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Effect of Embryo Extract on Growth of Fibroblasts Previously Irradiated with X-rays.

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Embryo extract is a powerful growth-stimulant for cell colonies *in vitro*. It is generally accepted that its influence is manifested in, and limited to, the activation of cell-division. Our observations on the effect of X-rays on cell cultures make it necessary to revise this view. The investigations reported here demonstrate that cell cultures deprived of mitotic capacity by previous irradiation with X-rays will also grow at a considerably increased rate after the addition of embryo extract.

The experiments were performed on standardized cultures of chicken fibroblasts derived from the hearts of 7-days-old embryos. The cell colonies were cultivated in hanging drops according to the method of Carrel. After the irradiation, they were planted into Carrel flasks. Growth curves of the cultures were constructed from planimetric measurements of outline drawings of the surface areas made every 24 hours. Irradiation was carried out using a demountable X-ray tube which worked at a tension of 35 KV on currents of 20 MA. The tube had a copper anticathode and a window of aluminium foil, 30 μ thick. Absorption analysis showed that the rays penetrating through the window foil and the mica-

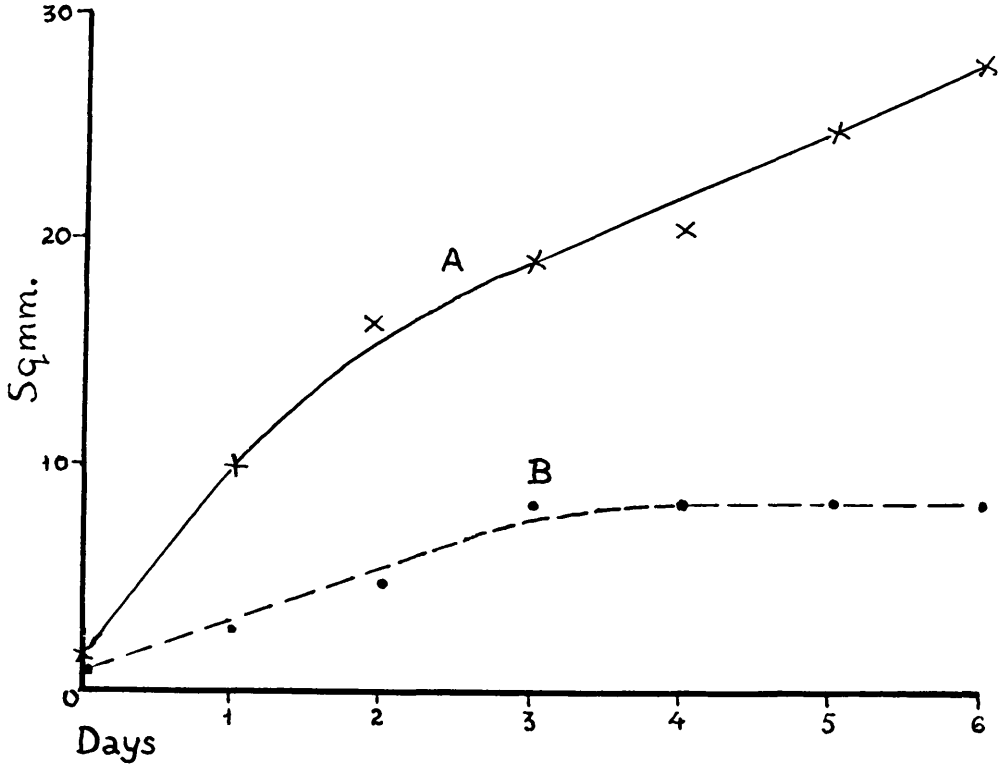

FIG. 1.
Experiment 13108/9b. Growth curves of sister halves of a fibroblast culture irradiated with 25000 r units: A, in medium containing embryo extract; B, in protective medium.

coverglass of the cultures were mainly copper K-rays.

The following procedure was adopted: Preliminary studies having shown that the X-ray dose necessary to inhibit completely cell multiplication is 10,000 r units, cultures of fibroblasts were irradiated with 25,000 r units, *i.e.*, a dose 2½ times greater than that required to arrest all mitoses. The cultures irradiated were divided into two equal parts: one fragment was planted into a medium which consisted of chicken plasma diluted with Tyrode's solution in the proportion 1:2; the other half was cultured in a medium of the same composition to which 30% embryo extract was added as a fluid phase.

In both fragments, the cells deprived by irradiation of their mitotic capacity migrated progressively and surrounded the explant. The growth rate of the culture that developed in a non-growth-promoting medium remained low. But the culture grown in a medium containing embryo extract showed considerable increase in area. After 6 days' cultivation the surface of cultures grown in a medium to which embryo extract was added exceeded by 200% (average of 17 experiments) the growth area of cultures developed in plasma alone. Fig. 1 represents this relation graphically.

The increase in area of a cell colony *in vitro* is the resultant of a number of factors of which the most important are cell multiplication and call migration. The culture in which no cell multiplication occurred enlarged by the migration of cells from explant into the culture medium. The fact that embryo extract promoted the rate of growth in cultures deprived of mitotic capacity proves that embryo extract not only possesses the property of stimulating cell divisions but also increases the migratory activity of the cells. These findings cast doubt on the validity of the idea that embryo extract activates the mitotic capacity of the cells selectively. Rather, they favor the view that embryo extract increases the general activity of the cells (probably by altering the cellular metabolism) and that this activation is reflected in both greater cell mobility and increased mitotic rate of the cells. The recent findings of Willmer and Jacoby[1] on the influence of various concentrations of embryo extract on cells *in vitro* would appear to lend support to this view. By means of cinematographic records, these workers were able to show that increased concentrations of extract caused an increase in the rate of migration as well as in the rate of mitosis of individual fibroblasts.

[1] Willmer, E. N., and Jacoby, F., *J. Exp. Biol.*, 1936, **13**, 237.

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Effect of Temperature on the Action of Penicillin* *In vitro.*

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Hobby *et al.*[1] reported that at low temperature penicillin showed very little bactericidal activity, but that this property was enhanced with a rise in the temperature, becoming very marked in that range favoring maximum growth of the organism. Their work showed the interrelation of the bactericidal action of penicillin and rate of bacterial growth. To support a hypothesis that penicillin acts upon organisms during the time of their division. Bigger[2] carried out almost the same type of experiment as the one of Hobby *et al.*, except that his technic of "one loop sterility" allowed the finding to be stated on a semi-quantitative basis. At almost the same time,

* Throughout this paper "penicillin" refers to material containing about 300 units per mg (Merck).

[1] Hobby, Gladys L., Meyer, Karl, and Chaffee, Eleanor, PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 281.

[2] Bigger, Joseph W., *Lancet*, 1944, **247**, 497.