strain of *T. pallidum*. In from 4 to 6 weeks all developed acute syphilomas with positive darkfield examinations. Two rabbits were kept as untreated controls while the remaining 6 were treated with tyrothricin administered by stomach tube once a day for 15 days in succession. Freshly prepared 1:100 suspensions of the compound in sterile distilled water were employed. The daily doses were 1 cc (0.0002 g), 5 cc (0.001 g) and 10 cc (0.002 g) per kg for each of 2 rabbits receiving the 3 different amounts. One of the 2 rabbits receiving 10 cc (0.002 g) died on the 8th day of treatment, apparently from the toxic effects of the compound. Clinical records and darkfield examinations were made at daily intervals. During and at the end of the period of treatment none of the animals showed any clinical evidences of healing of the testicular lesions as compared with the untreated controls and all showed persistently positive darkfield examinations. Under the circumstances lymph node transfers were regarded unnecessary.

Summary. The maximum tolerated dose of tyrothricin for adult white mice, administered by stomach tube 3 times a day for 10 days in succession, was about 0.0008 g per mouse. By this route of administration 3 doses of tyrothricin daily in dose of 0.0004 g per mouse were very slightly effective in the treatment of mice inoculated with virulent *Streptococcus hemolyticus* (group A) and type III pneumococcus, while definitely more effective in the treatment of mice inoculated with virulent types I and II pneumococci and especially type I. Tyrothricin in daily doses of 0.0002, 0.001 and 0.002 g per kg, administered by stomach tube for 15 days in succession, was completely ineffective in the treatment of acute testicular syphilis of rabbits.

15586 P

Inhibition of Bacterial Growth by Auxins.

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We have observed that a number of synthetic unsaturated ring-containing acids endowed with auxin (plant hormone) activity can exert a bacteriostatic effect on the growth of certain bacterial species. The following substances were selected as representatives of different chemical types of auxins: indole-3-acetic acid, β-(indole-3)-propionic acid, -(indole-3)-n-butyric acid, α-naphthaleneacetic acid, β-naphthoxyacetic acid, 2,4-dichlorophenoxyacetic acid, 2,3,5-triiodobenzoic acid.[1]

The order of activity of these different substances on a few bacterial species is illustrated in Table I in which are given the concentrations of auxins required to cause a 50% inhibition of growth in a medium containing by weight 0.1% enzymatic hydrolysate of casein and 0.02% yeast extract. It should be emphasized that the inhibitory concentrations recorded in Table I have only a relative value since, as will be indicated later, tryptophane and peptone can antagonize the bacteriostatic action of auxins. It is also worth recording that the antibacterial activity of 2 of these substances had been recognized before anything was known of their plant hormone activity. Thus, it was known that indole acids (indole acrylic acid in particular) inhibit the growth of certain Gram-negative bacilli in synthetic media, and that this bacteriostatic effect can be reversed by tryptophane;[2] the growth of mycobacteria in synthetic Long's medium has been shown to be inhibited by 2,3,5-triiodobenzoic acid although the same substance stimulates oxygen uptake of these organisms

[1] Zimmerman, P. W., *Indus. and Eng. Chem.*, 1943, **35**, 596.

[2] Fildes, P., *Brit. J. Exp. Path.*, 1941, **22**, 20.

TABLE I.
Comparative Bacteriostatic Activities of Auxins Against Different Bacterial Species in Casein Hydrolysate-Yeast Extract Medium at pH 6.0.

Inoculum 10^{-4} cc culture per cc medium	Approximate concentration of auxin (in %) required for 50% inhibition of growth*						
	Indole acetic acid	Indole propionic acid	Indole butyric acid	Naphthalene acetic acid	Naphthoxy acetic acid	2-4 dichloro-phenoxyacetic acid	Triiodo-benzoic acid
Mycobacterium tuberculosis (human strain)	.01	.0005	.01	.005	.003	.003	.002
Streptococcus hemolyticus (H69D)	.01	.015	—	.01	.01	.015	.003
" *salivarius* (MG)	.001	—	—	.01	—	—	.001
Shigella paradysenteriæ (Sonne)	.003	.008	.01	.01	.01	.01	.01

— = not done.

* Readings of growth were made after incubation at 37°C for 1 day (streptococci and dysentery bacilli) or 10 days (tubercle bacilli). In general, growth did not increase significantly upon further incubation.

Concentrations of auxins 3 to 5 times greater than shown in the table are required for complete inhibition of growth.

when their metabolism is studied by the conventional Warburg technic.[3]

As shown in Table I the bacteriostatic activity of the different auxins varies greatly from one bacterial species to another, each substance appearing to exhibit a selective inhibitory effect against one or several groups of organisms. With the strains and preparations used in our tests, indole-3-acetic acid is the most active substance against the Gram-negative bacilli, whereas mycobacteria are peculiarly susceptible to β-(indole-3)-propionic acid; triiodobenzoic acid exhibits highest activity against certain strains of streptococci.

The inhibitory concentration of auxin is not markedly affected by the size of the inoculum (the inhibitory concentration increases 2- to 4-fold as the inoculum is increased 100- to 1000-fold). It is also independent of the addition of serum albumin to the medium (0.5% was the highest concentration of albumin tested). Peptone and tryptophane can reverse in part or completely the bacteriostatic effect of auxins, tryptophane being by far the most active in this respect. Reversal of bacteriostasis usually requires concentrations of tryptophane approximately 10 times higher than those of auxin added to the medium.

In the case of most organisms, the bacteriostatic activity of all substances increases as the medium becomes more acidic: in fact, it is often difficult to detect any inhibition of growth at slightly alkaline reactions. On the other hand, inhibition of growth of tubercle bacilli by auxins appears to be almost independent of the reaction of the medium.

When the concentration of auxin in the medium is insufficient to produce complete inhibition of growth, bacterial multiplication proceeds at first at a rate similar to that in the untreated culture. However, the presence of auxin results in an interruption of multiplication before maximal development has occurred; the density of the final cul-

[3] Saz, A. K., and Bernheim, F., *J. Pharm. and Exp. Ther.*, 1941, **73**, 78; Saz, A. K., Johnston, F. R., Burger, A., and Bernheim, F., *Am. Rev. Tub.*, 1943, **48**, 40.

ture is inversely related to the concentration of auxin. In other words, the antibacterial mechanism under consideration affects the total amount of bacterial protoplasm synthesized rather than the rate of growth of the culture.

15587

Chemotherapy of Certain Iodonium and Sulfonium Compounds.*

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In the following experiments diphenyliodonium chloride and several of its derivatives were tested for bacteriostatic and fungistatic action *in vitro* upon the following organisms: *M. tuberculosis, Staphylococcus aureus, E. coli, Proteus vulgaris, Streptococcus pyogenes, Ps. pyocyaneus, Cl. histolyticum,* and Monilia *(Candida krusei)*. Methyl diphenylsulfonium nitrate was also evaluated *in vitro*. In addition, several of the diphenyliodonium derivatives were investigated *in vivo* for their therapeutic action on tuberculous animals.

The sulfonium and iodonium compounds discussed in this paper are members of a class of compounds having large positive ions as an integral part of their structure. This class includes many organic phosphonium, arsonium and ammonium compounds and many derivatives of elements on the right-hand side of the periodic table. Certain compounds in this group are powerful cationic detergents, for example the aryl or aralkyl-alkyl-dimethyl-ammonium salt, and these compounds are generally and actively bacteriostatic by virtue of their surface-active character. The compounds discussed in this paper, however, are not notably surface active but do show strong and selective biostatic actions.

Methods. In Vitro Experiments. For the *in vitro* studies the tubercle bacilli were grown on Proskauer and Beck synthetic media, pH 7.0. The pyogenic bacteria were grown on beef heart broth, pH 7.2. *Cl. histolyticum* was grown in Brewer's fluid thioglycollate medium and in standard beef heart broth containing 0.5% glucose. For the culture of the fungus, Monilia, Sabouraud's agar was used. The chemical compounds were dissolved in water to make 0.1% solutions; subsequent dilutions were made directly into the media without further heating. Dilutions into agar were made at approximately 60°C. Two strains of tubercle bacilli were used, the virulent H37Rv and the avirulent strain No. 607. The media were inoculated with one loopful of fresh culture of tubercle bacilli. The beef heart broth was inoculated with 0.1 cc of a 24-hour culture of the pyogenic bacteria. For the fungistatic tests, the agar slants were streaked with one loopful of a 3-day culture of Monilia. All the cultures were incubated at 37°C. The cultures were read at the following periods of time: virulent tubercle bacilli—3 weeks; avirulent tubercle bacilli—4 days; pyogenic bacteria—1 day and Monilia—3 days. The highest bacteriostatic or fungistatic dilution was taken as that dilution in which there was no or very slight growth.

Animal Experiments. Nine guinea pigs, averaging 350 g in weight, were used to evaluate each compound for its therapeutic effect in animals infected with tuberculosis. The animals were inoculated in the left

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