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alkaloid ryanodine, by way of contrast, causes the endothelial cells to round up while the fibroblasts remain extended (unpublished data).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*).

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

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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.

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The fork of two large blood vessels on the C.A. membrane is located, and a circular area in the crotch is marked on the shell with a lead pencil. This area is disinfected with 95% alcohol, and a small circle ground through the shell with a dental engine equipped with a fine corborundum disk. Care must be taken not to damage the shell membrane. The circle of shell is removed with fine-pointed forceps, leaving the shell membrane intact.

The eggs are then inoculated by injecting 0.05 ml of the washed bacterial suspension just under the shell membrane and onto the C.A. membrane by means of a tuberculin syringe equipped with a ½-in., 27-gauge needle. The exposed shell membrane is then covered with sterile, melted paraffin-vaseline mixture, and the eggs are returned to the 37° C incubator.

The inoculated eggs are examined daily by transillumination, and embryos which appear to be dead are removed from the shell and examined grossly. The typical appearance is marked engorgement of blood vessels, and hemorrhage in the embryo, C.A. membrane, and sometimes in the yolk membrane. The fluids are usually clear, but the amniotic fluid may be tinged with hemolyzed blood. Cloudy fluids or embryos showing evidence of decomposition indicate contamination.

Toxigenic strains of *Coryn. diphtheriae* kill the embryos with remarkable uniformity. Ninety-nine per cent of 424 embryos were dead in 96 hr under the conditions described here. On the other hand, the majority of the embryos inoculated with nontoxigenic strains survived; i.e., 84% of 84 embryos were alive at 96 hr. Survival to the date of hatching gave essentially the same results (0.24% survival for embryos inoculated with toxigenic strains, and 81% survival for those inoculated with nontoxigenic strains).

A total of 32 strains was tested by this method. Of these, 9 were known *Coryn. diphtheriae,* and 23 had been isolated from cases of clinical or suspected diphtheria. Toxigenicity, as determined in guinea pigs, corresponded exactly with the results in the embryos. From these results, it appears that death of 80% or more of the embryos in test lots of 10 or more for each culture may be regarded as a specific indication of toxigenicity.

The response of the 9–10 day chick embryos is sufficiently uniform that it could be used as a preliminary method of differentiating between toxigenic and nontoxigenic cultures suspected of being *Coryn. diphtheriae.* The potential usefulness of this method lies in the fact that it may be used in small laboratories or in those not having provisions for the care and maintenance of animals.

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A Reevaluation of Steroid Nomenclature

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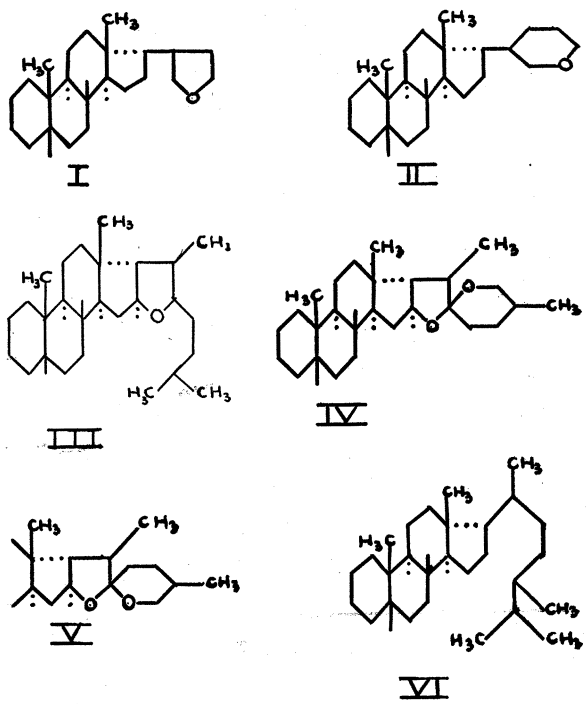
It must be obvious to those actively engaged in steroid studies that the various nomenclatures currently in use are by no means consistent and are not particularly facile in operation. The difficulties are noticeable when unnatural or unusual compounds are encountered, especially those differing in bridgehead configuration, those that have larger or smaller rings than usual, those that have one or more opened rings, and the genins, sapogenins, and their transformation products.

To attempt to bring some sort of order to this rather bewildering picture, a synthesis of the naming systems commonly used by authoritative workers has been made, which, with certain modifications, seems to lend itself to an adequate delineation of most compounds encountered in steroid chemistry.

As an initiation into the system, it is suggested that the parent substances commonly met with be named as follows: estrane,[1] androstane, and etiocholane; pregnane and 5-allopregnane; genan (I) and the 5-allo derivative, cholane and 5-allocholane; *E*-homogenan (II) and the 5-allo derivative, cholestane and coprostane; pseudosapogenan (III) and the 5-allo derivative, sapogenan (IV); 22-isosapogenan (V) and the 5-allo derivatives, ergostane and copro-



[1] Configuration undetermined at positions 5 and 10.