cates that boron-neutron treatment offers little promise for malignant tumors other than those of the brain.

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Action of Hydrocortisone on Cells in Tissue Culture.* (21011)

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Since it was established that cortisone inhibits production of granulation tissue in experimentally produced wounds(10), much work has been conducted by numerous investigators studying the direct effect of cortisone on cells in tissue culture. The results have been diverse and in some instances have been contradictory. To mention only a few, inhibition of growth of fibroblasts by cortisone acetate in suspension in concentrations of about 200 to 50 μg/ml (reviewed in 4,2,6), and also by doses as small as 0.5 and 0.3 μg/ml(1,4) has been reported. On the other hand, negative results have been obtained with doses as high as 500 μg/ml (reviewed in 4,8). Stimulation of growth of fibroblasts(6), but also inhibition(8) by desoxycorticosterone have been reported.

In view of the above, attempts have been made to ascertain whether an easily reproducible effect of cortisone on growth in tissue culture, if any, could be demonstrated.

Methods. Primary cultures were used since such growth seemed to be more comparable to growth in regenerating wounds than would be the case if older laboratory strains were used which in long generations may have acquired different qualities. When the first experiments appeared inconclusive, it was thought results might be more clearcut, if embryonic extract (EE) was omitted from the culture medium. It appeared unreasonable to add so potent a growth stimulator as EE, to compete with the substance to be tested, particularly since the active principle of EE is unknown. A medium of amniotic fluid alone, without EE, has been found very satisfactory for obtaining adequate growth in control cultures. In all experiments the hanging-drop method on coverslips was used. In one series of experiments cultures of chick embryo tissue were explanted on coverslips in

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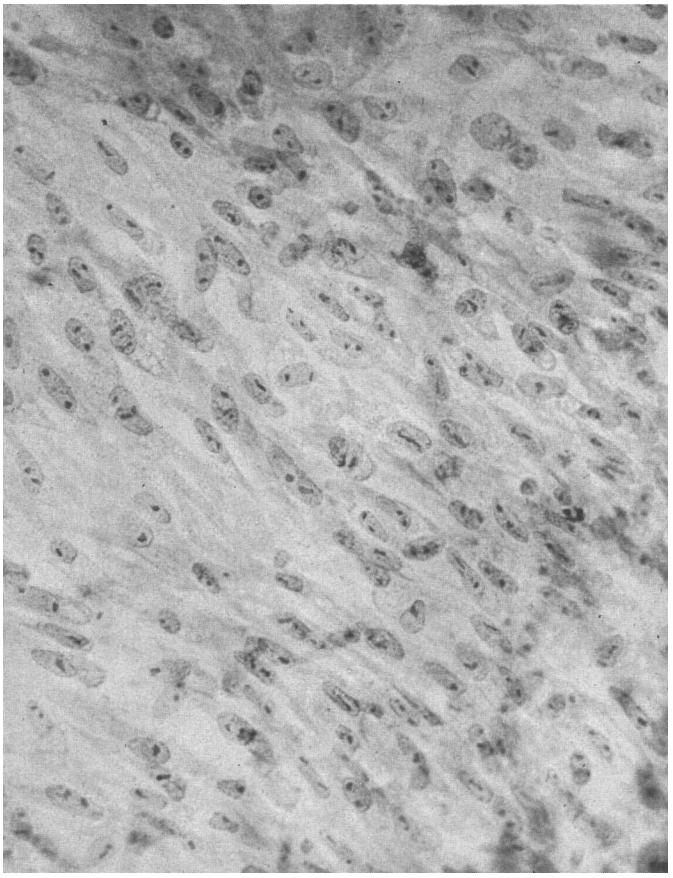

FIG. 1. Fibroblasts from chick embryo heart grown in chick plasma and amniotic fluid. Magn. 350 ×.

a liquid medium without plasma, the medium consisting of equal parts of chick amniotic fluid(3) and chick EE. Growth was obtained in about 70% of cultures and in general was not inferior to growth in a plasma clot. For all other experiments a plasma clot medium was used, to which either chick amniotic fluid plus 30% chick EE, or chick amniotic fluid alone, was added. In most experiments tissue fragments from hearts of 10- to 12-day-old chick embryos were used for explantation; chick embryo stomach and intestine were also used. The substance to be tested was added to the medium at the time of explantation. Whenever possible, water soluble compounds of steroids were employed since suspensions may be inhomogeneously distributed in individual cultures. In most experiments, the free alcohol of hydrocortisone† (17-hydroxycorticosterone, compound F, hereafter referred to as F alc.) was tested. To one ml containing 25 mg F alc., after removing the original vehicle (0.9% benzyl alcohol) and replacing it by saline, 1.25 cc 95% ethyl alcohol and 0.75 cc distilled water were added in the order mentioned. A clear and stable solution containing 8.33 mg F alc. per ml was obtained; this was diluted in 9 parts of Ringer solution. The final solution for testing in tissue culture was made with chick amniotic fluid. When the vehicle for F alc. was diluted in a similar fashion, no effect on cultures was noted. Four hundred and sixty preparations were made in studying F alc. in solution; F alc. as a suspension was employed in 80; cortisone acetate† in suspension in 80; desoxy-

† Kindly supplied to us by Dr. Elmer Alpert of Merck & Co., Rahway, N. J.

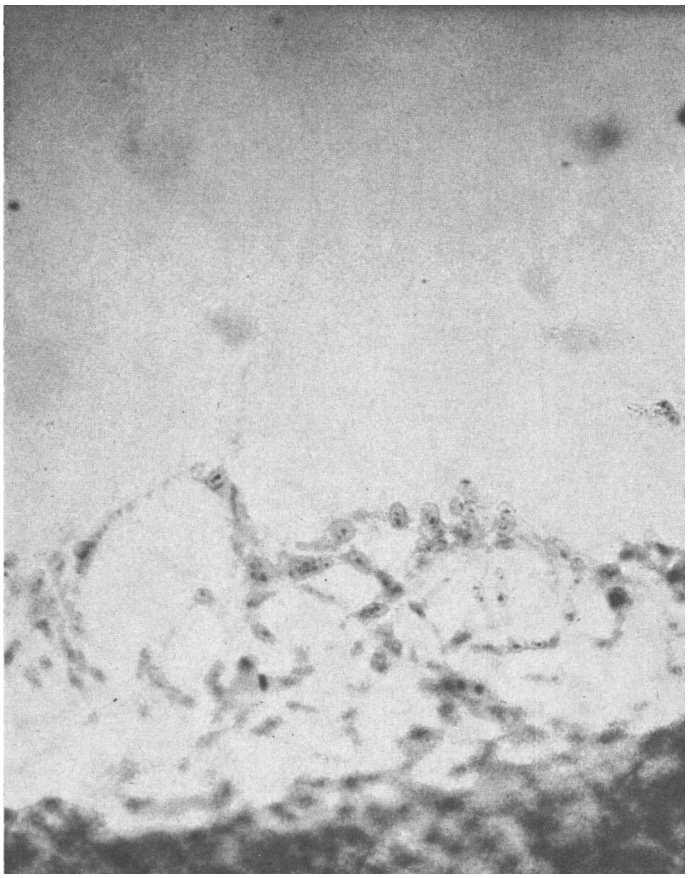

FIG. 2. Fibroblasts from chick embryo heart grown in chick plasma and amniotic fluid containing 250 μg/ml soluble hydrocortisone. Magn. 350 ×.

cortisone glucoside in suspension‡ in 40; and in aqueous solution§ in 40; and cholesterol in suspension† in 80. A smaller number of controls was run with each experimental set. Estimations of growth rate were made daily for 4 days. Area was estimated by recording the number of visual fields at a given microscopic magnification occupied by outgrowth, starting from the edge of the explant. Two zones in each culture were recorded, the largest and the smallest. Estimation of density was recorded by numbers 0 to 4. The average outgrowth area and density of a set of cultures gave a number characteristic for the medium used. Such results appear to agree with those obtained by measuring projectoscopic drawings with a planimeter, insofar as area is concerned; more emphasis, however, has been put on density. The described method is satisfactory for establishing large effects on growth in tissue culture, but is not sufficient for finding small differences which do not exceed considerably the fluctuations of normal cultures.

Results. At first, a liquid medium consisting of equal parts of chick amniotic fluid and chick EE was used with soluble F alc. in a final concentration of 250 μg/ml. Reproducible growth inhibition occurred in this medium containing soluble F alc. Growth of control cultures underwent such large fluctuations, however, that use of a liquid medium was abandoned and a medium containing plasma used. With chick plasma containing 20 to

‡ Kindly supplied to us by Dr. Yonkman of Ciba Pharmaceutical Co., Summit, N. J.

§ Kindly supplied to us by Dr. Kenneth Thompson of Roche-Organon, Nutley, N. J.

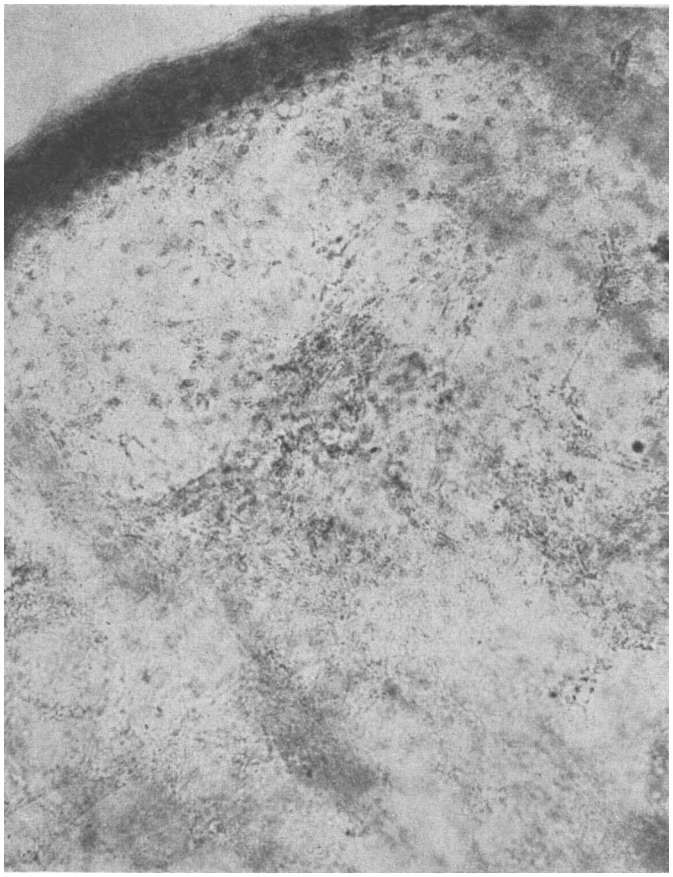

FIG. 3. Epithelium from chick embryo stomach mucosa grown in plasma and amniotic fluid containing 250 μg/ml soluble hydrocortisone. Magn. 150 ×.

50% of EE in amniotic fluid, the effect of F alc. varied greatly from experiment to experiment and often no effect was seen. In media containing equal parts of plasma and amniotic fluid without EE, with addition of 200 to 400 μg/ml of soluble F alc. in final concentration, the results were clearcut and easily reproducible. The difference in growth area and density between F alc.-treated and control cultures was large and the growth inhibition considerable. Higher concentrations of 400 to 500 μg/ml inhibited growth completely or almost completely. Concentrations of 100 and 50 μg/ml often showed slight inhibition which, however, was not always reproducible with this method.

Mitotic figures were encountered, but more rarely than in control cultures. Though mitotic indices were not counted at this time, no arrest of mitosis in a particular phase could be noted and there was no accumulation of one type of mitotic figure at any day of cultivation.

The presence of large fat vacuoles in an unusually early stage of growth was noted in cultures submitted to the action of soluble F alc. These have been found by others (9,11) and have also been seen in cells in tissue sections(1,9).

When cultures, treated with soluble F alc., 250 to 400 μg/ml, showing typical growth inhibition, were transferred on the 3rd or 4th day of cultivation into a medium of amniotic fluid without F alc., complete recovery of the cultures and excellent growth equal to that of control cultures ensued.

Aqueous suspension of cortisone acetate tested in a medium of plasma and amniotic fluid in the same concentrations as soluble F alc. caused slight inhibition of growth, con-

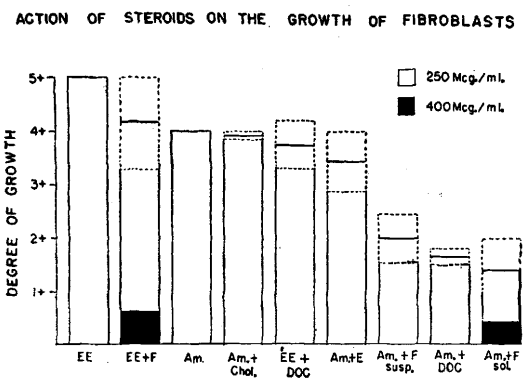

FIG. 4.

Straight lines at top of each column represent avg degree of growth, and fluctuations in growth are represented by broken lines. EE—embryo extract, F—compound F alcohol, AM—amniotic fluid, DOC—desoxycorticosterone glucoside, E—compound E.

siderably smaller than the inhibition by soluble F alc. Inhibition by F alcohol in suspension was greater than by cortisone acetate in suspension indicating also that factors other than water solubility make compound F more effective than cortisone acetate.

Two preparations of desoxycorticosterone glucoside (DOC), one in aqueous suspension and one in aqueous solution, were tested in the same medium and concentrations as above. The extent of growth inhibition caused by these steroids was quite similar to that obtained when soluble F alc. was used. It would thus appear that, as far as the action on fibroblast growth is concerned, DOC is not antagonistic to cortisone, but exerts a strong inhibitory effect similar to that of soluble F alc. Differences in cell morphology between DOC-treated cells and F alc.-treated were noted which are now under study. Cholesterol in suspension produced a slight degree of growth inhibition in tissue culture. Most of the experiments were carried out with soluble F alc. The inhibition produced by this compound was typical and reproducible. Hyaluronidase did not interfere with the F alc. inhibition.

Addition of various amounts of EE to a medium containing soluble F alc. in concentrations of 200 to 250 μg/ml, as mentioned above, gave better growth than a medium with soluble F alc. in the same concentrations without EE. (In most instances, the difference in growth between soluble F alc.-treated cultures with and without EE was greater than the differences in growth between control cultures with and without EE. The F alc. inhibition appeared thus to be absolutely smaller in the presence of EE. This would imply that EE partly removes the inhibitory effect of F alc., and suggests that EE may contain some principle antagonistic to the F alc. inhibition.) It should be emphasized, however, that the action of F alc. with EE depends on relative concentrations of both. When the concentration of soluble F alc. was raised to 400 μg/ml, inhibition was as great in the presence of EE as in amniotic fluid only.

Experiments with explants of stomach and intestinal epithelium were carried out in 2 media: first, one containing soluble F alc., 250 μg/ml amniotic fluid without EE, and second, in a medium of 400 μg/ml of soluble F alc. per ml amniotic fluid containing in addition 15% EE. Control cultures were grown in the same media without F alc. In the F alc.-treated cultures, growth of fibroblasts was considerably inhibited, whereas growth of epithelium in large sheets was abundant. In the control cultures, fibroblasts grew as usually, often abundantly, where the medium was not liquefied. (Stomach epithelium may cause liquefaction of the plasma clot. Under these circumstances, fibroblasts grew poorly and these cultures were discarded.) Big sheets of epithelium also grew irregularly in control cultures.

The same results were obtained when, in the same clot containing soluble F alc. in amniotic fluid, 2 tissue fragments were explanted, one from chick heart, and one from stomach or intestine. Controls were made with similar pairs of explants without F alc. Suppression of growth of fibroblasts in heart as well as in explants from stomach and intestine, and growth of large epithelial sheets in explants from stomach and intestine were noted. In control cultures there was normal growth of fibroblasts from all explants, and less vigorous growth of epithelium from stomach and intestine explants. It seems probable that F alc. could be practically used for suppressing growth of fibroblasts and indirectly enhancing

growth of epithelium in tissue culture.

Discussion. The first question suggested is whether the growth inhibition effected by F alc. in tissue culture is related to known effects of cortisone *in vivo, e.g.,* retarding granulation in experimentally produced wounds in animals. At first glance, the effect of a water soluble steroid—F alcohol—appears to mimic *in vitro* its accepted *in vivo* action. Chick embryo growth *in vivo* is inhibited by 11-oxysteroids; compound F acetate is the most potent(5). The selectivity of the growth inhibition on growth of fibroblasts with an absence of growth inhibition of stomach and intestine epithelium correlated with *in vivo* experience. Reversibility of inhibition when the steroid is removed is also compatible with *in vivo* findings.

On the other hand, DOC (granted in relatively high concentration) exerted an effect similar to F alc. on growth inhibition; even cholesterol apparently effected a minimal inhibition. This, particularly the DOC experiments, is contrary to *in vivo* work. The effect, however, is exerted upon the fibroblast and not upon the epithelial cell. The role of EE in this pattern is not completely clear. It does seem to negate partly the effect of compound F but the reason for its interference with the action of compound F has not been elucidated.

Summary. 1. In a medium consisting of chicken plasma and amniotic fluid, growth inhibition by soluble F alc. in concentrations from 200 μg/ml can be demonstrated consistently. 2. When EE is added to the culture medium, growth inhibition occurs only in a higher concentration of soluble F alc. The suggestion that EE contains a principle antagonistic to the inhibitory action of F alc. is discussed. 3. Growth of epithelium from stomach and intestine is not affected by F alc., while growth of fibroblasts is suppressed. 4. Reversibility of the effect of F alc. after its removal from the medium, and its selective inhibitory action on fibroblasts indicate that its inhibitory action *in vitro* is related to its known action *in vivo,* in particular to the retardation of regeneration in wounds experimentally produced in animals. 5. Aqueous soluble DOC in similar concentrations has an effect similar to soluble F alc. and cholesterol in suspension has a minimal inhibitory effect on cell growth. This observation differs from the *in vivo* findings and warrants further investigation.

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