number of lesions occurred adjacent to feeding areas numbered from one to six. The occurrence of local lesions did not follow a definite pattern. In some cases they were consecutive, and in others as many as 16 feeding areas occurred between those producing lesions.

Another experiment consisted of feeding single grasshoppers on the virus source for 5 min and then immediately transferring them to individual hybrid plants to feed at will from 4:00 P.M. until 9:00 A.M. the following day. The amount of feeding ranged from single bites to almost complete consumption of the leaves. Twenty grasshoppers were tested, and 50% transmitted the virus, producing a total of 15 local lesions.

Local infection of the hybrid plants was not always adjacent to a feeding area. Approximately 3% of the total local lesions that developed in all tests occurred elsewhere. This might be due to transfer of the virus by the feet of the insects; however, grasshoppers do scar or make depressions in the leaf tissue with their mandibles while in search of a suitable feeding site. The latter seems to be the more plausible explanation. The feeding on a vein, or the development of a local lesion adjacent to one of the larger veins, brought about systemic infection of the hybrid plants in several instances.

In one experiment, single grasshoppers were transferred to individual healthy tobacco (*N. tabacum*) plants immediately after feeding on the virus source and were allowed to feed approximately 10 times in different locations. Fifty-seven (54.8%) of the 104 insects tested transmitted the tobacco mosaic virus, causing systemic infection of the plants. After a waiting period of 2 hr, 9 (45%) of the 20 insects tested transmitted the virus; and 9 (42.9%) of the 21 individuals tested after a 4-hr waiting period brought about systemic infection of the test plants.

Tobacco was also used as the test plant for the transmission of potato virus X (potato ringspot type) and tobacco ringspot virus. Single grasshoppers were transferred to individual healthy plants immediately after feeding on the virus source and were allowed to feed 6–8 times in different locations. The insects were disturbed between feedings, making the leaf perforations appear as isolated holes. Local infection appeared within 5–6 days. For the potato virus X, 18 (18%) of the 100 grasshoppers tested transmitted the virus to tobacco plants, which developed local lesions and later became systemically infected. Four of the initial infections did not occur adjacent to apparent feeding areas. With tobacco ringspot virus only 6 (6%) of the 100 insects tested transmitted the virus. Initial infection, with one exception, occurred adjacent to known feeding areas. In a majority of the cases, with both viruses, initial infection developed adjacent to feeding areas near one of the larger veins.

These experiments show that tobacco mosaic virus, potato virus X, and tobacco ringspot virus can be transmitted with the differential grasshopper under conditions as described above. This is possibly a simple mechanical transmission and nonspecific. The importance of grasshoppers transmitting these viruses under field conditions has yet to be investigated. A more detailed account of the work relating to this problem will be published elsewhere.

### References


1. ALLARD, H. A. *U. S. Dep. Agr., Bull. 40,* (1914).
2. ———. *J. Agr. Research,* **10**, 615 (1917).
3. HOGGAN, I. A. *Phytopathology,* **19**, 109 (1929).
4. ———. *J. Bact.,* **19**, 21 (1930).
5. ———. *Phytopathology,* **21**, 199 (1931).
6. ———. *J. Agr. Research,* **49**, 1135 (1934).
7. GIGANTE, R. *Boll. staz. patol. vegetale,* N.S., **18**, 93 (1938).


## Selective Damage to Fibroblasts by Desoxycorticosterone in Cultures of Mixed Tissues


Ivor Cornman[1]

*George Washington University School of Medicine, Department of Anatomy,* and *Warwick Memorial for Cancer and Allied Diseases, Washington, D. C.*


The remarkable selectivity which adrenocortical steroids appear to show for mesenchymal tissue, as demonstrated in pathological and clinical studies (*1*), raises the question as to whether this represents a direct effect on the cells of connective tissues. In rheumatic fever the connective tissues of the heart are profoundly altered by necrosis and fibrosis, whereas the changes in endothelium and in muscular activity appear to be secondary. Desoxycorticosterone (DOC) induces similar changes, but not in hypophysectomized animals (*2*). In this study it is proposed to explore the possibility of comparable *in vitro* effects of adrenal steroids on heart tissues, particularly with a view toward establishing whether there is one target tissue.

Hearts were removed from newborn line C white mice, cut into fragments about a millimeter across, and cemented into roller tubes with chicken plasma. Such fragments continued to pulsate in the cultures and at the same time provided outgrowths of two kinds of cells. The most abundant cells were of the fibroblast type, elongate bipolar cells arranged in radiating strands or in irregular networks (Fig. 1, right). The cells believed to be endothelium grew as continuous sheets of polygonal cells (Fig. 1, left). The initial growth was in nutrient solution: balanced saline (Gey's) + serum + embryo extract. The nutrient was then replaced by balanced saline containing the experimental agent. Desoxycorticosterone was used in doses sufficient to produce cytological changes in 6–24 hr. At this time the experimental solution was replaced with nutrient solution. It was thus possible to follow the relative rapidity of response of the two types of


[1] I am indebted to Clifford Grobstein, of the National Cancer Institute, for mice used in these experiments. This investigation was financed from an institutional grant to the Helen L. and Mary E. Warwick Memorial for Cancer and Allied Diseases by the American Cancer Society, Inc.

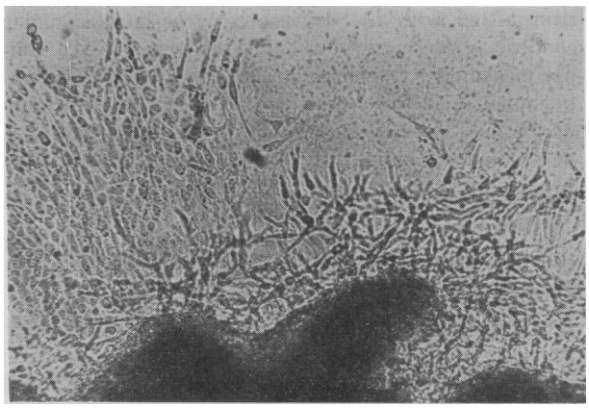

FIG. 1. Two-day growth from a heart fragment. Endothelium to the left, fibroblasts right (approx. x 100).

tissue, and later to compare the recovery as revealed by new growth from the explant.

For most of these studies DOC purchased from Delta Chemical Works was used. The results have been checked with DOC contributed by Ernst Oppenheimer, of Ciba Pharmaceutical Company. The two products differ in physical and biological behavior. The Delta sample, received as a lump of slightly yellow material, when dispersed in balanced saline at 1:100 from a 1% acetone solution, formed an opaque suspension which later coated the side of the tube. The Ciba material was white and finely divided. The 1% acetone stock solution diluted 1:10 in balanced saline was faintly opalescent and later became milky and precipitated loose crystals. DOC acetate (Delta), cortisone (Merck), and cortisone acetate (Merck) were also tested.

In the 2–4 days following planting, 12 (2%) heart fragments had grown only endothelium, 144 (25%) had grown both endothelium and fibroblasts, and 414 (73%) had produced only fibroblasts. Upon exposure to 0.02 mg/ml of Delta DOC or 0.1 mg/ml of Ciba DOC, cytological changes became visible by 16 hr. At 0.03 mg/ml of Delta DOC or 0.2 mg/ml of Ciba DOC, changes were seen at 6–7 hr. Some cells became rounded, as is characteristic of the response to most noxious agents, but the typical DOC-induced alteration is best described as a moderate shriveling. The fibroblast cell membrane lost its smooth contours and became angular; the cytoplasm became granular, and the cells narrowed to a half or a third their usual width, although the filamentous processes remained extended. Endothelium similarly became granular, and the cells sometimes shrank, separating the membrane into isolated or grouped cells.

At these doses the original outgrowth usually never recovered. In one experiment, however, exposure to 0.1 mg/ml of Ciba DOC for 25 hr did not damage the endothelium. The rounded and shriveled fibroblasts (Fig. 2) never recovered, but the endothelium which lay between the fibroblasts and the DOC solution merely became granular and upon return to nutrient resumed normal morphology and growth. In the more severely damaged cultures about a fourth of the DOC-treated explants produced new fringes of cells. In contrast to the initial low percentage of endothelial growth, the regenerated tissue comprised 34 (57%) explants with endothelium alone, 14 (24%) with endothelium and fibroblasts, and 11 (19%) with fibroblasts alone. These same explants before treatment included only 1 (2%) with pure endothelial growth, 28 (47%)

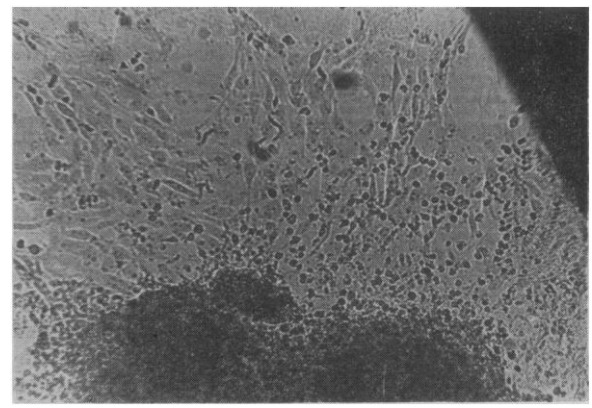

FIG. 2. Original outgrowth of cells after 24-hr exposure to 0.1 mg/ml DOC (Ciba) followed by 2-day recovery in nutrient solution. The endothelial cells (left) are expanded and transparent. The fibroblasts (right) are rounded or shriveled and densely granular. The endothelium is growing, but the fibroblasts have remained unchanged for 2 days (approx. x 100).

with endothelium and fibroblasts, and 30 (51%) with fibroblasts alone. All those that on recovery produced pure endothelium had some fibroblasts originally. Thus, fibroblasts were eliminated from 34 explants, whereas endothelium was lost from only 3. Chi-square analysis shows the frequencies of the tissues in the treated cultures to be significantly different from the frequencies in the same explants before treatment, and from the frequencies in all untreated cultures. In untreated cultures the distribution of endothelium and fibroblasts did not change during the 2–3 weeks the cultures were followed, but the area occupied by fibroblasts increased more rapidly than did the endothelial area.

Cortisone at 0.05–0.15 mg/ml added to DOC accelerated and increased the visible cytological alterations (which included more rounding of cells than caused by DOC alone), but did not interfere with the favoring of endothelium in explants which survived. When 10% of the saline was replaced by serum, DOC (Delta) exerted no visible effects in doses up to 0.03 mg/ml. DOC acetate at 0.031 mg/ml, or cortisone acetate at 0.026 mg/ml (both in excess of saturation), had no effect on the 30 explants tested with each.

In most DOC cultures where selective damage to fibroblasts was obtained, the acetone used as the initial solvent for DOC did not exceed 0.2%. Where high doses of steroids were tested, the concentration of acetone sometimes reached 1%. Acetone or ethanol alone at 1% had no perceptible effect on the cells. Increasing the dosage slowly over a period of hours until 2.3%

acetone or 2.6% ethanol had been added caused both the fibroblasts and endothelial cells to round up.

The pulsation of the heart fragments was diminished or stopped by concentrations of DOC which damaged the cells, and not by DOCA at saturation (*3*). The pulse returned when the DOC was removed.

Isolated heart tissues in culture are damaged by DOC but not by its acetate. This may result merely from the low solubility of the acetate, or may indicate that these tissues lack the enzymes by which the intact body is able to utilize DOCA. The DOC effect, elimination of fibroblasts from tissue cultures, is quite the reverse of the trend in untreated cultures. Usually the fibroblast is the hardiest of cells in culture and outgrows most other cell types, although poisons specific for mesenchymal cells are known (*4*). Indifferent poisons such as ethanol or acetone affect endothelium and fibroblasts equally, causing rounding of both at threshold doses. The alkaloid ryanodine, by way of contrast, causes the endothelial cells to round up while the fibroblasts remain extended (unpublished data). Nonadrenal steroids must be tested in cultures, however, before any correlation with systemic effects of adrenocortical hormones can proceed very far.

It is tempting to draw a complete parallel between the selective damage to fibroblasts in culture by DOC and the necrosis of vascular and cardiac fibrous tissue in rheumatic diseases. Selye and co-workers have induced periarteritis (*5*) and Aschoff bodies (*6*) in rats by injection of DOCA. Moreover, the dose (1 mg/day) used by Selye to produce periarteritis nodosa in sensitized rats is close to the levels employed in tissue culture on a mg/kg basis.

There are obstacles to this line of reasoning, however. Although cortisone induces an atrophy of the dermis when applied directly to the skin (*7*) and inhibits formation of granulation tissue (*8*), early arthritic lesions induced in rats by DOC acetate are highly cellular (*5*). Also, granulation tissue is well populated with large, many-branched fibroblasts in rats receiving DOCA, whereas rats treated with sex hormones produce a granulation tissue sparse in both cells and fibers, and the cells are thin and small (*9*). The DOC-treated fibroblasts in culture cease to proliferate and shrink to a narrow or even threadlike configuration. The similarity of these two pictures suggests that the adrenal hormone effects reported here are nonspecific steroid influences. There remains some reason for doubting, however, that such a nonspecific steroid effect occurs in the absence of adrenal hormones, inasmuch as the local inhibition of hair growth induced by estrone is dependent upon the adrenals (*10*).

At least, the picture of adrenal cortical influence is somewhat clarified by a demonstration of a direct selective effect of adrenal steroids upon fibroblasts isolated from all systemic influences. The lethal effects observed in tissue culture are probably in excess of inhibitory phenomena within the body, but high concentrations of DOC were employed in order to get as close as possible to an all-or-none selection of the resistant tissue. Possibly at lower doses more subtle effects reflecting the physiological specificities and antagonisms of adrenal steroids will be detected in tissue cultures. Also, fixed cultures should be employed in an attempt to follow cytological changes such as those reported by Schneebeli (*11*).

### References


1. HENCH, P. S., *et al. Proc.. Staff Meetings Mayo Clinic,* **24**, 181 (1949).
2. SELYE, H. *J. Clin. Endocrinol.*, **6**, 117 (1946).
3. CORNMAN, I. *Proc. Soc. Exper. Biol. Med.*, **75**, 355 (1950).
4. MEDAWAR, P. B., ROBINSON, G. M., and ROBINSON, R. *Nature,* **151**, 195 (1943).
5. SELYE, H., BELAND, E., and SYLVESTER, O. *Exp. Med. Surg.*, **2**, 224 (1944).
6. SELYE, H., *et al. J. Am. Med. Assoc.*, **124**, 201 (1944).
7. BAKER, B. L., and CASTOR, C. W. *Anat. Record,* **106**, 173 (1950).
8. RAGAN, C., *et al. Proc. Soc. Exp. Biol. Med.*, **72**, 718 (1949).
9. TAUBENHAUS, M., and AMROMIN, G. D. *Endocrinology,* **44**, 356 (1949).
10. WHITAKER, W. L. *Anat. Record,* **106**, 257 (1950).
11. SCHNEEBELI, G. I. *Ibid.*, 244.


## A Simple Presumptive Test for Toxigenicity of *Corynebacteria*[1]


Florence L. Evans[2]

*Department of Microbiology, Louisiana State University School of Medicine, New Orleans*


Laboratory methods of determining toxin production among the *Corynebacteria* depend upon the use of animals. As a result, many laboratories report on morphology of such cultures only. Previous work has indicated that chick embryos are susceptible to pure toxin (*1*), and to whole broth cultures (*2, 3*). However, only 3 strains of *Coryn. diphtheriae* and one of *Coryn. hoffmannii* were used. It occurred to the author that a method being used in an investigation of this group of organisms, now in progress, was sufficiently sensitive and reliable to warrant a brief report. The simplicity of the method, and ready availability of the test animal, should make it practical even in small laboratories.

Essentially, the procedure consists of inoculating a light suspension of the culture on the chorio-allantoic membrane of 9–10-day-old embryonated hen's eggs. The culture is purified by plating, and a loopful of growth from a 24-hr-old blood agar plate is suspended in broth and washed twice, centrifuged and resuspended in fresh broth. The sediment from the last centrifugation is emulsified in a few drops of broth, and a loopful is transferred to about 10 ml of broth. The turbidity should be just visible in a bright light. Such suspensions give reproducible results with the same culture and from one culture to another.

Eggs at the 9–10-day stage of incubation are candled to determine the viability and location of the embryo.


[1] Aided in part by a grant from Eli Lilly and Company.


[2] Present address, Department of Microbiology, Baylor University College of Medicine, Houston, Texas.