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Abstract

An attempt was made to isolate the cell proliferation stimulation factors in the supernatant of embryo carcases and adult muscles of chickens. Evidence was obtained for the presence of at least two or more stimulating factors in both the embryonic and adult muscular supernatants. These factors did not require a supplement of sera or other supporting agents. Furthermore, the use of the salting-out method with ammonium sulfate revealed two or more growth stimulants in the supernatant of chick cells.

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BRIEF NOTE

PLURAL GROWTH FACTORS IN THE SUPERNATANT OF EMBRYOS AND ADULT MUSCLES OF CHICKENS

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Abstract. An attempt was made to isolate the cell proliferation stimulating factors in the supernatant of embryo carcasses and adult muscles of chickens. Evidence was obtained for the presence of at least two or more stimulating factors in both the embryonic and adult muscular supernatants. These factors did not require a supplement of sera or other supporting agents. Furthermore, the use of the salting-out method with ammonium sulfate revealed two or more growth stimulants in the supernatant of chick cells.

Many investigators have attempted to purify the active components from chick embryo extracts that promote cell proliferation but the successful isolation and purification of the stimulating factors as protein have not been reported (1, 2, 3). We have previously investigated (4) the reasons for the difficulty in purifying the cellular stimulants and have tried to purify such active components from the supernatants of embryo carcasses and adult muscles of chickens using various preparation methods. Under ethanol fractionation, two or more cell proliferation stimulating factors were tentatively found in the supernatant of embryo carcasses and adult muscles (4). The changes in stimulating activities and physicochemical properties were remarkable during embryological development, but the stimulating activities were not fractionated. To further elucidate whether there are two or more stimulants in the supernatant under ethanol fractionation and to determine whether ethanol fractionation is unsuitable for purification of such active components, we used the salting-out method with ammonium sulfate. In the present paper, we report on the findings of this probe. The results indicated that there were at least two or more cellular proliferation stimulating factors in the supernatant of embryo carcasses and adult muscles of chickens.

The supernatant (S_2) of chick embryo (Em) carcasses (10-day-old) and adult chicken muscles (M) were prepared by the procedures described in a previous

paper (4). The S_2 was slowly saturated by addition of solid ammonium sulfate up to 30% and 60% in the presence of 0.5 mM 2-mercaptoethanol, and the precipitates were collected stepwise by centrifugation and were lyophilized after extensive dialysis against 0.5 mM 2-mercaptoethanol and 1 mM Tris-HCl, pH 7.4. The stimulating effects of these fractions were assayed by secondary cultured chick embryo cells (CEF) as described in the previous papers (4, 5).

Table 1 shows that the Em supernatant(EmS_2) contained stimulating factor(s) of CEF proliferation and that the active components were independently precipitated in 30% fraction (Em30), 60% fraction (Em60) and unprecipitated fraction (EmSup) by salting-out with ammonium sulfate. The relative stimulating activities in each fraction were similar to each other. The muscular fractions also contained stimulants in all three fractions (Table 2). These results strongly

TABLE 1. STIMULATING EFFECTS OF EMBRYONIC FRACTIONS ON CHICK EMBRYO CELL (CEF) PROLIFERATION

Systems	Cell number $\times 10^4$[a]	Stimulation percent[b]
Resting control (RT)	4.90	0
RT + EmS_2[c]	7.75	+ 58
RT + Em30[c]	8.04	+ 64
RT + Em60[c]	8.78	+ 79
RT + EmSup[c]	8.54	+ 74

a CEF in resting media (RT) composed of MEM + 2% TPB were incubated for 48 hours at 37°C in a CO_2 gas incubator with or without sample fractions (50 μg/ml). The cells were counted by a Coulter counter after trypsinization. Triplicate plastic dishes were used for one assay system and the standard errors were within 7.8 to 9.6% of the mean value ($\bar{M}$).

b Stimulation percent was calculated by : [Cell number in experimental system—Cell number in RT] ÷ Cell number in RT × 100.

c EmS_2 was the original supernatant of Em; Em30 was fractionated with 30% saturation of ammonium sulfate; Em60 was 60% saturation fraction; and EmSup was unprecipitated fraction after 60% saturation of ammonium sulfate.

TABLE 2. STIMULATING EFFECTS OF MUSCULAR FRACTIONS ON CEF PROLIFERATION

Systems	Cell number $\times 10^4$[a]	Stimulation percent[b]
Resting control (RT)	6.6	0
RT + MS_2[c]	9.6	+ 45
RT + M30[c]	9.6	+ 45
RT + M60[c]	9.0	+ 36
RT + MSup[c]	8.4	+ 27

a Same as footnote *a* in the legend of Table 1.

b Same as footnote *b* in the legend of Table 1.

c MS_2 was the supernatant fraction of M; M30 was 30% precipitated fraction; M60 was 60% precipitated fraction; and MSup was unprecipitated fraction after 60% saturation of ammonium sulfate.

support the possibility that there are two or more stimulants in the supernatant, and these results coincide with those obtained by ethanol fractionation. Both methods of fractionation revealed the presence of plural stimulating factors in the supernatant. Isoelectric precipitation also showed two stimulants in the same supernatant (data not shown).

Fig. 1 shows the electrophoretic pattern of Em30, Em60, M30 and M60 in 7.5% polyacrylamide gel, and common components are found in each fraction.

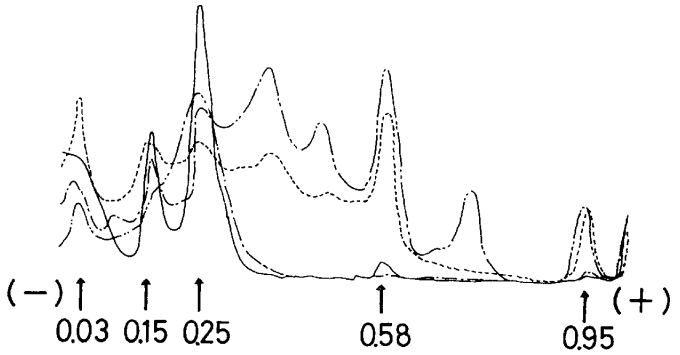

Fig. 1. Densitometrical pattern of embryonic and muscular fractions in polyacrylamide gel electrophoresis.
Polyacrylamide gel (7.5%) electrophoresis was performed by the method of Davis (7) in Tris-glycine buffer, pH 8.5. The samples (100 μg) were charged and electrophoresed for 150 min at room temperature. M30, —•—; M60, ——; Em30, ·····; Em60, – – –

The Rfs were 0.03, 0.15, 0.25, 0.58 and 0.95. The active components in the banded patterns are possible subjects of future investigations on stimulating activity.

These stimulating factors may be different from the fibroblast growth factor (FGF) reported by Gospodarowicz (6), because FGF required supplements of hydrocortisone and insulin to stimulate cell growth in absence of sera, but our factors stimulated growth without any supplement.

It is concluded that there are at least two stimulating factors of CEF proliferation in the supernatant of embryo carcasses and adult muscles of chickens.

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