TABLE II.
Precipitative and Protective Antibodies Removed from Immune Serum by Absorption with Specific Antigen and Hapten.

Immune serum	Antibodies removed as determined by: Quantitative estimations on immune precipitates %	Quantitative estimations on supernates %	Mouse protection tests %
Absorbed with specific antigen	95	94	97
Absorbed with hapten	—	90	77

the polysaccharide hapten corresponded to 90% of that precipitable by the complete antigenic complex. From the mouse protection tests recorded in Tables I and II it can be seen that absorption of the serum with hapten removed approximately 77% of the protective antibodies.

The pooled antiserum was also absorbed with a large excess of Type III hapten (3000 μg per ml of serum). The results of the mouse protection tests with this serum are recorded in Table I. These results do not differ significantly from those obtained with the serum absorbed with a slight excess of hapten.

From the above experiments it is evident that the immune bodies in dysentery antiserum which confer passive immunity on mice are those primarily directed against the somatic antigen. The fact that some 77% of the protective antibodies can be absorbed with the polysaccharide hapten suggests that the major portion of these immune bodies are directed against the carbohydrate component of the complex antigenic molecule.

Summary. 1. Antisera prepared by prolonged immunization of rabbits with Type III *Shigella paradysenteriae* (Flexner) contain mouse protective antibodies which can be removed by absorption with the chemically purified type specific antigen. Absorption of the serum with the polysaccharide portion of the antigenic complex removes approximately 80-90% of the precipitating and protective antibodies.

2. Most, if not all, of the protective antibodies present in Flexner Type III antiserum are directed against the homologous specific antigen. Any protective antibodies reactive with other constituents of the bacterial cell appear to have little or no significance in the immune response. The polysaccharide hapten is the component of the antigenic complex most important in orienting the protective and precipitative antibodies.‡

‡ Similar results are reported by Smolens, J., Halbert, S. P., Mudd, S., Doak, B. W., and Gonzalez, L. M., *J. Immunol.*, in press.

15091

Subtilin—An Antibiotic Produced by *Bacillus subtilis*. I. Action on Various Organisms.*

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Although it has been known for many years[1,2] that cultures of *Bacillus subtilis* are antagonistic to the growth of other organisms,

* This and subsequent investigations on subtilin are part of a cooperative study undertaken by the Department of Pharmacology and Experimental Therapeutics, University of California Medical School, San Francisco, under the direction of Dr. H. H. Anderson; the Department of Bacteriology, University of California, Los Angeles, under the direction of Dr. A. J. Salle; and the Biochemical

comparatively few studies are recorded in the literature. Several workers[3–7] found that *B. subtilis* produced a lytic action on a number of bacteria. The organism has been shown to be antagonistic to *Mycobacterium tuberculosis*,[8] the virus of vesicular stomatitis,[9] and a number of other pathogenic and saprophytic fungi.[10–12] In general, Gram-negative organisms were not appreciably affected by *B. subtilis*.

The subtilin used in these investigations was a purified product prepared from *B. subtilis* grown in a synthetic medium and

TABLE I.
Organisms Susceptible to the Action of Subtilin.

Alcaligenes viscosus
Bacillus anthracis
" *cereus*
" *megatherium*
Corynebacterium diphtheriæ
Diplococcus pneumoniæ I (parent strain)
" " I (sulfa-resistant)
" " II CH (parent strain)
" " II CH (sulfa-resistant)
Gaffkya tetragena
Lactobacillus casei
" *delbruckii*
" *fermenti*
pentosus
Micrococcus ureæ
Mycobacterium phlei
" *smegmatis*
Neisseria catarrhalis
Rhodococcus roseus
Sarcina lutea
" *ureæ*
Staphylococcus aureus (sulfa-resistant)
" "
" " (hemolytic)
" " (Oxford)
" *citreus*
Streptococcus fæcalis
" *lactis*
" *pyogenes* (hemolytic)

TABLE III.
Organisms Not Susceptible to the Action of Subtilin (1:1000).

Aerobacter aerogenes
Alcaligenes fecalis
Brucella abortus
" *suis*
Eberthella typhosa
Escherichia coli
" " *communior*
Klebsiella pneumoniæ
Pasteurella avicida
Proteus X 19
" *vulgaris*
Pseudomonas aeruginosa
" *fluorescens*
Salmonella paratyphi
" *schottmuelleri*
Serratia marcescens
Shigella alkalescens
" *dysenteriæ*
" *paradysenteriæ*
" *sonnei*
Vibrio comma

TABLE II.
Diameters of Zones of Inhibition of Organisms Susceptible to Subtilin.

Organism	Concentration of subtilin used and diameter of zone of inhibition measured in mm				
	1:1,000	1:10,000	1:100,000	1:1,000,000	1:10,000,000
Alcaligenes viscosus	12	0	0	0	0
Bacillus cereus	17	14	0	0	0
Neisseria catarrhalis	14	12	0	0	0
Rhodococcus roseus	35	29	23	12	0
Sarcina lutea	29	25	20	12	0
Staphylococcus aureus (hemolytic)	17	13	0	0	0
" " (Oxford)	22	18	0	0	0
Streptococcus fæcalis	22	18	10	0	0
" *pyogenes* (hemolytic)	17	12	0	0	0

Division, Western Regional Research Laboratory, Albany, California, under the direction of Dr. Howard D. Lightbody.

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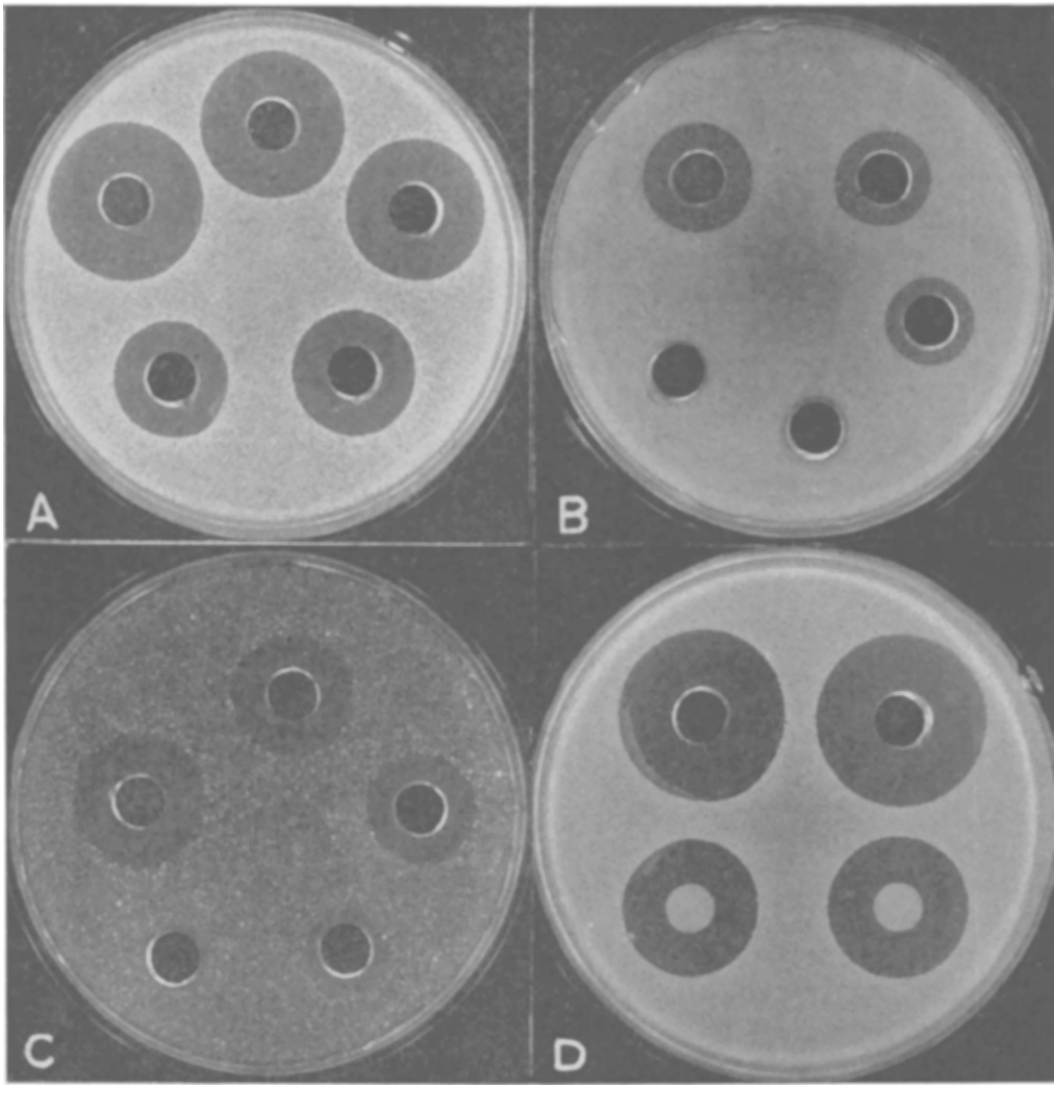

FIG. 1.

Action of subtilin on bacteria by the agar cup-plate and filter paper methods. A. *Sarcina lutea* antagonized by subtilin in concentrations of 1:10,000, 1:50,000, 1:100,000, 1:500,000, and 1:1,000,000. Into each cup was placed 0.1 cc of the subtilin dilution. B. *Staphylococcus aureus.* Dilutions same as in A. C. *Corynebacterium diphtheriæ.* Dilutions same as in A. D. *Sarcina lutea* treated with a 1:10,000 dilution of subtilin. Comparison made between the agar cup-plate and the filter paper methods.

tested for potency against several Gram-positive and Gram-negative organisms.[13] It was prepared by the Biochemical Division of the Western Regional Research Laboratory, Albany, California.

Experimental. Various procedures were

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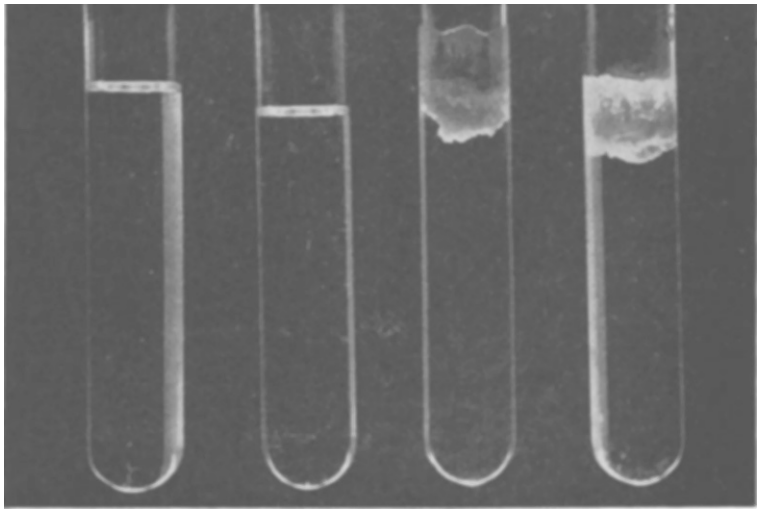

A B C D

FIG. 2.

Action of subtilin on *Mycobacterium tuberculosis* inoculated into Long's synthetic medium. A. subtilin concentration, 1:33,333; B. subtilin concentration, 1:50,000; C. subtilin concentration, 1:666,666; D, subtilin concentration, 1:100,000.

TABLE IV.
Effect of Subtilin (1:1000) on a Group of Higher Fungi.

Susceptible organisms	Non-susceptible organisms
Actinomyces species (unidentified)	Actinomyces species (unidentified)
Actinomyces asteroides	*Candida albicans*
Actinomyces pelletieri	*Cryptococcus neoformans*
Nocardia mexicana	*Penicillium notatum*
	Sporotrichum schenckii
	Trichophyton gypseum

used to test the activity of subtilin. The method employed depended upon the organism being investigated. To test the action of subtilin on bacteria which grew readily, the following method was used: to 20 cc of melted and cooled agar was added 0.1 cc of a 24-hour nutrient broth culture of the test organism. The inoculated agar was poured into a sterile Petri dish, thoroughly mixed and allowed to harden. The effect of the antibiotic was shown by (1) pipetting 0.1 cc of subtilin dilution into a cup 10 mm in diameter in the agar, or (2) placing on top of the agar a 10 mm disc of filter paper (Whatman No. 2), previously soaked in the subtilin dilution. The inhibitory action of subtilin manifested itself as a clear zone around the agar cup or the disc of filter paper (Fig. 1).

Organisms which were found to be susceptible to the action of subtilin are given in Table I. The concentrations of purified subtilin used ranged from 1:1000 to 1:10,000,000. With the exception of *Neisseria catarrhalis* and *Alcaligenes viscosus*, the organisms are Gram-positive.

Measurements of the diameters of the zones of inhibition of a number of the organisms given in Table I are recorded in Table II.

Organisms not susceptible to the action of subtilin in a concentration of 1:1000 are grouped in Table III. All of the species included in this group are Gram-negative.

Neisseria gonorrhoeae was tested by streaking the organism over the surface of chocolate blood agar, then placing a 10 mm disc of filter paper, previously soaked in a 1:1,000 dilution of subtilin, in the center of the plate. Subtilin was strongly antagonistic to the growth of this organism.

Mycobacterium tuberculosis was inoculated

into tubes of Long's synthetic medium and subtilin added in concentrations ranging from 1:5000 to 1:250,000. The tubes were incubated at 37° C for 3 weeks. The highest dilution of subtilin showing no growth was 1:50,000. At the end of this period of time transfers were made to new medium. The highest dilution showing no growth in the transfer tubes was 1:10,000. These results appear to indicate that subtilin is bacteriostatic in high dilution and germicidal in greater concentration (Fig. 2).

A number of higher fungi were tested by streaking the organisms over the surface of Sabouraud's glucose agar, then placing discs of filter paper soaked in 1:1,000 dilution of subtilin in the centers of the plates. The results are recorded in Table IV.

Summary. The antibacterial product, subtilin, obtained from *Bacillus subtilis* was found to be active chiefly against Gram-positive bacteria. Two notable exceptions to the rule were *Neisseria catarrhalis* and *N. gonorrhoeae,* both Gram-negative, but also antagonized by subtilin. Acid-fast organisms, including *Mycobacterium tuberculosis,* were also found to be susceptible to the antibiotic. The agent produced a bacteriostatic action in high dilution and a germicidal effect in greater concentration. A number of pathogenic higher fungi were also found to be susceptible to subtilin.

15092

Action of Atropine on the Turtle Heart.

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Atropine has at least two significant modes of action on the heart, an early effect due to stimulation of the vagus center, and its well known action as an antagonist of acetylcholine. Concerning an independent direct action on heart muscle, the literature contains conflicting statements. This paper reports a study of the direct action of atropine on the turtle heart *(Pseudemys elegans).* The results indicate that the only significant direct effect of atropine on heart muscle is to depress fiber conduction when the rate of beating is abnormally high. Details of technic have been described in connection with observations on the action of digitalis.[1] The influence on rhythmicity and on contractility was observed when whole auricles or ventricular strips were suspended in a bath and the beat recorded on a smoked drum. Changes in refractory period and conductivity were followed in rhythmically stimulated ventricular strips placed on moistened filter paper; potential differences were amplified, and recorded by a piezoelectric ink writer. Two receiving electrodes were placed on the muscle strip about 15 mm apart, and each was paired with an electrode placed on the paper at a distance of about 2 cm. It has already been shown that the Q-T interval of the electrogram so recorded is an accurate measure of the refractory period, since its duration corresponds closely with measurements made by a direct method.[2] The tissue was observed for an adequate control period before the drug was added.

Effect on Rhythmicity. The effect on a natural pacemaker was studied only in the whole auricle. In the freshly removed auricle, concentrations of atropine varying from 1:200,000 to 1:50,000 caused an increase in rate of beating from 10 to 15% in 4 experiments, while in 3 no change occurred. Con-

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