reversibly the proteolytic activity of fibrinolysin resembles the antiproteolytic properties of some natural peptides, such as the pepsin inhibitor and the trypsin inhibitors (*12*). The interaction of the natural as well as the synthetic peptides with the different proteolytic enzymes is probably determined in both cases by some specific groups present in the enzyme and the inhibitor, as well as by the electrostatic forces prevailing between the enzyme and the relatively high molecular weight inhibitor. Further studies with the synthetic amino acid polymers may contribute to our basic knowledge of the mode of action of naturally occurring polypeptides on enzyme behavior.

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Manuscript received January 24, 1952.

Action of Penicillin on Streptococci: Enhancement of Sensitivity *in vivo?*

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The bacteriostatic and bactericidal action of penicillin on growing bacterial cells may under certain conditions continue after the complete removal of the penicillin (*1*). On the other hand, it has been stated by Grunberg, Unger, and Eldridge that streptococci exposed to penicillin *in vivo* are temporarily more susceptible to the action of penicillin than are cells of the same culture not so exposed (*2, 3*). Because of the evident significance of this phenomenon, and because of the increasing use of the method of sensitivity testing upon which the conclusion was based, an attempt has been made to confirm the observation.

In brief, the experimental procedure used by Grunberg, Unger, and Eldridge was as follows. Mice were injected subcutaneously with a broth culture of a hemolytic streptococcus and were immediately treated by injection into the same site of sodium penicillin G. At intervals thereafter the mice were sacrificed, cultures were made of the tissues at the site of inoculation, and the sensitivity of the surviving organisms tested by the use of "gutter" plates. It was found that with advancing time the susceptibility to penicillin of the surviving streptococci progressively increased. The increase in sensitivity was deduced from the fact that with a constant concentration of penicillin in the gutter, the zone of inhibition, or distance from the edge of the gutter to the nearest streptococcal colony, became greater. It was stated, however, and examination of the protocols confirms it, that with time there was a marked decline in the number of streptococci in the lesion, and therefore in the number of streptococcal colonies on the test plate.

TABLE 1

EFFECT OF DENSITY OF INOCULATION ON DIAMETER OF "ZONE OF INHIBITION"

Expt. 1		Expt. 2	
Dilution of culture	Diameter of zone of inhibition (mm)	Dilution of culture	Diameter of zone of inhibition (mm)
Undiluted	32	Undiluted	42
10^{-1}	34	10^{-1}	40
10^{-2}	43	10^{-2}	44
10^{-3}	48	10^{-3}	62
10^{-4}	50	10^{-4}	76
10^{-5}	55		

In the present experiments the same strain of hemolytic streptococcus was used as was employed by Grunberg *et al.* It was used, however, without exposure to penicillin or injection into mice. Experiments were designed to test the effect of dilution of the culture on the apparent sensitivity of the organism to penicillin. Constant 0.1 ml portions of serial decimal solutions of an 18-hr broth culture were spread over the surface of a blood agar plate. A ceramic porcelain cup ("Penicylinder"®) was then fixed in place on the surface of the agar and filled with a solution containing 100 units penicillin/ml. After overnight incubation the diameter of the zone of inhibition which surrounded the penicillin cup was measured. The results are set forth in Table 1. It is evident that a very direct relation exists between the diameter of the zone of inhibition and the density of the inoculum.

These data indicate, therefore, that in order to explain the results which were seen following *in vivo* exposure of streptococcus to penicillin it is not necessary to invoke damaging effects associated with the *in vivo* situation. A simple decrease in number of streptococci present would serve equally well to explain them. The data also indicate the possible hazards associated with the use of the various disk and tablet methods for assaying the sensitivity of bacteria to various antibiotics in the clinical laboratory. Difficulties in interpretation of results would most easily arise when the disk method is applied to an initial mixed culture in which the absolute numbers and the proportions of various organisms are uncontrollable.

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Manuscript received January 25, 1952.

The Detection of Crystals Following Local Injection of Cortisone and Compound F into the Skin of Man

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In the course of investigative studies (*1*) with the local application and injection of four oxysteroids, a cortisone acetate,[1] a water soluble ester[1] of cortisone, Compound F free alcohol, and Compound F acetate,[1] it was found necessary to attempt to control some of the observations by the examination of crystals of these compounds in the skin.

In routine paraffin-fixed sections crystals of these steroids cannot be found. The purpose of such studies on crystals is to determine the morphologic distribution patterns of such crystals in relationship to possible local mechanisms (epidermis, dermis, etc.) and the persistence in relationship to possible persistent local therapeutic effects from local injections. Moreover, an effort should be made to determine whether the crystals remaining in the skin are the same as those injected. Finally, a study of these crystalline masses may serve to explain the tissue reactions produced in normal tissue by the local injections of suspensions of cortisone and Compound F.

In his study of skin irritations in animals, Ake Nilzen (*2*) in our department, together with Knut Schmidt-Nielsen, was able to detect crystals presumably of cortisone acetate in the skin of guinea pigs following local injection. Their technique, in brief, was the preparation of frozen sections without fixation of tissue. We have modified the technique of Nilzen and Schmidt-Nielsen in our recent experiments with cortisone acetate and Compound F.

The steroids used in the work reported here included only cortisone acetate, Compound F, and cholesterol (plate type only). The vehicles used included, first, the suspension mixture of benzyl alcohol and polyoxyethylene sorbitan monooleate, sodium carboxymethylcellulose; and, second, saline as the only suspending agent. It was necessary to use saline suspensions since the other vehicle had definite reactions on local injection (*1*).

At first, suspensions of the steroids in the two vehicles were observed under varying conditions such as room temperature, freezing, and heating to attempt to determine any changes in the size of the crystals. At the suggestion of Schmidt-Nielsen, we incubated crystals of cortisone with minced human tissue (skin and subcutaneous tissue) at 37° for 24 and 48 hr. There was no change in the size of the crystals. The crystals of Compound F acetate measured in our experiments 1.25 μ and are smaller than those of cortisone acetate, which varied in length from 6 to 9 μ (Fig. 1). The crystals of the free alcohol of Compound F are 30–45 μ. The heavy saline suspensions of cortisone acetate gave larger crystals than the suspension of cortisone acetate in benzyl alcohol and the other suspending agents.

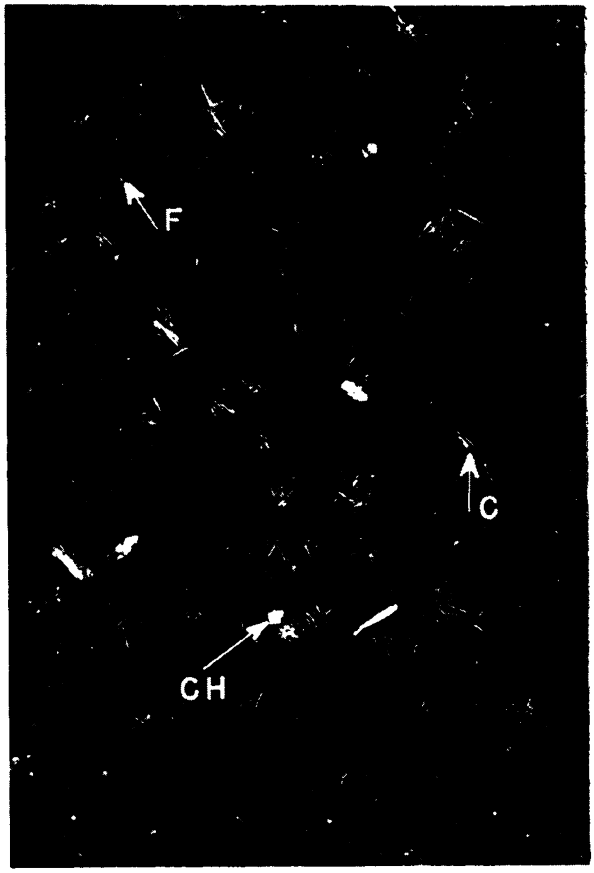

FIG. 1. Mixture of crystals of cortisone acetate (*C*), Compound F acetate (*F*), and cholesterol (*CH*). Vehicle, benzyl alcohol, polyoxyethylene sorbitan monooleate, and sodium carboxymethylcellulose; polarized light; approx ×100.

The following materials were injected intradermally into the normal skin of man: (1) vehicles without the steroid; (2) cortisone acetate suspensions, 25 mg/cc; (3) Compound F suspension, 25 mg/cc; (4) cholesterol, 25 mg/cc.

Injections of cortisone acetate and Compound F were done also with the Hypospray jet injection apparatus. Subsequently, injections of the steroids were made into pathologic skin and into normal skin which,

[1] Supplied by Augustus Gibson, Merck & Co., Inc., Rahway, N. J.