of Presidon which protects mice from procaine convulsions is about one-fourth the hypnotic dose but the protective and hypnotic doses of pentobarbital are almost identical. In the other species the protective dose of Presidon is about one-third the hypnotic dose whereas the protective dose of pentobarbital is about one-half the hypnotic dose. There is large species variation in the sensitivity to procaine convulsions and also to the hypnotic and anticonvulsive potency of pentobarbital and Presidon. The mouse is most resistant to both procaine and the hypnotics. The dog is more resistant than the guinea pig or rabbit to procaine convulsions but is most sensitive to the hynotics. The smallest protective doses of the hypnotics were required by the dog.

Discussion. In cases of procaine intoxication, there is an initial period of respiratory stimulation which is followed by depression which may lead to terminal respiratory failure(2,7,8). Most deaths following the subcutaneous administration of procaine were caused by respiratory failure even though, in some instances, the convulsions had been controlled.(9,10) In view of the strong depressant action of the barbiturates upon respiration, it would seem more feasible to employ a prophylactic drug which could abort the convulsive effects of procaine HCl without causing additive effects on the already depressed respiration. Presidon and pentobarbital have been compared as central nervous system depressants using hypnotic activity as an index. That Presidon has a wider margin of safety than representative barbiturates has been previously reported.(3,4) It is important to note that pentobarbital confers protection against the convulsive effects of procaine HCl only in doses which are considerably more depressant than equally protective doses of Presidon. It would seem, therefore, that the ability of a drug to prevent local anesthetic intoxication does not parallel its degree of depressant action on the central nervous system.

Summary. Presidon and pentobarbital administered intraperitoneally, prevent the convulsions produced by procaine in dogs, rabbits, guinea pigs, and mice.

Presidon confers protection against the convulsive effects of procaine at one-fourth to one-third the hypnotic dose, whereas the protective dose of pentobarbital is from one-half to the full hypnotic dose.

7. Dragstedt, C. A., and Long, V. F., *J. Pharm. and Exp. Therap.*, 1928, v32, 215.

8. Beutner, R., *Anesthesia and Analgesia*, 1943, v22, 121, 205.

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Relationship of Type of Growth of *M. tuberculosis* to Antituberculous Activity of Subtilin.*† (17574)

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In the course of an investigation of the antimicrobial activity of subtilin it was observed that although this substance possessed a high degree of inhibitory activity on tubercle bacilli *in vitro*, it failed to afford protection to mice experimentally infected with tuberculosis,

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TABLE I.
Increase in Activity of Subtilin on Tubercle Bacilli Associated with the Diffuse Growth in the Presence of Tween 80.

Concentration of Tween, %	Min. inhibitory conc. of subtilin, μg/ml	Type of growth in control
.0	>100	Clumped
.001	>100	"
.005	50	"
.01	3.12	Partially clumped
.02	3.12	Diffuse
.05	3.12	"
.1	1.56	"
.2	1.56	"
.4	1.56	"
.8	1.56	"

despite prolonged administration of relatively large doses. These findings were in accord with those of other investigators, Anderson and Wong,(1) and Wong, Hambly, and Anderson,(2) who had noted similar activity of subtilin *in vitro*, but failure to modify the course of experimental tuberculosis in hamsters. There are numerous examples of drugs which exhibit antibacterial activity *in vitro* but lack therapeutic value. Nevertheless, many of the recognized causes of loss of antibacterial activity *in vivo* appeared to be excluded in the case of subtilin by virtue of the demonstration in several laboratories, including our own, that subtilin was highly active *in vivo* on other bacteria such as pneumococci and streptococci.(3-5) Further study, however, disclosed that the activity of subtilin *in vitro* was maintained only in the presence of a suitable detergent, such as Tween 80,§ which was contained in the culture medium employed in the tests mentioned above. Fischer(6) has reported that certain strains of tubercle bacilli recovered from patients receiving streptomycin may be inhibited by low concentrations of that drug in a medium containing Tween, but are resistant to as much as 1000 μg per ml in other culture media. Enhancement of the action of penicillin on tubercle bacilli by Tween has been reported by Kirby and Dubos,(7) who suggested the possibility that the increase in activity of penicillin might be correlated with the degree of growth dispersion of the bacilli. The findings with subtilin suggested that this factor might also be operative in determining the degree of activity exerted by this drug on tubercle bacilli.

Accordingly, an investigation was made of the effect of the type of growth of tubercle bacilli on the antituberculous activity of subtilin, under various conditions which modify the degree of dispersion of the microorganisms.

Materials and methods. The *in vitro* tests were carried out in the Tween-albumin medium described by Dubos and Davis,(8) which was modified in certain experiments by variations in the concentration of Tween, or by the addition of serum. The test organism employed was *M. tuberculosis* (H37Rv), and the results reported are those recorded after incubation for seven days. Further incubation was continued, but caused no significant differences in the results. Bioassays of subtilin in serum were performed with a conventional serial dilution technic using as test organism *Streptococcus hemolyticus* (C203 MV) and *Staphylococcus aureus* (Oxford H

1. Anderson, H. H., and Wong, S. C., *Tuberculology*, 1946, v8, 77.

2. Wong, S. C., Hambly, A. S., and Anderson, H. H., *J. Lab. and Clin. Med.*, 1947, v32, 837.

3. Salle, A. J., and Jann, G. J., PROC. SOC. EXP. BIOL. AND MED., 1946, v62, 40.

4. Salle, A. J., and Jann, G. J., PROC. SOC. EXP. BIOL. AND MED., 1946, v63, 41.

5. Salle, A. J., and Jann, G. J., PROC. SOC. EXP. BIOL. AND MED., 1946, v63, 519.

§ Tween 80 (sorbitan monooleate), is a product of the Atlas Powder Co., Wilmington, Del.

6. Fischer, M. W., *Am. Rev. Tuberc.*, 1948, v57, 58.

7. Kirby, W. M. M., and Dubos, R. J., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 120.

8. Dubos, R. J., and Davis, B. J., *J. Exp. Med.*, 1946, v83, 409.

strain). The subtilin used in this study was obtained from two sources‖ and the results with all samples were in substantial agreement.

Results. The first group of tests was devised to determine the activity of subtilin on tubercle bacilli under conditions in which the type of growth was varied by cultivation in graded concentrations of Tween 80. The inhibitory concentrations of subtilin were determined in a group of serial dilution tests carried out in the Dubos-Davis medium, which was modified for the purpose of this experiment only in its concentration of Tween 80. In Table I are presented the results of a representative experiment. As may be seen in Table I, in the control without Tween and in the lowest concentrations of Tween, the growth of tubercle bacilli was in the form of macroscopic clumps, and under these conditions the subtilin had little or no effect. The presence of Tween in concentrations sufficient to permit diffuse growth of the organisms resulted in a striking increase in the inhibitory action of subtilin, to the extent that growth was inhibited by 1.56 μg per ml. As previously mentioned, the description of the types of growth reported in Table I is based on macroscopic appearance. When viewed in a hanging drop preparation an even more striking difference was observable. In Fig. 1a may be seen clumped growth of tubercle bacilli in the absence of Tween, as seen in such a preparation. The clumps are seen to consist of many bacterial cells arranged in branching skeins or cords. This type of growth has been described in detail by Middlebrook, Dubos and Pierce.(9) For comparison, in Fig. 1b is shown a hanging drop preparation of diffuse or dispersed growth of tubercle bacilli in liquid synthetic medium containing 0.05% Tween. Its appearance was in striking contrast with clumped growth and may be seen to consist of a uniform single cell suspension of bacilli.

It has been previously noted that in the Dubos-Davis medium the action of Tween could be antagonized by the addition of serum and this phenomenon appeared to afford an additional means of examining the relationship between subtilin activity and the type of growth of tubercle bacilli. The following experiments were thus designed to test the effects of serum on this relationship. To Dubos-Davis medium modified to contain Tween in concentrations from 0.05% to 0.8%, serum was added to give final concentrations ranging from 2.5 to 20%. The inhibitory concentration of subtilin was then determined in each of these media and the type of growth of tubercle bacilli in each test noted. The results are shown graphically in Fig. 2. The percentage of serum and the percentage of Tween in the individual tests are expressed as the serum-Tween ratio. A low ratio reflects a low concentration of serum or a high concentration of Tween in a particular test. On the ordinate is plotted the inhibitory concentration of subtilin, and along the base is recorded the type of growth in the control tubes at the various ratios of serum and Tween.

As may be seen in Fig. 2, serum antagonized the action of Tween, so that when the serum-Tween ratio was high, growth was clumped and subtilin activity slight. As the ratio decreased, growth became diffuse and subtilin activity correspondingly increased. In these tests, it was observed that the effect of serum in concentrations as high as 20% was overcome by the presence of 0.4% Tween or more (serum-Tween ratio of 50 or greater), with resulting diffuse growth and a high degree of subtilin activity.

In Fig. 1 is shown the appearance of tubercle bacilli in representative cultures from this experiment as seen in hanging drop preparations. As may be seen, (Fig. 1c) in 20% serum growth was clumped if the medium contained 0.05% Tween, the usual concentrations employed, and appeared identical with the type of growth seen in liquid medium which contained no Tween (Fig. 1a). In the same concentration of serum, however, diffuse

‖ Preparations of subtilin were obtained through the courtesy of the Wallerstein Company, New York City, and from the Antibiotics Study Section of the National Institutes of Health, U. S. Public Health Service, Bethesda, Md. The latter preparation, Lot 200 A-1, was produced by the Western Regional Research Laboratory of the U. S. Department of Agriculture at Albany, Calif.

9. Middlebrook, G., Dubos, R. J., and Pierce, C., *J. Exp. Med.*, 1947, v86, 175.

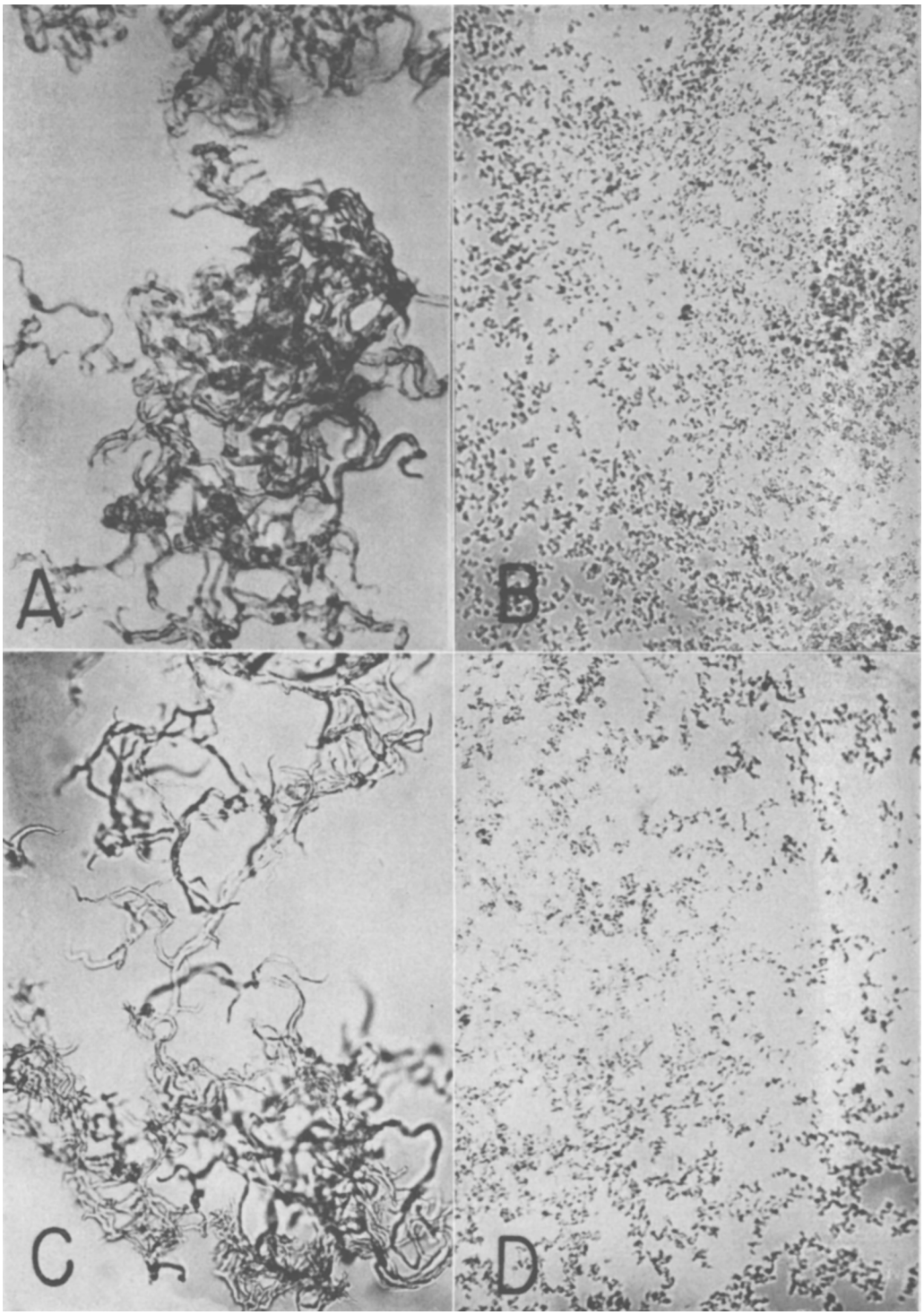

FIG. 1.
Types of submerged growth of tubercle bacilli ($H_{37}RV$) in Dubos-Davis medium (hanging drop preparation, 100 ×).

a. Clumped growth in medium containing *no Tween*. One may observe a ropy or corded appearance of the clumps.

b. Diffuse or dispersed growth in Dubos-Davis medium which contains 0.05% Tween.

c. Clumped growth in medium containing 0.05% Tween but, in addition, 20% serum. This type of growth strikingly resembles the growth in a, in which no Tween is present.

d. Diffuse growth in medium containing 20% serum. The Tween concentration, however, has been increased to 0.8%. This type of growth strikingly resembles that seen in b, in which Tween but no serum is present.

growth occurred (Fig. 1d) in 0.8% Tween, and resembled the type of growth customarily observed in the Tween-albumin medium with 0.05% Tween but without the antagonism of serum (Fig. 1b).

Further experiments were performed to discover whether this mechanism was sufficient to explain the failure of subtilin to be effective *in vivo*. Serum was obtained from rabbits at intervals following intravenous injection of 22 mg per kg of subtilin and was tested for inhibitory activity on *Str. hemolyticus* and *Staph. aureus*. Parallel assays of these sera were made with *M. tuberculosis* (H37Rv) as test organism, and were carried out in media containing 0.4% and 0.05% Tween as well as one without Tween. In Fig. 3, the results are recorded as the inhibitory concentration of subtilin in micrograms per ml. It may be seen that the activity of serum tested with tubercle bacilli in 0.4% Tween paralleled that found in assays with streptococci and staphylococci. There was no demonstrable inhibition of the tubercle bacilli in the two control media (not shown) containing 0.05% and no Tween.

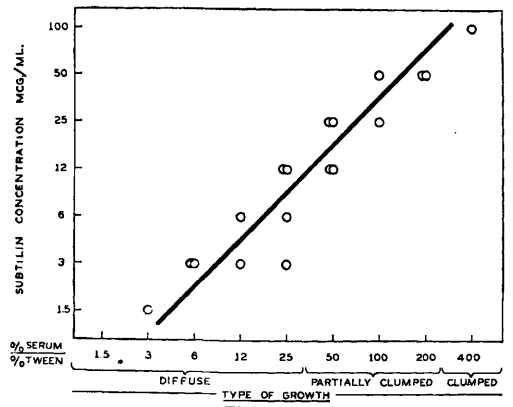

FIG. 2.

Effect of alterations in the ratio of serum and Tween on the inhibition of *M. tuberculosis* by subtilin.

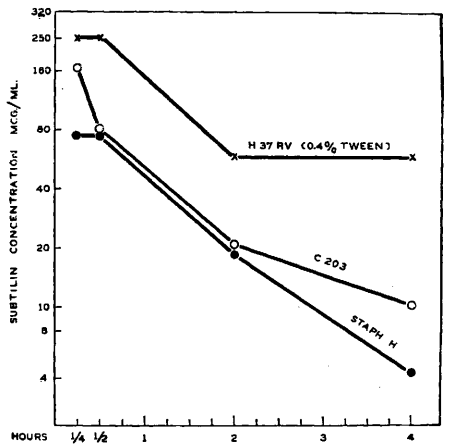

FIG. 3.

The antibacterial activity of serum of rabbits subsequent to intravenous administration of subtilin, as measured with 3 test organisms.

Discussion. It appears from these observations that the factors operative in the production of growth of tubercle bacilli in clumps or cords in a liquid medium afford these microorganisms a high degree of protection from the action of subtilin. Moreover, at least several other antimicrobial agents are considerably more active against tubercle bacilli which are growing diffusely than when the microorganisms are growing in clumps. This suggests the possibility that the clumped growth of tubercle bacilli reflects the presence of a mechanism which serves to protect the microorganisms from the harmful effects of certain antimicrobial drugs to which they would otherwise be sensitive. The question immediately arises as to the nature of the growth of tubercle bacilli *in vivo*, for if these findings may be more broadly applied, one might anticipate the failure of subtilin to affect tuberculosis in animals if the microorganisms existed as aggregate rather than as individual cells. Dubos has called our attention to the original reports of Koch(10) on the etiology of tuberculosis in which may be found draw-

10. Koch, R., 1884, *Die Aetiologie der Tuberkulose. Mittheilungen aus dem Kaiserlichen. Gesindheitsamte*, Bd. 2, 1-88.

ings made from histological sections of necrotic foci of tuberculosis in man and animals. In these drawings the presence of tubercle bacilli in large aggregates is clearly illustrated. Moreover, Middlebrook, Dubos and Pierce(9) have also observed clumped growth of tubercle bacilli in the brain tissue of guinea pigs infected intracerebrally with virulent human strains. It is of interest that the occurrence of this phenomenon has not been more widely discussed. It is possible that modern methods of preparing tissues for histologic examination may not be ideally designed to reveal the nature of the type of growth of *M. tuberculosis in vivo*. On examination of conventionally prepared tissue sections of experimental tuberculous lesions of the lung, virtually all of the microorganisms during the early stages of the infection appear to be intracellular, principally within the monocytes. Under these conditions the tubercle bacilli are present as aggregates but the role of the phagocyte in producing the aggregation cannot be discounted. The principal situation where tubercle bacilli are seen to be extracellular is within areas of caseation where they are also present in clumps. In this situation, also, it is difficult to exclude the possibility that the aggregations were originally formed by the monocytes and were released with disruption of the latter cells.

Thus it cannot be considered as established at present that the clumping of bacilli observed *in vivo* has the same physiologic implications in regard to antimicrobial therapy as the clumping of bacilli *in vitro*.

Steenken and Wolinsky(11) have recently confirmed the observation that subtilin activity *in vitro* is dependent upon the presence of Tween (sorbitan monooleate). They further observed that guinea pigs infected with a virulent human strain, H37Rv, of *M. tuberculosis* were not benefitted by an extensive course of treatment with subtilin.

Summary. It has been observed that the degree of inhibition of tubercle bacilli by subtilin *in vitro* parallels the degree of growth dispersion of the organisms. In a medium permitting diffuse growth, subtilin was highly active. Conversely, in a medium in which growth was clumped, whether because of omission of Tween or from antagonism of Tween by serum, no significant inhibition of growth by subtilin was demonstrable. When serum from rabbits injected with subtilin was tested against tubercle bacilli in a medium containing sufficient Tween, the observed antituberculous activity paralleled the activity against streptococci and staphylococci. If the amount of Tween added to such tests was insufficient to produce diffuse growth of tubercle bacilli, subtilin activity was greatly diminished or absent.

11. Steenken, W., Jr., and Wolinsky, E., *J. Bact.*, 1949, v57, 453.

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Relation of Low Temperatures to X-ray Injury of Hematopoietic Tissue of Tadpoles.* (17575)

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Tadpoles of *Rana catesbiana*, from 70-90 mm total length, were subjected to x-ray treatment of definite dosage and killed at the end of specific periods of time during which some were refrigerated and others kept at normal temperature. When tadpoles were refrigerated during irradiation, they were previously kept in the refrigerator for 2 hours. During irradiation, tadpoles were kept in water 7 mm deep, leaving the dorsal surface

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