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Rate of Degeneration in Pure Strains of "Fibroblasts" Possessing Varying Growth Potencies in vitro.

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In a previous paper the author in collaboration with Richardson[1] described variations in the cytolytic changes occurring in undifferentiated and differentiated tissues which were induced to undergo prolonged proliferation in an unchanged medium *in vitro*. The present study was undertaken to extend similar comparative observations upon the rate of cytolysis in pure isolated strains of cells, each possessing different inherent growth rates. Under experimental conditions Parker and Fischer[2] depicted different strains of "Fibroblasts" possessing varying growth potencies, and, for the purpose of these experiments similar tissues were selected and isolated according to the methods described by these investigators. All tissues employed were taken simultaneously from the same embryo of 12 days' incubation and throughout this investigation were explanted under exactly identical conditions from the moment of their isolation *in vitro*. The selected fragments were obtained from the following regions: osteoblasts from the supra-orbital; chondrioblasts from the periostium of the sphenoid (fast growing); and fibroblasts from heart and leg muscles (slow growing).

The first cytolytic response to the accumulations of cyto-toxins in the unchanged medium was morphologically expressed in the slow growing strains after 65-70 hours incubation *in vitro*. Similar examinations, however, of the fast growing strains after the same periods of growth showed them to be more resistant to the factors incurring cytolysis, as degenerative phenomena were not yet apparent. Even when the fast growing osteoblasts and the slow growing muscle-fibroblasts were implanted side by side in the same culture medium, they not only showed the same relative difference in their growth rates, but exhibited the same time variations as when cultivated in their isolated conditions. This experimental evidence suggests that cells are endowed with an inherent intrinsic mechan-

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[1] Horning, E. S., and Richardson, K. C., *Aust. J. Exp. Biol. and Med. Science*, 1929, **6**, 229.

[2] Parker, P. C., and Fischer, A., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 580.

ism and, moreover, that cell-behavior *in vitro* is not solely determined by the extrinsic physiological conditions of the culture medium.

After 65-80 hours' growth, a very interesting reaction to the toxicity of the unchanged medium was first observed in the slow growing cultures. Following general hypertrophy of the protoplasmic inclusions, many of the cells, especially those on the peripheral margins of the areas of new growth, underwent an unequal division of the cytoplasm. The nucleus played no apparent rôle and remained in the resting phase during this abnormal process. As the 'daughter portion' contained no nuclear material, and was crowded with hypertrophied inclusions, it might be assumed that this is an effort on the part of the degenerating cell to restore the nucleo-cytoplasmic ratio. When the apparent karyoprotoplasmic relations have been restored the cell survives while the 'daughter portion' disintegrates within the culture medium. Evidence of such a phenomenon does not become apparent in the fast growing strains until after 90-95 hours' growth. As far as could be estimated the phenomenon described above is more or less specific to the slow growing strains, but has, nevertheless, occasionally been detected in the osteoblast cultures after 100 hours incubation.

Other evidences of cytolysis such as chondriolysis, vacuolation, and lipoid formation which have previously been described in detail[3] were prevalent degenerative features common to all strains, but appeared first in the cells of the heart and muscle fibroblasts.

As cytolysis proceeds after 90 hours' incubation the cells of the slow growing fibroblasts further respond to the exhaustion of the culture medium by a marked hypertrophy and intense pseudopodial outgrowths and so much alter their contours that a differentiation is immediately suggested. The cells at this period presented a totally different morphological picture, having completely lost their spindle-shaped fibroblast-like characters. In order to observe the mechanism underlying this apparent differentiation, pure cultures of heart and muscle fibroblasts of 48 hours' growth were cut into equal portions and after fresh media had been added were sealed to a concavity slide upon which a film of H_2O_2 had been previously made. An equal number of remaining cultures were employed as controls. Examination after 90 hours in an unchanged medium revealed a striking difference between the experimental and control explants. The former retained to a large degree their typical spindle-like structure, while the controls displayed marked alterations in

[3] Horning, E. S., and Richardson, K. C., *Aust. J. Exp. Biol. and Med. Science*, 1929, **6**, 229.

their morphology. This experiment suggests that the "differentiation" was purely a superficial process, and was probably an effort on the part of the cell to increase its surface area owing to lack of oxygen, through cultivation in an unchanged medium. This might possibly explain the results of earlier workers who held that fully differentiated tissue during growth *in vitro* reverts back to an indifferent cell-type.[4]

Hyperchromasy which is generally followed by chromidiosis was frequently observed in the slower growing "fibroblasts" after 100 hours growth *in vitro,* and was very rarely detected in the osteoblast and chondrioblast cultures. These observations support the contentions of previous authors who regard it as representing a pathological rather than a normal state. This phenomenon might be interpreted in view of the hypothesis of Popoff, who contends that this is a function wherein the mass relation between the nucleus and the cytosome might be restored, if for any reason the amount of chromatin relating to the cell protoplasm has increased. Comparative observations of a large series of cultures showed in all cases that the fast growing strains are more resistant to pathological conditions than the slow growing tissues. The final evidence of cytolysis is manifested in the heart and muscle fibroblasts after 10 days' incubation in the unchanged medium. Fusion of fat globules occurs and tension forces within the cell interior are responsible for the extremely bizarre morphology of the cultures. Chromatolysis which becomes apparent on the ninth or tenth day, is invariably followed by plastin hypertrophy of the nucleolus. This association of nucleolar change with absorption of chromatin material is significant, as it suggests the possibility that this structure is built up of waste nuclear material. As soon as the nucleus collapses cell death occurs, and in order to verify this fact the cultures were transferred to fresh media and in all cases they failed to exhibit signs of renewed growth; while disintegration of the faster growing osteoblasts and chondrioblasts did not set in until the fourteenth or fifteenth day *in vitro.*

In conclusion it might be assumed that tissue cells which exhibit similar morphological characteristics and behavior under normal conditions *in vitro,* differ however in their reactions to similar induced pathological conditions, as the rate of cytolysis was in all cases found to be dependent upon the inherent growth energy of the given strain.

I wish to express my indebtedness to Dr. Albert Fischer for his interest and suggestions during this investigation.

[4] Champy, C., *Bibliog. Anat.,* 1913, **33**, 184.