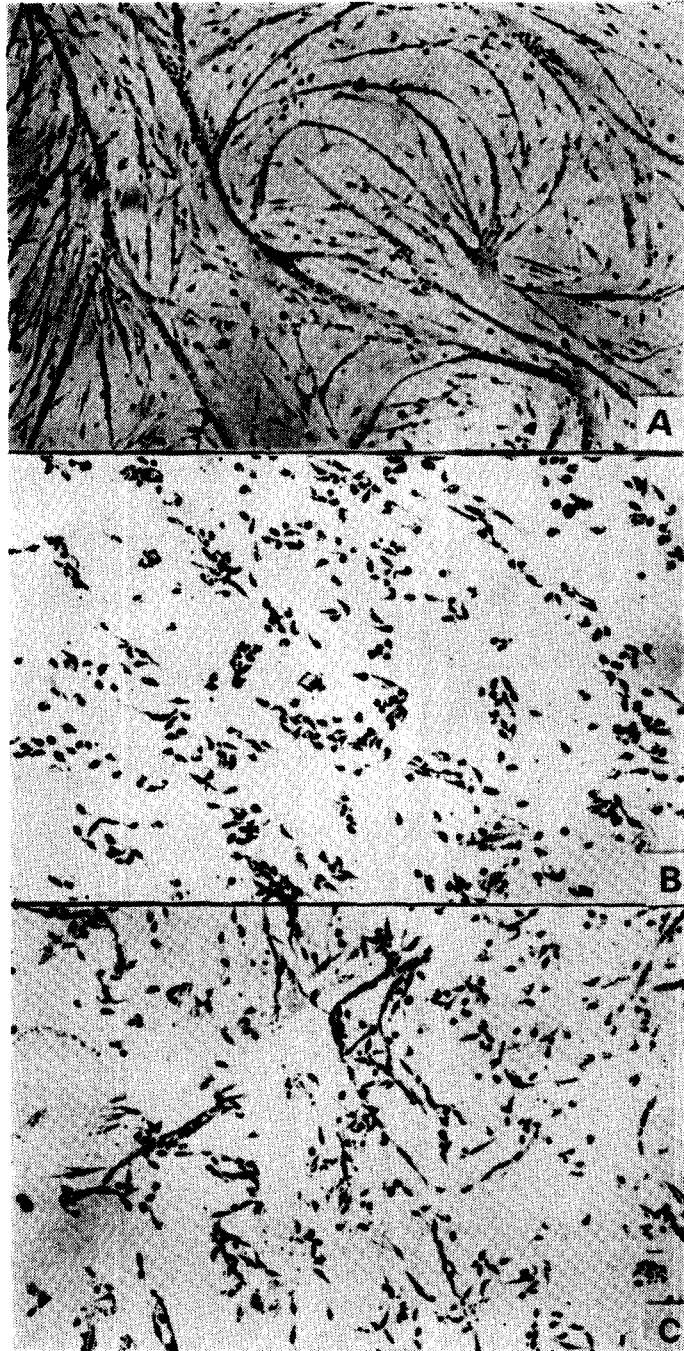
Fig. 3. Effect of catecholamine on myoblast dfferentiation. (A) control;   (B) 3×10^{-5}M dopamine; (C) 3×10^{-5}M epinephrine.
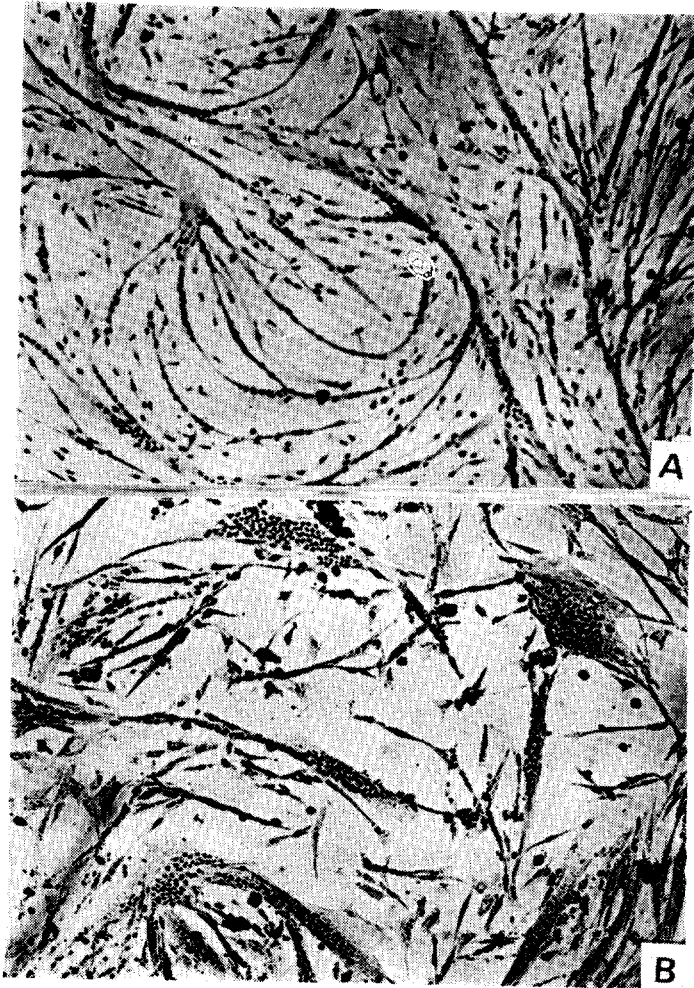
Fig. 4. Morphology of myoblasts cultured with control (A) and with depleted medium by passage over lysine-Sepharose column (B).

inhibited myoblast fusion. The inhibitory effect of dbcAMP was weaker than that of catecholamine. On the other hand, PGE_1 $(3 \times 10^{-6}M)$ had no inhibitory effect but enhanced myoblast fusion by about a few percent (Fig. 2).

Since myoblast fusion was enhanced by PGE_1, aspirin, an inhibitor of PG synthetase, was used to test if PGE_1 indeed involved in myoblast differentiation. When aspirin was added to the cultures at a concentration of $1 \times 10^{-3}M$, myoblast fusion was markedly inhibited. However, aspirin did not seem to prevent the cell alignment which normally preceded myoblast fusion. This result suggests that PGE_1 is intimately related to myoblast fusion.

cAMP Assay

To ascertain the inhibitory effect of catecholamine that is mediated by an increase in intracellular cAMP, it was examined whether dopamine or epinephrine induce cAMP accumulation. Both dopamine and epinephrine had no effect on intracellular cAMP level (Fig. 5). The basal levels of cAMP were 30 pmole cAMP/mg DNA. It fell in the range of 10~200 pmole cAMP/mg DNA, which is the result of Reporter (1972) obtained from differentiating rat myoblasts.

The Effect of the Depleted-medium

It was reported that passage of chick embryo extract on lysine-Sepharose columns largely reduced its ability to promote myoblast fusion (Hatzfeld *et al.*, 1982). Since the active group of lysine-Sepharose was primary amino group, it was speculated that the primary amino group of catecholamine might be responsible for the inhibition of myoblast fusion. To test this possibility the myoblast was cultured in this depleted-medium and the inhibitory process in the depleted-medium was compared with that in the culture added by catecholamine.

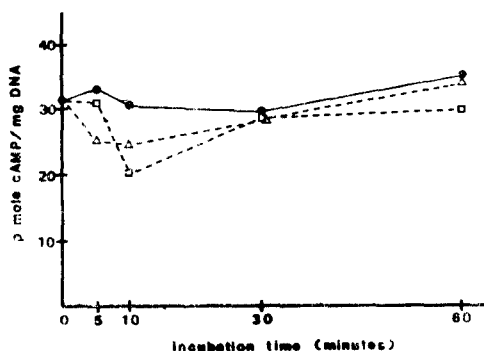During the first 60 hr, the myobast in the depleted-medium normally proceeded proliferation

Fig. 5. Effect of catecholamine on the intracellular cAMP level. ●—●, control; △—△, $3 \times 10^{-5}M$ dopamine; □—□, $3 \times 10^{-5}M$ epinephrine. The chemicals were added at 34 hr after cell plating.
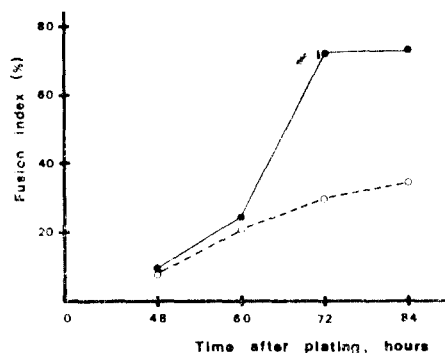
Fig. 6. Effect of depleted medium (○···○) by passage over lysine-Sepharose column on myoblast fusion as compared to control (●—●).

and cell alignment. After 60 hr, however, a large portion of them did not fuse, and then degenerated (Fig. 6). Although some of them fused, they failed to form normal myotube. Instead of elongated myotubes, abnormal multinucleated cells similar to the myosacs described by Bischoff & Holtzer (1968) were formed (Fig. 4).

DISCUSSION

Catecholamine produces inhibitory or stimulatory effect on exocytosis of secretory vesicles in various kinds of cells (Birge, 1970; Bower et al., 1974; Miller & Nelson, 1977; Dunlap & Fischbach, 1978; Horn & McAffe, 1980), and myoblast membrane fusion is hypothesized to occur by the mechanism similar to that of exocytosis of secretory vesicles (Kalderon & Gilula, 1979). Furthermore, it has been proposed that neuron has some effect on the differentiation of myoblast (Bonner, 1978; Filogamo et al., 1978). These observations suggest that the differentiation of myoblast may be affected by catecholamine.

The present experiments showed that dopamine and epinephrine had inhibitory effect on myoblast fusion. Since our finding shows that catecholamine had no effect on cell proliferation and the catecholamine-treated myoblast advanced fusion in spite of the addition of catecholamine at 72 hr, the inhibitory effect on myoblast fusion by catecholamine is unlikely to be due to toxic effects. Thus, it is likely that neuron and myoblast have some interrelationship in the process of myoblast fusion.

Zalin (1973) reported that exogeneous cAMP delayed the onset of myoblast fusion. This was confirmed in the present culture condition. However, dopamine and epinephrine were shown to have no effect on the intracellular cAMP level in cultured myoblast. Thus, the inhibitory effect by dopamine or epinephrine was not due to the intracellular cAMP accumulation. Bower et al. (1974) reported that dopamine (10^{-5}M) and epinephrine (10^{-5}M) inhibited melanophore stimulating hormone (MSH) release, and proposed that the D-2 receptor was involved in the action of dopamine and epinephrine (Munemura et al., 1980). Dopamine receptors can be divided into two general categories (designated D-1 and D-2) on the basis of biochemical and pharmacological criteria (Kebabian & Calne, 1979). The D-1 receptor in various tissues regulates a specific dopamine-sensitive adenylate cyclase, and therefore to increase the level of cAMP (Brown et al., 1977), whereas the D-2 receptor neither enhances adenylate cyclase activity nor increases the level of cAMP. Thus, it is possible that the interaction of dopamine or epinephrine with the D-2 receptor which may exist in myoblast results in the inhibition of myoblast fusion. In addition, it is also possible that the α-adrenoreceptor may be involved in this inhibitory effect, but it remains to be elucidated.

Curtis & Zalin (1981) reported that epinephrine (10^{-5}M) and isoproterenol (10^{-5}M) maximally stimulated cell fusion with concomitant increase in the intracellular cAMP level in cultured myoblasts. However, in our studies epinephrine added at the same concentra-

tion stimulated slightly the cell fusion, but showed rather an inhibitory effect on the fusion at the later stage of the culture (data not shown). Furthermore, at higher concentration of epinephrine (e.g., at 3×10^{-5}M), this neurotransmitter consistently inhibited the fusion process of myoblast.

In addition, it was reported that epinephrine interacted with the β-adrenoreceptor at lower concentration (10^{-6}–10^{-7}M), whereas at higher concentration epinephrine interacted with the α-adrenoreceptor or the D-2 receptor in the intermediate lobe of the rat pituitary gland (Bower *et al.*, 1972; Munemura *et al.*, 1980). The conflicting results of epinephrine effects on myoblast fusion, therefore, can be due to the presence of both the D-2 receptor (or the α-adrenoreceptor) and the β-adrenoreceptor.

How does dopamine or epinephrine inhibit myoblast fusion is uncertain. There are, however, some evidence that catecholamine affects Ca^{2+}-influx. Horn & McAffe (1980) proposed that activation of an α-adrenoreceptor inhibits a voltage-sensitive Ca^{2+} conductance, thereby reducing the inward Ca^{2+} current. Douglas & Taraskevich (1978) reported that dopamine decreased the frequency of spontaneous action potentials. Calcium normally enters a cell through a relatively specific calcium channel that is independent of membrane potential, or through a potential-dependent calcium channel (Rasmussen & Goodman, 1977). Both channels may be activated by hormonal interaction with the plasma membrane. The fusion of myoblast is also known to be dependent on Ca^{2+} (Bischoff, 1978; Ha *et al.*, 1979). Little is known about the molecular action of Ca^{2+} until now, but David *et al.* (1981) have recently observed that a measureable increase in net calcium influx occurred just prior to fusion. This finding indicates that Ca^{2+} may play a role in myoblast differentiation. On the basis of all these observations, it can be speculated that dopamine or epinephrine interacts with their receptor, and their interaction blocks Ca^{2+} influx, which is responsible for either (or both) the myoblast differentiation and/or the membrane fusion of myoblast.

Zalin (1977, 1979) has reported that PGE_1 is an effective promoter of precocious fusion in cultured myoblast. This was confirmed in the present culture condition. Addition of PGE_1 enhanced myoblast fusion, whereas aspirin, an inhibitor of PG synthetase, inhibited. This result suggests that PGE_1 has a normal role in the fusion process, perhaps as the synchronizing agent. It was proposed that the effect of PGE_1 was mediated by an increase in the intracellular cAMP level (Zalin & Leaver, 1975). However, how exogeneous dbcAMP delays myoblast fusion remains to be elucidated.

In depleted medium, abnormal multinucleated cells that are similar to the myosacs described by Bersten *et al.* (1983), who observed that lysosomotropic amines prevented elongation of myotubes, were formed. Bersten *et al.* (1983) proposed that transglutaminase, an intracellular enzyme, was responsible for the formation of myotube, but the present result indicates that some components of embryo extract are also involved in the formation of myotube. The multinucleated cells were not observed in the culture added with catech-

olamine. Thus, it is unlikely that catecholamine inhibits myoblast fusion by the same way as the depleted medium.

There are some debates about the control of fusion process *in vitro*. One hypothesis is that cells in culture deplete a substance that stimulates cell proliferation and inhibits fusion (Konigsberg, 1982). This model assumes that there exists (a) differentiation-inhibiting factor(s) in embryo extract and this is generally believed to be a protein (Evinger-Hoges *et al.*, 1982).

In the present experiment, catecholamine was shown to inhibit myoblast fusion and therefore it is also possible that neuron has some inhibitory effect on myoblast differentiation *in vivo*. If so, it can explain the nature of the synchronous differentiation of myoblast *in vitro*.

ABSTRACT

In order to investigate the effect of neurotransmitter on myoblast differentiation *in vitro*, the effects of dopamine and epinephrine on myoblast fusion and on the intracellular cAMP level in cultured myoblasts were examined.

Dopamine $(3 \times 10^{-5}M)$ and epinephrine $(3 \times 10^{-5}M)$, when added at 34 hr after cell plating, markedly inhibited myoblast fusion, and dopamine was more potent than epinephrine. Both dopamine and epinephrine had no effect on intracellular cAMP level.

At the same time, exogenous dbcAMP, PGE_1, and aspirin were used to examine whether cAMP is involved in myoblast differentiation. dbcAMP $(1 \times 10^{-4}M)$ inhibited myoblast fusion, whereas PGE_1 $(3 \times 10^{-6}M)$ had no inhibitory effect, rather enhancing myoblast fusion. Aspirin, an inhibitor of PG synthetase, was shown to inhibit myoblast fusion.

Possible mechanism by which dopamine or epinephrine at a specific concentration used inhibits myoblast fusion is discussed.

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