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Influence of Allantoin on Growth of Chicken Epithelial Cells and Chondroblasts in Culture.

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Robinson[1] reported that he had found allantoin in the secretion of maggots which are used in the treatment of chronic wounds. While allantoin may play a rôle in the therapeutic result of maggot treatment, Robinson himself pointed out that allantoin alone could not fully take the place of living maggots from the therapeutic standpoint. Recently Shipp and Hetherington[2] published the results of their study on the influence of allantoin on the growth of chicken fibroblasts in tissue culture. They found a slight but not significant stimulation by allantoin. In studying the literature regarding the effect of allantoin on chronic wounds, one gets the impression that the growth of the epithelial cells is especially stimulated. For this reason, it seemed of interest to study the influence of allantoin on the growth of epithelial cells in culture. Since pure cultures of chondroblasts (fibroblasts) were also available, the experimental series with epithelial cells was supplemented by another series on chondroblasts.

The cultures were epithelial cells from the retina and chondroblasts (fibroblasts) from the cartilage of a femoral epiphysis of a 12-day- and 9-day-old chicken embryo. The epithelial cells had been transferred 9 times and the chondroblasts 8 times before they were used for the experiment. Cultures were carried out by the coverslip-hanging-drop method and kept for 4 days. A freshly prepared 1% allantoin solution in distilled water was used as stock solution from which concentrations of 1/200 and 1/300 were made with Tyrode's solution. All solutions were sterilized by filtration through a Seitz filter, heating being avoided. The experiment for each concentration was carried out 3 times with epithelial cells and chondroblasts.

Epithelial Cells. Well growing cultures (9th passage) were divided in halves, one half being used for the experiment and the other one as control. The experimental cultures were placed in one

[1] Robinson, W., *J. Parasitol.*, 1935, **21**, 354.

[2] Shipp, M. E., and Hetherington, D. C., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 180.

drop of chicken plasma plus one drop of a mixture of equal parts embryonic tissue juice (2nd centrifugate) and allantoin solution (either 1/100, 1/200, or 1/300). The respective controls were kept in one drop of chicken blood plasma plus one drop of a mixture of equal parts embryonic tissue juice (2nd centrifugate) and Tyrode's solution. As mentioned before, each experiment with one of the 3 concentrations of allantoin solutions was carried out 3 times with epithelial cultures of the 9th, 10th and 11th passages.

Chondroblasts. Corresponding experiments were carried out with chondroblast (fibroblast) cultures of the 8th, 9th and 10th passages; for chondroblasts the fourth centrifugate of embryonic tissue juice was used.

The hanging-drop cultures were kept at 37°C. for 4 days and every day, outline drawings were made with the help of a projectoscope.

Epithelial Cells. A definite retardation of growth in the experimental cultures as compared with the controls was noticeable. This retardation was pronounced in the 3 cultures with allantoin 1/100 and was very slight in the cultures with allantoin 1/300.

Chondroblasts (Fibroblasts). All cultures which had received allantoin in solutions 1/100 and 1/200 showed a definite retardation of growth which was in proportion to the allantoin concentration. Cultures treated with allantoin 1/300 either were of the same size as the controls or only very slightly smaller. The present reaction towards allantoin of the chondroblast (fibroblast) material, therefore, was different from that of the fibroblasts used by Shipp and Hetherington. It must be remembered, however, that their technique and the source of the cell material were slightly different.

Summary. Pure cultures of chicken epithelial cells and pure cultures of chicken chondroblasts (fibroblasts) under the influence of allantoin in different concentrations showed a slight retardation of growth which was in proportion to the allantoin concentration. In higher dilutions of allantoin neither a retardation nor a stimulation of growth could be observed.