THE ANTIBACTERIAL PROPERTIES OF SULFUR

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In the course of attempts to purify the antibacterial agent we had extracted from Spanish moss (*Tillandsia usneosides*) (1), by adsorption on charcoal, we found that a powerful antibacterial substance for certain organisms could be recovered from charcoal itself. Blood charcoal and previously acidified animal charcoal contained it, but norit did not. This antibacterial substance recovered from charcoal proved to be elemental sulfur.[1]

Although sulfur is one of the oldest remedies known to man surprisingly little experimental work has been carried out with it. Colloidal sulfur was first prepared in 1888 (2), and since then many preparations have been used in the treatment of different conditions with varying degrees of success. It has been administered orally and by injection in the treatment of mental disease (3–5), by injection in the treatment of arthritis (6–8), and topically, as a fungicide in the treatment of cutaneous infections (9, 10). In both the precipitated and the colloidal forms, sulfur has been used extensively with notable success in the treatment of certain plant diseases (11, 12).

It is generally assumed that the size of the sulfur particles is basic in the activity of a preparation, the smaller the particles the more active the preparation obtained. In the skin it is thought that the particles of sulfur are converted through the medium of certain cells in the epidermis to sulfur-containing compounds (for example sulfides or pentathionic acid) and that these compounds are responsible for the antimicrobial action (9, 10).

We are aware of only two reports of experiments showing the *in vitro* antimicrobial activity of sulfur upon human pathogenic organisms. The first was of experiments conducted in 1934 by Lawson (13), who investigated the effect of precipitated sulfur incorporated into Corper's mashed-potato medium on the growth of tubercle bacilli. He found that the addition of as little as 3 mg. of sulfur to 100 cc. of medium completely inhibited the growth of this organism. He stated that sulfur appears to have no inhibitory effect upon the growth of some of the ordinary pathogenic bacteria, not specified by him.

The second report was of experiments conducted in 1935 by Kingery (14) who stated that colloidal sulfur was fungicidal and fungistatic for *Trichophyton interdigitale* and tinea corporis. The fungicidal experiments were carried out by adding a broth suspension of the organism to be tested, to a 1 per cent or 5 per cent dilution of colloidal sulfur. The mixture was shaken for 2 minutes and then several loopfuls were streaked on a suitable agar medium. No growth occurred in the streaks from the preparation

[1] We are indebted to Dr. E. G. Miller, Jr., for these analyses.

containing sulfur while growth occurred in the control streaks having no sulfur. The fungistatic tests were carried out in a similar way except that the organisms were left in contact with the sulfur for longer periods.

The present paper is a report of our experiments demonstrating the antimicrobial properties of elemental sulfur.

Materials and Methods

Two different preparations of sulfur were used: (1) a saturated alcoholic solution of sulfur which we shall call "alcohol-sulfur" and (2) a carbowax (15) preparation of sulfur which we shall call "carbowax-sulfur."

Preparation of Alcohol-Sulfur.—0.5 gm. of sublimed flowers of sulfur U.S.P. was added to 100 cc. of absolute ethyl alcohol, in a 300 cc. Erlenmeyer flask. After 2 days' standing, with intermittent shaking in a cork stoppered flask at room temperature, the water-clear supernatant was separated from the undissolved residue by centrifuging or by filtering through paper. This saturated solution was the alcohol-sulfur solution used in the tests.

Preparation of Carbowax-Sulfur.—12.5 gm. of flowers of sulfur U.S.P. and 500 gm. of carbowax 1540 pH 4.4 were heated together in a 2 liter Erlenmeyer flask, with cotton stopper, for 1½ hours, at 150–170°C. in a hot air oven. The hot clear yellow supernatant obtained was the carbowax-sulfur solution used in the tests below, the excess of sulfur at this temperature remaining at the bottom of the flask. On cooling, this preparation remained clear until the temperature reached about 70°C. at which time the sulfur began to precipitate out visibly and the carbowax solidified slowly. Our carbowax-sulfur preparations were made up fresh for each experiment since we found that on reheating solidified carbowax-sulfur to 170°C. all the sulfur did not again dissolve in the carbowax and preparations weaker in antibacterial activity were obtained.

Preparation of Culture.—Unless stated otherwise, *Staphylococcus aureus* (Oxford strain) was used in this work. Each day a fresh 3½ hour culture in bacto nutrient broth (inoculum 0.5 cc. of culture to 9.5 cc. of broth) was prepared from a similar culture of the previous day. Microscopic examination showed that these 3½ hour cultures contained Gram-positive organisms growing singly, in pairs, and in very small clumps only. The cultures were diluted with broth so as to contain from 200,000 to 300,000 organisms or groups of organisms per cc. and 0.05 cc. of this dilution was added to each tube in the tests described below. Since the activity of a preparation is, roughly, inversely proportional to the number of organisms present initially, it is of course important to have a constant inoculum.

Two methods of titrating the activity of sulfur were used; (1) serial dilution method in broth, (2) agar streak method.

1. Serial Dilution Method of Titration.—(*a*) For the alcohol-sulfur the method employed was as follows:—

The alcohol-sulfur was diluted 1–100 in bacto nutrient broth. From this slightly cloudy preparation a series of twofold dilutions were made in broth and 1 cc. of each was placed in 10 × 1.2 cm tubes. They were inoculated with 0.05 cc. of the suitably diluted freshly grown staphylococcus culture and incubated at 37°C. Readings were taken after 1 and after 2 days. The twofold dilutions of alcohol-sulfur ranged from 1–100 to 1–3,200.[2]

(*b*) For the carbowax-sulfur the method was as follows:—

[2] When greater accuracy was desired such as in tests to determine the weight of sulfur per unit, additional dilutions were interpolated between the dilutions of 1–1,600 and 1–3,200.

Half a cc. of the carbowax-sulfur at about 90°C. was taken up in a heated pipette and added to 4.5 cc. of broth at the same temperature to make a dilution of 1–10. This mixture formed a thick milk-white suspension. From it dilutions up to 1–10,000 were made in multiples of 10, using unheated broth and a fresh pipette for each dilution. For dilutions higher than 1–10,000 the spacing between dilutions was varied, depending upon the expected titre of a preparation.

2. Agar Streak Method of Titration.—This method was used only with carbowax-sulfur. Dilutions of carbowax-sulfur were made in broth and the proper amounts of such dilutions were added to and mixed with hot melted agar to obtain the desired concentrations of the carbowax-sulfur. When blood plates were desired 2 per cent sheep blood was added to the various dilutions of carbowax-sulfur in agar after cooling to 45°C. Appropriate controls were included.

EXPERIMENTAL

Titration by the Serial Dilution Method: Alcohol-Sulfur

Under the conditions of our tests,[3] alcohol-sulfur diluted 1–1,600 to 1–2,000 in bacto nutrient broth invariably completely inhibited the growth of *S. aureus* for 2 days at 37°C. (cultures sterile[4]), but when diluted 1–3,200 it merely delayed the growth of staphylococcus. As a basis for roughly assaying the potency of other sulfur preparations and for comparing the inhibitory action of sulfur upon various microorganisms, we have taken as one unit the volume of alcohol-sulfur necessary to inhibit completely the growth of *S. aureus* for 2 days (the quantity contained in 1/1,600 to 1/2,000 cc. of alcohol-sulfur). These alcohol-sulfur preparations therefore contained from 1600 to 2000 units per cc. In numerous samples the sulfur contained in one unit of alcohol-sulfur was found to weigh between 0.24 and 0.34 gamma.

Titration by the Serial Dilution Method: Carbowax-Sulfur

When proper precautions are taken in making the dilutions of carbowax-sulfur and in carrying out the tests, the sulfur content and the activity of a preparation run parallel. Any procedure which increases solution of sulfur in the carbowax, such as shaking the flask containing the carbowax and the sulfur during the heating period, results in higher potency of the preparation. At 90–100°C., the temperature at which carbowax-sulfur is added to the broth, a 1 per cent sulfur solution in carbowax is approximately saturated. If higher concentrations of sulfur in carbowax than this are utilized a constant proportion between sulfur content and activity may not obtain unless the temperature of carbowax-sulfur is kept high enough so that there is no precipitation of sulfur until it reaches the broth. This means that the temperature of the pipette must be well over 100° C. during the manipulations.

[3] It is emphasized that in order to obtain constant results the initial number of organisms per cc. must be between 8,000 and 15,000.

[4] No growth from one loop (0.002 cc.) inoculum.

Table I presents data on eleven different preparations of carbowax-sulfur containing between 0.25 and 2.15 per cent sulfur.[5] Except for the varying quantities of sulfur heated in the carbowax, these samples were prepared and the dilutions and titrations carried out in exactly the same manner and with the precautions we have indicated above.

The table shows that the potency of a preparation varies almost directly with the concentration of sulfur, when the sulfur content is varied between 0.25 and 2.15 per cent sulfur. Such variations as were encountered were probably due to slight uncontrollable differences in procedure of preparing the dilutions. In the eleven preparations recorded in Table I the weight of sulfur per unit ranged between 0.1 and 0.2 gamma.

TABLE I

Titration of Carbowax-Sulfur Preparations

	Sulfur per 100 gm. carbowax	Units per cc.	Sulfur per unit
	gm.		*gamma*
1	0.25	15,000	0.17
2	0.5	30,000	0.17
3	0.75	35,000	0.2
4	1	60,000	0.17
5	1.53	150,000	0.1
6	1.56	120,000	0.13
7	1.8	150,000	0.12
8	1.8	150,000	0.12
9	1.88	150,000	0.13
10	2	150,000	0.14
11	2.15	150,000	0.14

It is to be noted that the weight of sulfur per unit in carbowax-sulfur is approximately one half the sulfur per unit in alcohol-sulfur. This result may be due to a stabilizing effect of the carbowax on the sulfur particles making them more effective.

Effects of Alcohol-Sulfur and Carbowax-Sulfur upon the Growth of Various Organisms in Broth

As we have said, the carbowax-sulfur solutions due to their higher content of sulfur invariably had a much higher unitage per cubic centimeter than did the alcohol-sulfur solutions. But when a representative series of organisms were tested for inhibition by the two types of sulfur preparation, it was noted that those organisms which were most sensitive to one preparation were also most

[5] We are indebted to Dr. E. Maechling for the calculation of sulfur content in these preparations.

sensitive to the other. This is exemplified in Table II where it may be seen that *S. aureus* and *Cryptococcus hominis* were most affected and the Gram-negative organisms[6] not affected by both preparations. This correlation suggests, as we shall discuss later, that the same substance is exerting the inhibitory effect in both preparations.

TABLE II

Inhibitory Effects of Alcohol-Sulfur and Carbowax-Sulfur upon the Growth of Various Organisms in Broth

Organism	Medium	Dilutions at which inhibitory effects were observed					
		Alcohol-sulfur			Carbowax-sulfur		
		Complete*	Partial‡	None§	Complete*	Partial‡	None§
Staphylococcus aureus	Bacto nutrient	1,600	3,200	6,400	40,000	160,000	—
Staphylococcus aureus	Meat infusion	400	800	1,600	10,000	20,000	40,000
Pneumococcus Type I	Meat infusion	<100	800	1,600	1,000	10,000	20,000
Pneumococcus Type II	Meat infusion	<100	800	1,600	1,000	20,000	40,000
Pneumococcus Type III	Meat infusion	<100	800	1,600	1,000	80,000	160,000
Streptococcus hemolyticus C203	Meat infusion	<100	200	400	1,000	10,000	20,000
Corynebacterium diphtheriae	Meat infusion	<100	200	400	<1,000	1,000	10,000
Cryptococcus hominis, 3 strains	1 per cent dextrose bacto nutrient	400–800	3,200	—	40,000	160,000	—
Candida albicans	1 per cent dextrose bacto nutrient	<100	1,600	3,200	<1,000	80,000	160,000

All Gram-negative organisms tested (*Bacterium coli; Salmonella typhosa, paratyphi* A and B; *Shigella flexneri, sonnei,* and *dysenteriae; Proteus vulgaris; Pseudonomas aeruginosa*) were completely resistant to the action of both sulfur preparations in 1–100 dilution.

* No growth. Tube clear after 2 days at 37°C.

‡ Delayed growth. Tube clear or only slightly cloudy, after 1 day at 37°C., but cloudy, after 2 days at 37°C.

§ Growth like that in control tube without sulfur.

Effect of Carbowax-Sulfur upon the Growth of Various Organisms on Agar

The inhibitory effect of a carbowax-sulfur preparation was studied by the agar-streak method. This preparation contained 40,000 units per cc. The experiment (Table III) shows that, depending upon the test organism used, carbowax-sulfur in dilutions of 1–500 to 1–2,000 completely inhibited the growth of Gram-positive cocci, three anaerobic bacilli, *C. hominis*, and various derma-

[6] See note under Table II.

TABLE III

The Inhibitory Effects of Carbowax-Sulfur upon the Growth of Various Organisms on Agar

Organism	Agar medium	Dilutions at which inhibitition effects were observed		
		Complete*	Partial‡	None§
Staphylococcus aureus	Bacto nutrient	2,000	10,000	100,000
Staphylococcus aureus	2 per cent sheep blood bacto nutrient	500	—	1,000
Pneumococcus Types I, II, III	2 per cent sheep blood bacto nutrient	1,000	2,000	10,000
Streptococcus hemolyticus C203	2 per cent sheep blood bacto nutrient	500	1,000	2,000
Corynebacterium diphtheriae	2 per cent sheep blood bacto nutrient	<100	100	1,000
Clostridium histolyticum	2 per cent sheep blood bacto nutrient	250	2,000	—
Clostridium novyi	2 per cent sheep blood bacto nutrient	500	2,000	—
Clostridium welchii	2 per cent sheep blood bacto nutrient	500	2,000	—
Cryptococcus hominis, 3 strains	1 per cent dextrose bacto nutrient	2,000	—	4,000
Cryptococcus hominis	2 per cent sheep blood bacto nutrient	500–1,000	1,000	2,000
Candida albicans	1 per cent dextrose bacto nutrient	<100	100–500	1,000
Candida albicans	2 per cent sheep blood bacto nutrient	500	1,000	2,000
Microsporum canis‖	1 per cent dextrose bacto nutrient	1,000	2,000	4,000
Microsporum audouini‖	1 per cent dextrose bacto nutrient	2,000	—	4,000
Trichophyton gypseum‖	1 per cent dextrose bacto nutrient	2,000	—	4,000
Trichophyton purpurium‖	1 per cent dextrose bacto nutrient	2,000	—	4,000
Epidermophyton‖	1 per cent dextrose bacto nutrient	2,000	—	4,000

All Gram-negative organisms tested (same organisms as those in Table I) were completely resistant to carbowax-sulfur in 1–100 dilution.

* No growth.

‡ Delayed growth.

§ Growth like that in control plates without sulfur.

‖ Saline suspension of organism from growth on honey agar slant used for making broth suspensions.

The plates were read after the following periods of time: (*a*) The anaerobes, *C. hominis* and *Candida albicans*, read after 2 days at 37°C. (*b*) The dermatophytes read after 2 weeks at room temperature. (*c*) Other organisms listed read after 24 hours at 37°C.

tophytes. It had a slight inhibitory effect upon *Corynebacterium diphtheriae* and *Candida albicans*. · All Gram-negative organisms tested were completely resistant to this material in 1–100 dilution. It is apparent that the carbowax-sulfur was generally less effective in solid media than in liquid media. (Compare Tables II and III.)

Effect of the Medium on the Inhibition of the Growth of S. aureus by the Sulfur Preparations

That the kind of medium used in the tests must be considered in evaluating the action of sulfur upon a particular organism, was seen from the results of the tests with *S. aureus* (Table II). Meat infusion markedly neutralized the activity of the sulfur. In other experiments (not given in Table II), bacto nutrient broth containing 1 per cent serum (human-inactivated) or a casein digest broth with niacin and thiamin added, also reduced the potency of the sulfur preparations by one-fourth to three-fourths. However, the addition of dextrose (0.1 per cent to 2 per cent) or *p*-aminobenzoic acid (0.1 to 100 gamma per cc.) to the bacto nutrient broth did not affect the titre of a sulfur preparation. These latter experiments were carried out only with alcohol-sulfur—utilizing *S. aureus* as test organism. It was also noted that sheep blood diminished the effect of sulfur upon the growth of *S. aureus* (Table III). Further study of the effect of the constituents of the medium upon the activity of sulfur has been left for future investigation.

Mechanism of Antibacterial Action of Sulfur

We have described experiments showing that (*a*) ethyl alcohol solutions of elemental sulfur diluted with broth, and (*b*) carbowax preparations of elemental sulfur diluted with broth or incorporated in agar after dilution with broth, inhibited the growth of certain bacteria and fungi. We proceed now to a study of the nature of the substance or substances which give this antimicrobial action and the possible mechanism of such action.

Before going into the question of the nature of the inhibitory properties of elemental sulfur in our preparations, we first excluded the possibility that an impurity existed in the sulfur preparations used (U.S.P. flowers of sulfur) which might be responsible for its activity. Tests by the serial dilution method in broth were set up with alcohol-sulfur preparations made from sulfur samples treated in the following ways: (1) crystallized five times from chloroform solutions, (2) crystallized five times from absolute alcohol solutions and, (3) sublimed.[7] These three preparations of alcohol-sulfur contained approximately 1600 units per cc. which was the unitage given by the unpurified flowers of sulfur preparations. We also found that alcohol-sulfur made separately from rhombic or monoclinic crystals obtained from the fifth crystallization from

[7] We are indebted to Dr. Hans Clarke for this preparation.

chloroform had the same 1,600 units per cc. These experiments appeared to justify the belief that the activity of the sulfur solutions was due to the sulfur itself and not to an impurity in the sulfur.

Concerning the nature of the inhibitory substance and the part the sulfur plays in it, one must ask the following questions:—

1. Is the sulfur in monomolecular dispersion or are larger sulfur particles responsible for the activity?

2. Must the sulfur be combined with some component of the broth to have activity? If so, what is this substance?

3. Is there any evidence suggesting that the two preparations, alcohol-sulfur and carbowax-sulfur, contain different antibacterial compounds?

The following evidence indicates that the activity of sulfur is dependent on fairly large aggregates of sulfur rather than on sulfur molecules dispersed in a true solution.

(*a*) When an alcohol-sulfur solution containing 1,600 units per cc. was diluted 1–10 with broth, a constant turbidity developed. If such a turbid preparation was centrifuged at 1,000 R.P.M. for 40 minutes a clear supernatant resulted containing less than 100 units per cc. and the bulk of the sulfur was in the sediment. (Activity of less than 100 units per cc. could not be measured because a concentration of alcohol greater than 1 per cent was inhibitory to the growth of *S. aureus*.) When the sedimented sulfur was redispersed by vigorous shaking the preparations again became cloudy but the sulfur particles separated out more quickly than those in the original suspension and they had only slight activity.

(*b*) A similar result was obtained by allowing a 1–10 broth dilution of alcohol-sulfur to stand undisturbed for 5 days at room temperature or for 2 days at 37°C., *viz.* the supernatants were clear and inactive and the preparations, when the tubes were shaken to redisperse the sediments, were only slightly active.

(*c*) When the alcohol-sulfur diluted 1–10 with broth was passed through a Berkefeld V filter the filtrate was clear and inactive.

(*d*) When carbowax-sulfur was centrifuged or allowed to settle as in (*a*) and (*b*) or passed through a Berkefeld V filter, as in (*c*) similar results were obtained, that is, loss of activity.

Nothing so far discussed has ruled out the possibility that the sulfur combines in some way with a component of the broth and that this combination is the active principle. Indeed, the following evidence suggests that such is the case. We have seen above that the activity of alcohol-sulfur diluted 1–10 with broth disappears when such a preparation is passed through a Berkefeld V filter. On the other hand when alcohol-sulfur was diluted 1–10 with distilled water, the Berkefeld filtrate was cloudy and the activity was retained (1,600 units per cc.). This experiment thus suggests that sulfur particles are held by adsorption or by actual combination to something in the broth. Strangely enough, the

carbowax-sulfur-water dilution did not retain its activity when passed through the Berkefeld filter. Perhaps this was due to the viscosity of the carbowax at the temperature at which it was filtered.

Another fact which suggests that sulfur combines with something in the medium before it can exert antibacterial action is that the activity varies considerably depending on the medium. (See Table II and III and previous dis-

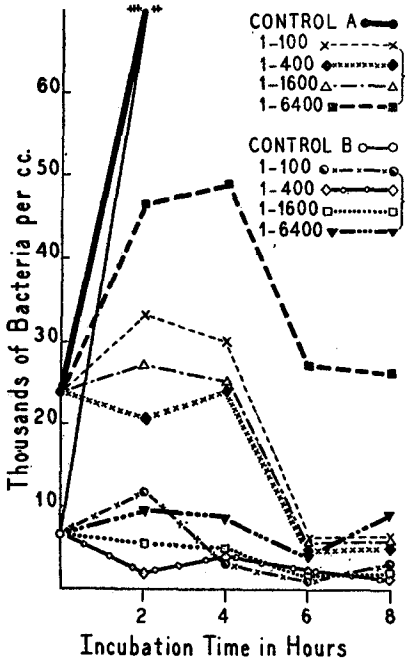

FIG. 1

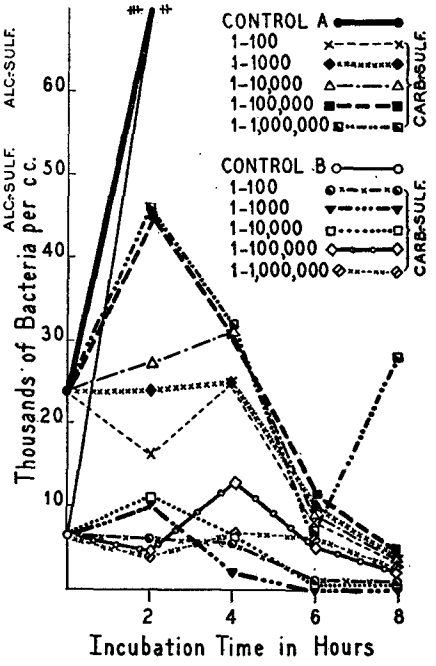

FIG. 2

FIGS. 1 and 2. Comparative inhibitory effects of alcohol-sulfur and carbowax-sulfur on the growth of *S. aureus*.

FIG. 1. Inhibitory effects of alcohol-sulfur. *A*, inoculum 24,000 organisms. *B*, inoculum 7,000 organisms.

FIG. 2. Inhibitory effects of carbowax-sulfur. *A*, inoculum 24,000 organisms. *B*, inoculum 7,000 organisms.

cussions.) At present we have no knowledge of the substance which might combine with the sulfur nor of the type of combination, whether physical or chemical, that may occur.

Evidence which indicates that the two preparations of sulfur have a similar mode of action and that such action probably depends on sulfur in the same form follows:—

(*a*) Except for the greater potency of carbowax-sulfur as compared to al-

cohol-sulfur, the two preparations have essentially similar antibacterial spectra (Table II).

(*b*) The activity of both preparations seems to depend on a visible dispersion of sulfur particles in the medium.

(*c*) A comparison of the effect of these two preparations of sulfur on the growth curves of *S. aureus* shows remarkable similarity (Figs. 1 and 2).[8] In this experiment, the effects of different dilutions of alcohol-sulfur (Fig. 1) and carbowax-sulfur (Fig. 2) upon the growth of *S. aureus* are compared. Each test dilution was run with inocula of two different sizes: A, 24,000 organisms, and B, 7,000 organisms. The figures from which the graphs were made represent the average of two separate counts. It is seen that the graphs show a striking similarity between the two sets of curves (Figs. 1 and 2) especially when the smaller inoculum of organisms (B) was used. It is also seen that for the same inoculum, although a wide range of concentrations of the preparation of sulfur was used (dilutions of 1–100 to 1–6,400 for alcohol-sulfur, and dilutions of 1–100 to 1–1,000,000 for carbowax-sulfur), the high and the low dilutions give essentially similar curves. All curves show bacteriostasis with no definite bactericidal effect appearing in this time (8 hours).[9] The wide range of bacteriostatic action, confirmed many times, is a distinguishing characteristic of alcohol-sulfur and carbowax-sulfur. This phenomenon has not been encountered in similar experiments with other antibacterial agents.[10]

This last experiment (Figs. 1 and 2) seems to indicate that the same principle of action governs the activity of alcohol-sulfur and carbowax-sulfur. If an antibacterial substance other than the sulfur particles were produced in the development of carbowax-sulfur one would expect some definite variation between the two sets of curves.

[8] A simple method of estimating bacterial growth by spreading 1 loopful of each test culture over an agar plate and counting the resulting colonies was used in this experiment (Figs. 1 and 2). A 3½ hour culture of known count made the previous day was diluted with broth so as to contain 480,000 (A) or 140,000 (B) organisms per cc. Then 0.05 cc. of each culture dilution was added separately to all the test fluids which contained varying amounts of either alcohol-sulfur or carbowax-sulfur, the volume of each test fluid being 1 cc. At the time intervals noted in the figures, one loopful from each test culture was removed and spread over an agar plate. The resulting colonies were counted after 18 hours' growth at 37°C. Since our standard loop holds 0.002 cc. of broth, the counts thus obtained were multiplied by a factor of 500 to convert them to bacteria per cubic centimeter and thus converted are shown in the figures.

We are indebted to Miss Natalie Pearlstein for the preparation of the figures.

[9] *Results after 8 hours:*

1. *Alcohol-sulfur:* Sterile after 24 to 48 hours in dilutions of 1–100 to 1–1,600 with both inocula A and B. Growth after 24 hours in dilution 1–6,400 with both inocula A and B.

2. *Carbowax-sulfur:* Sterile after 24 to 48 hours in dilutions of 1–100 to 1–100,000 with inoculum A and in dilutions of 1–100 to 1–1,000,000 with inoculum B. In the dilution of 1–1,000,000 with inoculum A, there were 10,000 organisms per cc. after 48 hours' incubation.

[10] Data to be published.

DISCUSSION

The foregoing results from the use of alcohol-sulfur and carbowax-sulfur, seem to justify the conclusion that in these experiments the inhibitory action of sulfur is due to the finely divided particles of sulfur, suspended in the nutrient medium, alone or in combination with some component of the medium. That insolubility may be a factor in the antibacterial action of other substances has been previously reported (16, 17).

The mechanism by which the particles of sulfur inhibit the growth of various organisms is a separate problem. There are many conceivable ways in which they might operate. For instance, they might block the enzyme systems of the organisms, and in so doing prevent division and growth; or they might react chemically with the lipoids of the cells (18) or with substances in the medium, resulting in the production of sulfur-containing compounds toxic to the organism; or they might make some nutrient in the medium unavailable to the organism. This problem; *i.e.*, the process by which the particles of sulfur cause inhibition is to be taken up in further study.

SUMMARY

1. Saturated solutions of sulfur in alcohol (alochol-sulfur) when diluted with broth are inhibitory to the growth of various Gram-positive bacteria and to *C. hominis*. By an arbitrary method of unitage with *S. aureus* as the test organism, our alcohol-sulfur contains 1,600 to 2,000 units per cc. and one unit contains between 0.24 and 0.34 gamma sulfur. The activity of a preparation is in general directly proportional to its sulfur content.

2. Solutions of sulfur in carbowax (carbowax-sulfur) when diluted with broth are likewise inhibitory to the growth of various Gram-positive bacteria and to *C. hominis*. When *S. aureus* is used as test organism, 1 unit contains between 0.1 and 0.2 gamma sulfur. The activity of these preparations is also in general directly proportional to their sulfur content.

3. Carbowax-sulfur when incorporated in agar in 1–500 to 1–2,000 dilution inhibits the growth of various Gram-positive aerobic and anaerobic bacteria, *C. hominis*, and certain dermatophytes.

4. Our experiments appear to show that both alcohol-sulfur and carbowax-sulfur owe their inhibitory properties to the sulfur particles that are dispersed throughout the medium when these sulfur preparations are diluted with broth. The inhibitory effect of these particles may or may not be due to a combination of the sulfur particles with substances in the medium in which they are suspended.

5. Evidence suggests that the activity of both alcohol-sulfur and carbowax-sulfur is due to sulfur in the same form. The inhibitory effect is characterized by prolonged bacteriostasis with similar activity over a wide range of dilutions.

There is no evidence of true bactericidal action even with the highest concentrations used.

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