# Effect of Extracts of Developing Muscle on Tissue Culture.* (22800)

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Gaillard(1) has shown that the growth promoting activity of extracts of whole chick embryos varies depending upon the age of the extracted embryos. From these experiments little could be concluded as to a possible correlation of growth promoting activity and the state of proliferation and differentiation of the donor tissue since whole embryo extracts include components of tissues at widely differing stages of development. Examination of extracts from adult tissues with different rates of cell proliferation did not indicate that proliferative activity in a tissue and growth promoting activity of its extracts are related (2). As a result of a more systematic study of the cytological and chemical changes in developing leg muscle of the chick(3,4), data have become available which invite a reconsideration of the relationships of the developmental stage in a single tissue and the growth promoting activity of its extracts. The results of tests of the growth promoting activity of extracts from developing chick leg musculature are reported in this paper.

*Materials and methods.* The muscle extracts to be tested were prepared by carefully dissecting the muscle tissue from the legs and passing it through a Latapie grinder. For each gram of pulp 1 ml of Pannett and Compton (P.C.) solution(5) was added. The mixture was immediately centrifuged (2,000 x g) and the supernatant decanted (designated as 50% extract). The tested extracts were obtained from leg muscle of the 12 day and 19 day chick embryo, and the 10 day and one-year chick because the growth characteristics of muscle tissue differ qualitatively and quantitatively at these stages of development. The mean protein concentration in muscle extracts, determined following Lowry's procedure(6), corresponds to 10.3, 19.7, 20.9 and 25.4 mg protein per ml of 25% extract, respectively for the extracts from the 4 ages given above. The embryo extract used for the first 4 passages was prepared in a similar way using the entire 12 day embryo as starting material. Coverslip cultures derived from leg muscle explants of 12 day embryos were used to assay the growth stimulating activity of muscle extracts. During 4 passages the cells were grown in a clot medium consisting of 2 drops of chicken plasma and 2 drops of 25% embryo extract with transfer to new medium every other day. At the fifth passage the whole embryo extract in the medium was replaced by P. C. solution (controls) or by muscle extracts of the desired concentration and donor age. Twelve hours and 48 hours after the last transfer the surface area of the cultures was recorded with a camera lucida, measured with a planimeter, and the relative increase computed according to Ebeling(7).

*Results.* The reported measurements of cell culture areas include both cell migration and cell proliferation in unknown proportions. Therefore, the term "growth" can be used in the context of this paper only with the reservation of this incomplete definition. Analysis of the results listed in Table I shows that addition of 0.125% extract from muscle of any of the 4 tested stages of development does not stimulate significantly‡ the "growth" of the test cultures. With extracts from 12 day embryos maximal activity is obtained only with the highest concentration tested (12.5%) whereas the extracts from muscle of the 12 day embryo and the 10 day chick give the same maximal "growth" stimulation with a 10 times lower concentration. The extracts of the one-year-old chick muscle remain sig-

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‡ Significance is attributed to differences with a statistical probability of chance occurrence of less than 1%.

TABLE I. Compiled Results of Clot Culture Experiments.

| Age of extract donor | Conc. of extract in medium, % | Mean relative increase,* $mm^2$ | No. of samples |
|---|---|---|---|
| 12-day embryo | .125 | 3.53 ± .5 | 15 |
| | 1.25 | 6.74 ± .4 | 47 |
| | 12.5 | 10.40 ± .5 | 53 |
| 19-day embryo | .125 | 3.27 ± .4 | 31 |
| | 1.25 | 11.87 ±1.1 | 29 |
| | 12.5 | 10.90 ± .5 | 42 |
| 10-day hatched chick | .125 | 5.14 ± .7 | 17 |
| | 1.25 | 11.80 ±1.0 | 44 |
| | 12.5 | 8.52 ± .6 | 53 |
| 1-year-old adult | .125 | 3.48 ± .6 | 10 |
| | 1.25 | 5.63 ± .4 | 33 |
| | 12.5 | 7.46 ± .4 | 27 |
| Controls | .00 | 4.71 ± .6 | 25 |

* $\frac{\text{Area}_2\ (48\ \text{hr}) - \text{Area}_1\ (12\ \text{hr})}{\text{Area}_1\ (12\ \text{hr})} \pm$ S.E. of mean.

nificantly below maximal activity even in the highest concentration. The decrease in the "growth" stimulation with the 19 day embryo extract (12.5%) is statistically insignificant, while the drop in the activity of the 10 day chick extract (12.5%) is significant.

Since the 19 day embryo extracts and the 10 day chick extracts give maximum "growth" promoting activity with the 1.25% extract concentration, the accumulation of the "growth" promoting substances apparently reaches an optimum during the last days of the pre-hatching and the first days of the post-hatching periods. Whether the extracts from the 19 day embryo or the 10 day chick are more active can not be conclusively stated at the present time. A reversal in "growth" promoting activity by excessively high concentrations of tissue extracts has been observed repeatedly(1,8) but its significance has been questioned(9) because of the frequently occurring liquefaction of the media. In the present series of experiments, cultures with liquefied plasma clot were not measured and, therefore, the marked decrease in growth stimulation with the highest concentration of 10 day chick extract may indicate a greater excess of "growth" promoting substances than in the muscle extracts from other stages of development.

*Discussion.* The period from the 12th to the 19th day of development of the chick embryo is a phase of development in which the rate of proliferation of the leg muscle declines. Correspondingly, total accumulation of DNA, as an index of cell proliferation, was found to come practically to a standstill on the 18th day of development(4,10). On the other hand, formation of specific cell proteins such as collagen (fibroblasts)(11) or of myosin(12) (myoblasts) takes place at a rapid rate during the 12th to the 18th day period of development and myosin production continues at a considerable rate for several weeks after this date. It is apparent that a maximal "growth" promoting activity of muscle extracts is observed when cell proliferation declines sharply and formation of specific cell proteins becomes the predominant growth component.

*Summary.* 1. Determinations were carried out of the effect of extracts from developing chick leg muscle on the increase in area of coverslip cultures derived from chick leg muscle cells. 2. Maximal stimulation with the lowest extract concentration was obtained with extracts from muscle of the 19 day embryo and the 10 day chick. Extracts from the 12 day embryo and the one-year chick were less effective. 3. The activity of the muscle extracts was found to be unrelated to the rate of cell proliferation in chick muscle tissue.

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