of the intramuscular medullated nerves.

The augmentation and aggregation of neurogenic substances in the voluntary muscle fibers affected by DDT parallels the abnormal response and subsequent paralysis through axonic exhaustion by chemical denervation of some muscle fibers. It is suggested that the normal dark and granular muscle fiber be designated as the neurosomic muscle fiber and that the light and agranular fiber be called the aneurosomic muscle fiber. This terminology is based on the hypothesis that there is a periodic discharge and disappearance of the neurogenic granules in the fibrillar protoplasm of the same voluntary muscle fiber observed at different periods of time, and the hypothesis itself is supported by the suggestive experimental evidence of the effects of DDT.

The observations reported in this paper are, in general, consistent with the views expressed, over one hundred years ago, by Doyère.[23] He observed the junction between the living nerve and muscle in the microscopic Tardigrade (Spallanzani) also called the "water bear." Its body is a transparent muscular bag. Doyère stated, page 346, that "the relation of the terminal nerve filaments with the muscles cannot be distinguished," and in Fig. 4, plate 17, he illustrates the junction of the granular nerve fiber and the granular muscle fiber as morphologically continuous.

Summary. The limited experimental evidence presented in this paper tends to support the statement that DDT increases the discharge of auriphilic neurogenic substances from some motor end plates into the myoplasm of some of the voluntary muscle fibers. These neurogenic substances form massive aggregates of neurosomes in parts of some voluntary muscle fibers. This results in a partial or complete dissociation of the neurogenic from the myogenic substances in the muscle fiber. Auriphilic granules are likewise found at the 2 poles of the elongated nuclei in the dark muscle fibers. The supporting evidence that some of the auriphilic granules and masses found after DDT toxicity are neurogenic in origin follows: (1) anatomical continuity of the auriphilic bodies in muscle with the auriphilic hypolemmal axons of the motor end plates; (2) similarity of staining reaction of the masses in muscle and axons of nerve endings with gold impregnation; (3) massive aggregation of auriphilic bodies in some muscle fibers contemporaneous with the centrifugal depletion of the related nerve axons and endings of their auriphilic substances. Presumptive evidence is presented that the normal fiber types in voluntary muscle are dependent, in part, upon the periodic alternation of the discharge and disappearance, by chemical action, of the neurogenic granules in the same muscle fiber at different time periods. The limited evidence also indicates that DDT poisoning may be produced in normal rats by feeding, and in chameleons by the intraperitoneal injection of the previously perfused, dehydrated, and emulsified muscles of rats manifesting toxicity with DDT.

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Diffusion of Sulfonamides and Penicillin into Fibrin.*

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Until the advent of penicillin, subacute bacterial endocarditis largely resisted all forms of therapy. The persistence of the disease is due to the survival of the bacteria in the valve locus. The organisms in the vegetations are separated from the blood stream by a layer of fibrin and it has been

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shown that antibacterial agents penetrate fibrin poorly or not at all. Friedman[1] studied the effect of 3 germicides, gentian violet, merthiolate and Vuzino-toxine camphorated on the *Streptococcus viridans* growing imbedded in a fibrin mass. He compared the effect with that occurring in ordinary broth cultures of the same organism and found that the fibrin mass had a striking retarding effect on the bactericidal action of these compounds. Friedman[2] also demonstrated the inability of sulfanilamide and sulfapyridine to eradicate a focus of streptococci growing in and protected by a fibrin-platelet mass. Duncan and Faulkner[3] were unable to demonstrate any appreciable penetration of sulfonamide compounds into blood clots in periods of 24 hours to 15 days. These observations suggest that failure of therapy in bacterial endocarditis may be due to an ineffective contact between chemotherapeutic agents and the bacteria on the valves as a result of impermeability of the fibrin barrier.

The sulfonamide compounds[4] and penicillin[5] inhibit the growth of *Streptococcus viridans* in the test tube. However, in bacterial endocarditis with the organisms lodged in the heart valve, penicillin has shown a striking therapeutic effect while the sulfonamides have at best exhibited only an occasional and usually temporary beneficial action. The present study is an attempt to determine whether the greater effectiveness of penicillin can be ascribed to a superior diffusion of this substance into fibrin. A comparison was made of the diffusibility into fibrin of the relatively ineffective sulfonamide compounds with that of penicillin.

Procedure. A modification of the cup assay method for penicillin was utilized. Agar plates were prepared using *Bacillus subtilis* as the test organism following the technic of Foster and Woodruff.[6] Fibrin plates were prepared in the following manner. Dried fraction I of human plasma† was reconstituted by the addition of saline, containing 10% glucose. Twenty ml portions were poured into petri dishes and 0.2 ml of a spore suspension of *B. subtilis* added to each plate.

The plates were shaken to permit an even distribution of the bacterial suspension. To each plate 0.2 ml of a solution of bovine thrombin was added. The thrombin was prepared by dissolving 5000 units in 5 ml of isotonic saline. The fibrin hardens within 2 minutes after the addition of the thrombin. Three penicillin assay cups were placed equidistantly on each agar and fibrin plate. The 3 cups were filled with the substance to be tested, permitting the reading of the results in triplicate. The plates were kept at room temperature as preliminary experiments showed that liquefaction occurred in the fibrin at incubator temperature. Measurements of the diameter of the zones of inhibition were made at 24-hour intervals.

Sulfonamide Compounds. Sulfathiazole and sulfadiazine were used in this study. Relatively dilute solutions of these compounds did not produce a definite inhibition in either the agar or fibrin plates. More concentrated solutions (M/25) of the sodium salts of these compounds showed definite zones of inhibition in the agar but there was no evidence of inhibition in the fibrin. To assay the possible effect of the alkalinity of these solutions, a control of disodium phosphate in M/25 solution, adjusted to the same pH as that of the sodium sulfonamides was utilized. One agar and one fibrin plate were set up with the 3 cups filled with the M/25 sodium sulfathiazole, another pair with M/25

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[3] Duncan, C. N., and Faulkner, J. M., *Am. J. Med. Sc.*, 1940, **200**, 492.

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† This plasma fraction was prepared under a contract between the Office of Scientific Research and Development, and Harvard University, from blood collected by the American Red Cross. This was supplied by the Department of Physical Chemistry, Harvard Medical School. In this fraction, 60% of the protein is fibrinogen and the remainder is albumin and globulin.

TABLE I.
Inhibition Zones Diameter in mm.
Diameter of zones of inhibition in mm on agar and fibrin plates produced by sodium sulfathiazole, sodium sulfadiazine, and disodium phosphate.

	M/25 Sodium-sulfathiazole		M/25 Sodium-sulfadiazine		M/25 Disodium-phosphate	
hr	Agar	Fibrin	Agar	Fibrin	Agar	Fibrin
24	0	0	0	0	0	0
48	12	0	0	0	0	0
72	15	0	14	0	0	0
96	18	0	18	0	0	0
120	18	0	18	0	0	0

TABLE II.
Diameter of Zones of Inhibition in mm.

Penicillin units/ml	0.5		1.0		2.0		4.0	
hr	Agar	Fibrin	Agar	Fibrin	Agar	Fibrin	Agar	Fibrin
24	9	9	9	9	11	9	11	10
48	10	9	11	9	12	9.5	15	12
72	11	9	12	10	12	10	18	12
96	12	10	13	12	14	13	18	14
120	14	10	16	12	16	14		
144	14	10	16	12	16	14		
168	14	10	16	12	16	14		

sodium sulfadiazine and the third with the control M/25 disodium phosphate. Readings were made at 24-hour intervals and the averages of the 3 readings taken. Table I shows the results of these experiments. It is evident that sodium sulfathiazole and sodium sulfadiazine diffuse into agar but show no reaction in fibrin. The control solution shows no inhibition in either the agar or fibrin plates indicating that the alkalinity of the sodium sulfonamides is not a factor in the reactions.

Penicillin. A number of experiments were carried out with varying concentrations of penicillin. A zone of inhibition appeared in both the agar and fibrin plates in 24 hours. Although the inhibition zones were somewhat larger in the agar as compared with fibrin plates, the difference was relatively slight, indicating a comparatively free diffusion of penicillin into fibrin. Table II shows the results of a typical experiment.

Discussion. These results indicate that penicillin diffuses freely into fibrin as compared with sulfonamide compounds. The conclusion seems justifiable that the greater effectiveness of penicillin in subacute bacterial endocarditis is at least in part due to this superior diffusibility. The fact that there is a relationship between the chemotherapeutic activity of a compound and its ability to permeate fibrin supports the concept of a fibrin barrier as the mechanism which permits the persistence of the infection on the valve. It is clear that in evaluating the efficiency of a drug in the treatment of bacterial endocarditis, the ability to penetrate fibrin requires important consideration.

A further implication of the present study relates to the use of anticoagulants in the treatment of subacute bacterial endocarditis. With the purpose of lessening the deposition of fibrin on the diseased valve, the administration of heparin has been used as an adjunct to antibacterial agents. Although some clinical reports suggest that heparin is beneficial, others indicate that the results of penicillin therapy are not improved by its use. The present experiments would tend to minimize the importance of heparin as it is shown that fibrin has little retarding effect on the activity of penicillin.

Summary and Conclusion. Using a modification of the cup assay method on agar and fibrin plates, there is no evidence of penetration of sulfathiazole and sulfadiazine into

fibrin. Penicillin diffuses almost as well into fibrin as into agar. It is suggested that the diffusibility of penicillin into fibrin is an important factor in the efficacy of this substance in the treatment of subacute bacterial endocarditis.

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Serum Levels After Repository Injections of Penicillin.

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There is considerable interest in the development of methods for administering penicillin which are both simple and efficient. Oral administration would be the obvious solution were it not for the marked irregularity of absorption as well as the low efficiency of this method with the materials thus far available.[1-4] Various attempts have been made to maintain and prolong the effective concentrations of penicillin after parenteral administration with the hope of reducing the number of injections necessary. Most promising at the present time is the use of a repository injection of a relatively large dose in a medium from which absorption takes place slowly. Among the various combinations proposed, the mixtures of beeswax in peanut oil have been studied most extensively.[5-9] With this method effective serum levels can be maintained quite regularly over a 12-hour period and in most instances for 18 to 24 hours, but the concentrations after more than 12 hours following a dose of 300,000 units or less may be quite irregular or not measurable. While the beeswax-peanut oil mixtures have been used successfully in several clinics, their high melting point and the marked viscosity of the preparations make the injections of these materials technically difficult for most inexperienced persons. Substances which are more easily manipulated at ordinary room temperatures would be much more desirable.

Freund and Thomson[10] proposed the use of a simple, rapid technic of preparing water-in-oil emulsions of penicillin. The penicillin is put into solution in a small volume of saline and added to a mixture of a lanolin-like substance (Falba) and peanut oil and thoroughly emulsified before the injection. This method has been used by Cohn *et al.*[11] in the treatment of 52 cases of acute gonorrhea by the single injection of the emulsion containing 150,000 units of penicillin, with only 2 failures. The serum levels obtained in 3 subjects given the penicillin in this manner were somewhat better sustained than in 2 others in which the same amount of penicillin was given in saline. Similar or identical materials have been prepared commercially (Solvecillin, Pendil, Emulgen, etc.) and have the advantage that they flow readily at or

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