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.

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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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groin with 0.01 mg of H37Rv tubercle bacilli. The drugs were administered either orally or hypodermically beginning on the day of infection. For oral administration the drug was dissolved in water and then mixed with ground rabbit pellets. For hypodermic administration the drugs were ground with a 1.5% beeswax-sesame oil mixture, and the resultant mixtures were injected in 0.3 cc amounts subcutaneously. All experiments were terminated at the end of 6 weeks. Pathology was evaluated numerically by the method of Sweany, Sher and Kloeck,[1] in which the maximum gross involvements of the organs were given the following numerical ratings: lymph nodes 8, spleen 24, liver 28, and lungs 40. Hemoglobin determinations were made at the end of the experiments.

Results. The *in vitro* bacteriostatic results are recorded in Table I. The parent compound, diphenyliodonium chloride, inhibited the growth of tubercle bacilli in very high dilutions; the virulent culture in a dilution of 1:800,000 and the avirulent strain in a concentration of 1:600,000. In the presence of 50% horse serum, diphenyliodonium chloride showed no decrease in bacteriostatic effect (1:600,000). The di-p-methyl derivative, di-p-tolyliodonium iodide, was equally effective. The di-p-amino derivative, di-p-aminodiphenyliodonium iodide, was somewhat less bacteriostatic. Acetylation of the amino groups decreased the tuberculostatic action considerably. (o)-Carboxydiphenyliodonium iodide was without inhibiting action. Methyl-diphenylsulfonium nitrate was also without tuberculostatic action. *p*-Aminobenzoic acid did not antagonize the inhibition of tubercle bacilli by diphenyliodonium chloride.

Diphenyliodonium chloride showed a moderate bacteriostatic action on *Streptococcus pyogenes,* being effective in a dilution of 1:80,000. This compound also inhibited the growth of *Staphylococcus aureus* in a concentration of 1:40,000. Diphenyliodonium chloride exhibited still less inhibiting effect

TABLE I.

Highest Bacteriostatic or Fungistatic Dilution *in Vitro.* (× 10,000.)

	Tubercle bacillus H37Rv	Tubercle bacillus No. 607	*Staphylococcus aureus*	*E. coli*	*Proteus vulgaris*	*Streptococcus pyogenes*	*Ps. pyocyaneus*	*Cl. histolyticum*	*Monilia krusei*
Diphenyliodonium chloride									
(<⟨⟩ I <⟨⟩)+ Cl.-	80	60	4	1	2	8	<1	20	40
Diphenyliodonium iodide		60	2	<1	2				
Di-p-tolyliodonium iodide		60	2	<1	1				
Di-p-acetylaminodiphenyliodonium iodide		2	2	<1	<1				
Di-p-aminodiphenyliodonium iodide		10	<1	<1	<1				
o-Carboxyldiphenyliodonium iodide		<1	<1	<1	<1				
Methyldiphenylsulfonium nitrate		1	2	1	<1	<1	<1		10

Controls with potassium iodide were run to exclude the effect of ionic iodine; these were negative for bacteriostatic action. Phenol showed negligible fungistatic action on Monilia (<1:10,000).

[1] Sweany, H. C., Sher, B. C., and Kloeck, J. M., *Am. Rev. Tuberc.,* 1946, **53**, 254.

TABLE II.
Summary of Therapeutic Efficacy in Tuberculous Animals.

Group	Compound	Dosage	Avg Path. Rating	Avg wt Gain	Hglb.
A	Diphenyliodonium chloride	8 mg daily oral	40.6	172	11.8
	Controls		49	223	10.9
B	Diphenyliodonium chloride	1 mg daily* hypo.	38.1	155	11.1
	Controls		46.4	101	10.8
C	Di-p-acetylaminodiphenyliodonium iodide	1 mg every other day hypo.	46.6	158	12.2
	o-Carboxyldiphenyliodonium iodide	2 mg every other day hypo.	42	161	11.9
	Controls		39.3	215	12.4

* After 3 weeks of therapy the drug was given only on alternate days.

on *P. vulgaris, E. coli* and *Ps. pyocyaneus*. On the other hand, it showed a pronounced inhibiting action on Monilia (1:400,000). None of the derivatives of this compound was significantly effective in inhibiting the growth of *Staphylococcus aureus, B. coli,* or *B. proteus*. Likewise the compound, methyldiphenylsulfonium nitrate, was ineffective against these organisms; however, this compound showed an excellent inhibiting action on Monilia.

Cl. histolyticum grown in the beef heart medium was inhibited by diphenyliodonium chloride in a dilution of 1:200,000. In the thioglycollate medium, however, a dilution of 1:10,000 was required for inhibition. The addition of concentrations of thioglycerol ranging from 1:6,000 to 1:120,000 to Proskauer-Beck medium had no significant effect on the inhibition of tubercle bacilli by diphenyliodonium chloride. The replacement of thioglycerol by cysteine or sodium thioglycollate also yielded negative results.

The *in vivo* results are summarized in Table II. Diphenyliodonium chloride and several of its derivatives were administered therapeutically to guinea pigs infected with tubercle bacilli. On oral administration the animals tolerated 8 mg daily of the compound, diphenyliodonium chloride, without signs of toxicity. However, only 1 mg could be given hypodermically and only 70% of the animals survived this therapy. None of the compounds showed any significant therapeutic action.

Discussion. The very large difference between the activities of the iodonium and sulfonium salts may be, in part, due to gross geometrical differences. However, more specific factors such as ionic radius, polarizability, and oxidizing power are probably more significant. For the iodonium compounds certain configurational factors of importance have been demonstrated. For example, in (o)-carboxydiphenyliodonium iodide there is an enormous drop in tuberculostatic activity. This may be due to the formation of a compact inner salt structure at neutral or alkaline pH's. Such a structure could effectively block the manifestation of the bacteriostatic activity of the iodonium group. The drop of activity in p,p'diaminodiphenyliodonium iodide may be due to the electron donor character of the p-amino groups tending to decrease the positive character of the iodonium groups. Alternatively, amino groups and especially carboxyl groups may block entry of the molecule to the site of the characteristic toxic action of the iodonium group.

As to the mechanism of action of the iodonium compounds the following observations may be cited. It has been noted by Sandin and others[2] that the stability of aryliodonium salts decreases with increasing polarizability of the anion. For example, di-o-tolyliodonium bromide decomposes at 178°C, the iodide at 155°C, and the sulfide at room temperature. The less stable onium compounds of other classes show similar behavior. Snyder and Speck[3] have shown that in hot solutions containing benzylphenyldimethylammonium ion, cleavage of the benzyl group is easily effected by HSO_3^-, $SO_3^=$, HS^-, $S^=$, $S_2O_3^=$ and CNS^- while

[2] Sandin, R. B., *Chem. Rev.*, 1943, **32**, 249.

[3] Snyder, H. R., and Speck, J. C., *J. Am. Chem. Soc.*, 1939, **61**, 668.

CN^-, OH^-, $OC_6H_5^-$, and $PO_4\equiv$ are ineffective. The released benzyl radicals arylate the anions. In a microorganism, if such arylation occurs, a free and functionally active mercapto group, essential to the metabolism of the organism, would be converted into a nonactive thioether group.

Conclusions. It may be suggested, tentatively, that the activity of iodonium compounds may, in some cases, be due to interaction with certain mercapto groups essential to the microorganisms. This formulation rests largely on observations of the high reactivity of iodonium compounds toward sulfide, hydrosulfide and similar ions in simple chemical systems. That the detail of the mechanism of inhibition is different for *Cl. histolyticum* and *M. tuberculosis* is apparent from the experiments involving thioglycollate. In the case of *M. tuberculosis* we have not found a mercapto compound which antagonizes the effect of the iodonium compounds. That both sulfonium and iodonium compounds inhibit Monilia, while the iodonium compounds alone inhibit the growth of tubercle bacilli, suggest that a similar mechanism of action may be involved with both compounds for Monilia, but not in the case of *M. tuberculosis*.

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Effect of Some Barbiturates on Tissues *In vitro*.

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It is well known to workers with tissue cultures that outgrowths from explants of brain, ganglia and spinal cord can be easily obtained in fluid or semisolid media even without the addition of embryonic extract. The cultivation of spinal ganglia has proved to be a valuable test material in a variety of experimental studies (Burt,[1,2] Meyer and Jablonski,[3] Murnaghan,[4] Weiss,[5-7] Weiss and Wang[8,9]). Relatively little use has been made of nerve cord cultures even though the technic for obtaining such preparations is considerably easier than that required for the dissection of ganglia.

The procedure for cord cultures which has been used in our laboratories is schematized in Fig. 1 to 4. The necks of embryos of suitable ages are cut (Fig. 1) at their base with scissors or knives. The spinal cord is expelled from the neural canal by compressing the tissue in the mid-dorsal line with 2 pairs of forceps moved alternately in the direction of the sectioned vertebral column. Thus a cervical (Fig. 2) and a thoracolumbar segment (Fig. 3) of cord can be obtained. These are washed in Tyrode's solution and then transferred to a ground glass slide and cut with sharp knives. For this purpose we have found fragments of wafer razor blades soldered in the eye of a needle more satisfactory than cataract knives (Fig. 4a). Transverse sections varying from ¼ to ½ mm can be cut easily by moving knife tips, placed at right angles to the cord segment, in opposite directions (Fig. 4 and 4a). After washing in Tyrode's solution hang-

1 Burt, A., *J. Cell. and Comp. Physiol.*, 1943, **22**, 145.

2 Burt, A., *J. Cell. and Comp. Physiol.*, 1943, **22**, 205.

3 Meyer, H., and Jablonski, W., *J. Anat.*, 1937, **72**, 62.

4 Murnaghan, D. P., *Anat. Rec.*, 1941, **81**, 183.

5 Weiss, P., *J. Exp. Zool.*, 1934, **68**, 393.

6 Weiss, P., *Anat. Rec.*, 1944, **88**, 205.

7 Weiss, P., *J. Exp. Zool.*, 1945, **100**, 353.

8 Weiss, P., and Wang, H., *Anat. Rec.*, 1936, **67**, 105.

9 Weiss, P., and Wang, H., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 273.