

effect of the dietary situations on the spreading reaction is most probably due to the local influences of nutritional fractions on the skin apparatus of the rat.

Summary. 1. The hyaluronidase spreading reaction was tested in intact, cortisone injected and adrenalectomized rats and the effect of various dietary situations was studied. In intact rats, a diet containing excessive amounts of vit. B₁₂ decreased the area of spread significantly. At present, there is no obvious explanation for this observation. 2. Both the administration of cortisone and adrenalectomy decreased the area of spread in rats. Sham operation also decreased the area of spread of hyaluronidase. It is suggested that the effect of adrenalectomy is pri-

marily a non-specific effect due to the operative procedure itself.

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Free Wandering Cells and Cortisone. (20428)

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After Bloom(1) reported that cortisone could produce a rapid regression of mast cell tumors in dogs, we began a study of the effects of cortisone on the mast cell in tissue culture. For reasons which are not clear to us, the rapidly destructive effects on the mast cell obtained by Bloom *in vivo* were only mildly demonstrable *in vitro*. Mast cell activity was inhibited, it is true, but this was only a facet of a broader observation, *i.e.*, cortisone can effectively block the nomadic proclivities of free wandering cells. This agrees with previous observations on leucocytes(2,3) but the fact seems to have been overlooked that not only white blood cells but free wandering cells in general are more sensitive to the hormone than are fibroblasts. All free wandering cell activity can be brought to a stop in cultures in which fibroblastic activity remains relatively undisturbed.

Materials and methods. Coverslips (43 x 50 mm) containing crystals of cortisone (free-alcohol) in amounts ranging from 0.0005 to 0.025 mg were prepared by permitting the hormone to precipitate by evaporation from meas-

ured volumes of an alcohol-cortisone solution. On these coverslips fragments of 9-10 day old embryonic chick skin were placed in equal parts of blood plasma and embryonic extract before the medium clotted. Controls contained either no hormone, or cholesterol in amounts similar to the amount of hormone used in the experimental cultures. More than 200 pairs of embryonic skin fragments were grown and observations were taken each day over a period of 4 days. We also studied about 200 pairs of spleen fragments obtained from 16-19 day old chick embryos. Here the technic was varied in that, with both the experimental and control cultures, the medium was permitted to clot before a spleen fragment was placed meticulously, with no lateral motion, upon the surface of the medium. This procedure was followed in order to minimize the spread of cells by mechanical means. The spleen fragments were taken from 16-19 day old embryos because, at the age chosen for the source of skin material, namely, 9-10 days, the spleen is both undesirably small and soft.

Results. The method of introducing the

hormone and the concentrations chosen permitted a graded study of cellular reactions ranging from those which showed no apparent response to the hormone to those in which all activity of all the cells was completely inhibited. The first and most constant effect observed in the skin fragments subjected to cortisone was the failure of the free wandering cells to migrate from the parent fragment into the surrounding medium. Significantly, this retardation of migratory activity of the free wandering cells occurred in many cultures in which there was no apparent interference with the growth and movement of fibroblasts. In brief, the free wandering cell is distinctly more sensitive to cortisone than is the fibroblast. Following the failure of free wandering cells to migrate, the first apparent reaction of the fibroblast to the hormone is the formation of cytoplasmic vacuoles. A few cells show distinct vacuoles at the end of one day of cultivation. By the end of 2 days this number has increased to the extent that, in some cultures, it is difficult to find cells free of vacuoles. Finally, the most drastic effect of the hormone is expressed by the complete failure of fibroblasts to leave the parent fragment.

The reaction of spleen fragments to cortisone completely confirms the above observations on skin. Spleen cultures exposed to the hormone showed, at the end of 24 hours, either a marked or a complete suppression of the migratory activity of free wandering cells. This contrasted strikingly with the wide halo of newly migrated hordes of wandering cells seen in the controls. Occasionally it was possible to identify ray-like masses of spindle-shaped fibroblasts extending into the medium

from spleen fragments treated with hormone. It is apparent that these rays were unmasked by the wandering cell inhibitory action of cortisone.

Discussion. The term free wandering cell as used in this report is a blanket term for any and all cells which leave the parent fragment to travel singly through the meshes of the medium, over the surface of the medium and between the groups of fibroblasts. It includes the various types of white blood corpuscles, the so-called lymphoid wandering cells, macrophages, and also the mast cell.

Regarding the experimental results, it does not necessarily follow that the unfavorable reaction which the free wandering cell exhibits toward cortisone *in vitro*, will occur *in vivo*. The amount of cortisone which reached the cells in the cultures is not known. All of the cortisone deposited upon a coverslip does not dissolve and the rate of diffusion is very slow.

Conclusion. In tissue cultures of embryonic skin it can be demonstrated that, of the cell types present, the free wandering cells are the most sensitive to the hormone. All wandering cell movement can be brought to a stop in cultures in which fibroblastic activity remains undisturbed. This observation is supported by the behavior of cortisone-treated spleen fragments.

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