THE GERMICIDAL ACTIVITY OF VAPORS FROM IRRADIATED OILS

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Received for publication, October 7, 1931

It has been found that surface smears of various microörganisms are killed when exposed to the atmosphere over certain oils previously treated with ultraviolet light.

A brief survey of the literature indicates some reports on the factors affecting the germicidal power of oils, but only a few papers dealing with emanations of a germicidal nature from oils. This survey also indicates a tendency to ascribe to invisible light the effects caused by any unseen emanations. Thus Reid (1930) explains a germicidal effect of irradiated oil vapor as due to secondary radiations from that oil. We consider that this explanation is not justified, and in this paper present our reasons for a different explanation of the same phenomenon.

LITERATURE REVIEW

Schmidt (1908) thought he observed a radiation effect when he exposed photographic plates to linseed oil and got a screening action by placing a diaphragm of brass, glass or mica between the oil and the plate. Celluloid, paper, gutta-percha and gelatin allowed the fogging material to pass through. Kugelmass and McQuarrie (1924), in work which was later confirmed by Hauxthausen (1925) concluded that the fogging effect of irradiated substances was undoubtedly due to ultraviolet rays emitted from oils. This conclusion has not been substantiated by other investigators and there seems to be almost complete agreement that the fogging effect is due to some volatile chemical compound.

Malowan (1931) found that those oils which contain aldehydes and alcohols rather than esters, are the most active germicidally. There are numerous papers on the germicidal activity of oils, especially essential oils (Gatti and Coyola (1922); Wiechowski (1926); Morel and Rochaix (1927); Anderson and Leitch (1927); Morel, Rochaix, and Sevelinge (1928); Kurgery and Adkisson (1928); Obst (1929)), but very few deal with the effects of the vapors evolved from these oils. Spolverini (1927) has written on the therapeutic value of inhalations of irradiated oils. Rhodes (1925) reported that the successful treatment of tuberculous abscesses with cod liver oil was possibly due to ultraviolet emanations from the oils. Wrenn (1927) exposed bacterial plates to irradiated oil vapors and observed bactericidal action in several cases. He drew the conclusion that this effect was due to light emanations in spite of the fact that he was unable to demonstrate the passage of these emanations through quartz glass. Schöbl and Kusama (1924) placed organisms near oils so that only the volatile constituents at 37°C. and not the oils, came in direct contact with the cultures. They found that the vapors of oils containing acids of the chaulmoogra series have no germicidal power and that the volatile constituents of certain essential oils are highly germicidal to acid-fast bacteria *in vivo*.

PRELIMINARY TESTS

Ten cubic centimeters of Patch's cod liver oil were placed in a Petri dish and irradiated for two hours at a distance of 75 cm. from a 6-inch horizontal quartz-mercury vapor arc. A surface smear of *Staph. aureus* on nutrient agar (Difco) was inverted above this irradiated oil and also above a 10 cc. sample of unirradiated oil and incubated at 37°C. At the end of twenty-four hours there was growth on the agar placed above the unirradiated cod liver oil but there was no growth on that over the irradiated oil. This experiment was repeated with a 4 mm. quartz plate placed between the sample of irradiated oil and the agar plate. There was growth on the agar after incubation. This clearly shows that the bactericidal substance was unable to pass through a quartz plate. It is logical to assume therefore that the sub-

stance evolved from cod liver oil is a chemical substance rather than a radiation as has heretofore been assumed.

This fact was demonstrated in still another way. A series of Petri dishes, each smaller than the one preceding it, were placed inside one another alternately inverted. Each stood on glass rods so that there was free air passage from the innermost dish containing the oil to the external inverted dish on which the smeared agar had been placed. After incubation, there was no bacterial growth on this plate. The germicidal substance had traveled from the interior outward over a devious path. The germidical effect, therefore, could not be due to a radiation, for radiations travel in straight lines.

BACTERIOLOGICAL TESTS

A series of oils was tested against *Staph. aureus* to determine how long each had to be irradiated before it became germicidal. All tests were run in duplicate. Five cubic centimeters of each sample were irradiated in the bottom of a Petri dish at a distance of 45 cm. from the arc. One-half cubic centimeter of a 1:100 dilution (750,000) of *Staph. aureus* was placed in the center of each agar plate and the plate tipped to insure complete surface covering. These agar-aureus plates were inverted over the irradiated oils and the two dishes sealed with adhesive tape. After incubation at 37°C. for twenty-four hours the plates were observed for growth. The bottom dish containing the oil was removed from those samples which showed no growth on the agar, replaced by a clean, sterile dish, and the culture again incubated. If there was no growth after twenty-four hours further incubation, the culture was considered killed. If there was growth after the second incubation, the organism was considered inhibited rather than killed.

From table 1 it is apparent that tuna fish, seal and high fatty-acid fish oils are germicidal to *Staph. aureus* without irradiation. Cod, cod liver, elast, L. C. P. fish, linseed, perilla, sardine, walnut, and whale oil give off germicidal vapors only after irradiation of less than two hours. The remaining oils are impotent in this respect even after four hours of irradiation.

A medium that was suitable for the growth of the organism employed was used in all cases. Nutrient broth and nutrient

TABLE 1

Effect of irradiation time on germicidal power of oil vapors toward Staph. aureus

OIL	TIME OF IRRADIATION OF OIL										
	0 minute	5 minutes	10 minutes	15 minutes	30 minutes	45 minutes	60 minutes	75 minutes	90 minutes	120 minutes	240 minutes
Chaulmoogra	++	++	++	++	++	++	++	++	++	++	++
Cocoanut	++	++	++	++	++	++	++	++	++	++	++
Cod	++	++	++	++	++	−	−	−	−	−	−
Cod liver (Murray)	++	++	++	−	−	−	−	−	−	−	−
Cod liver (Patch)	++	++	++	−	−	−	−	−	−	−	−
Cod liver (Tech)	++	++	++	−	−	−	−	−	−	−	−
Elastoil-D	++	++	++	++	++	++	++	−	−	−	−
Elastoil-R	++	++	++	++	++	++	++	−	−	−	−
Fish (fatty acid)	−	−	−	−	−	−	−	−	−	−	−
Fish (L. C. P.)	++	+	+	−	−	−	−	−	−	−	−
Lime	++	++	++	++	++	++	++	++	++	++	++
Linseed	++	++	++	++	++	++	++	−	−	−	−
Mineral	++	++	++	++	++	++	++	++	++	++	++
Mustardseed	++	++	++	++	++	++	++	++	++	++	++
Oleic acid (U. S. P.)	++	++	++	++	++	++	++	++	++	++	++
Olive	++	++	++	++	++	++	++	++	++	++	++
Orange	++	++	++	++	++	++	++	++	++	++	++
Palmetto	++	++	++	++	++	++	++	++	++	++	++
Palm kernel	++	++	++	++	++	++	++	++	++	++	++
Peanut	++	++	++	++	++	++	++	++	++	++	++
Perilla	++	++	++	++	++	++	++	+	−	−	−
Poppyseed	++	++	++	++	++	++	++	++	++	++	++
Rapeseed	++	++	++	++	++	++	++	++	++	++	++
Sardine	++	+	+	+	−	−	−	−	−	−	−
Seal	−	−	−	−	−	−	−	−	−	−	−
Soyabean	++	++	++	++	++	++	++	++	++	++	++
Sperm (crude)	++	++	++	++	++	++	++	++	++	++	++
Sunflower	++	++	++	++	++	++	++	++	++	++	++
Tuna	−	−	−	−	−	−	−	−	−	−	−
Tung	++	++	++	++	++	++	++	++	++	++	++
Walnut	++	++	++	++	++	++	++	++	++	++	−
Whale	++	+	+	−	−	−	−	−	−	−	−

++, normal growth; +, growth only after the removal of oil; −, no growth.

agar were used for *Strep. lactis, Staph. pyogenes-aureus, Sar. lutea, Esch. coli, Eb. typhi, B. subtilis* and *Serratia marcescens.*

Glucose broth and glucose agar were employed with *Strep. lactis* and *Cl. sporogenes*. Czapek's media, both broth and agar, were used for *Asp. niger*. Malt broth and malt agar were used with *Sacch. anomolous*.

Each organism was grown at its optimum temperature. *Strep. epidemicus*, *Strep. lactis*, *Staph. pyogenes-aureus*, *Esch. coli*, *Eb. typhi*, *B. subtilis* and *Cl. sporogenes* were grown at 37.5°C. *Asp. niger* and *Sacch. anomolous* were incubated at 30°C.

TABLE 2

Effect of oil vapors on typical bacteria and fungi

ORGANISM	TIME OF IRRADIATION OF OIL						
	0 minute	5 minutes	10 minutes	15 minutes	20 minutes	25 minutes	45 minutes
I. Cod liver oil							
Strep. epidemicus	++	++	++	−	−	−	−
Strep. lactis	++	++	++	−	−	−	−
Staph. pyogenes-aureus	++	++	++	−	−	−	−
Sar. lutea	++	++	++	−	−	−	−
Esch. coli	++	++	++	−	−	−	−
Eberthella typhi	++	++	+	−	−	−	−
B. subtilis	++	++	++	+	−	−	−
Cl. sporogenes	++	++	++	++	+	−	−
Serratia marcescens	++	++	++	−	−	−	−
Asp. niger	++	++	++	−	−	−	−
Sacch. anomolous	++	++	+	−	−	−	−

	20 minutes	25 minutes	45 minutes	60 minutes	75 minutes	90 minutes	120 minutes
II. Linseed oil							
Strep. epidemicus	++	++	++	++	−	−	−
Strep. lactis	++	++	++	++	−	−	−
Staph. pyogenes-aureus	++	++	++	++	−	−	−
Sar. lutea	++	++	++	++	+	−	−
Esch. coli	++	++	++	++	+	−	−
Eberthella typhi	++	++	++	++	−	−	−
B. subtilis	++	++	++	++	++	+	−
Cl. sporogenes	++	++	++	++	++	+	−
Serratia marcescens	++	++	++	++	−	−	−
Asp. niger	++	++	++	++	−	−	−
Sacch. anomolous	++	++	++	+	−	−	−

III. Perilla oil

	25 minutes	45 minutes	60 minutes	75 minutes	90 minutes	120 minutes	240 minutes
Strep. epidemicus	++	++	++	−	−	−	−
Strep. lactis	++	++	++	−	−	−	−
Staph. pyogenes-aureus	++	++	++	−	−	−	−
Sar. lutea	++	++	++	+	−	−	−
Esch. coli	++	++	++	−	−	−	−
Eberthella typhi	++	++	++	−	−	−	−
B. subtilis	++	++	++	++	+	−	−
Cl. sporogenes	++	++	++	++	+	−	−
Serratia marcescens	++	++	++	−	−	−	−
Asp. niger	++	++	++	−	−	−	−
Sacch. anomolous	++	++	+	−	−	−	−

IV. Oleic acid, U. S. P.

	25 minutes	45 minutes	60 minutes	75 minutes	90 minutes	120 minutes	240 minutes
Strep. epidemicus	++	++	++	++	++	++	++
Strep. lactis	++	++	++	++	++	++	++
Staph. pyogenes-aureus	++	++	++	++	++	++	++
Sar. lutea	++	++	++	++	++	++	++
Esch. coli	++	++	++	++	++	++	++
Eberthella typhi	++	++	++	++	++	++	++
B. subtilis	++	++	++	++	++	++	++
Cl. sporogenes	++	++	++	++	++	++	++
Serratia marcescens	++	++	++	++	++	++	++
Asp. niger	++	++	++	++	++	++	+
Sacch. anomolous	++	++	+	+	+	+	+

++, normal growth; +, growth only after the removal of oil; −, no growth.

Cl. sporogenes was grown "anaerobically" in glucose broth by sealing the tube with sterile vaseline. When exposed to the vapor of the oil it was grown in a Spray culture dish, alkaline pyrogallol being used to produce anaerobiosis. The irradiated oil was placed in a separate dish inside the Spray culture dish.

In a paper which will be published shortly the cause of this germicidal action of irradiated oil vapors will be explained as being very likely due to a peroxidic-oxygen compound and data to prove this contention will be presented.

CONCLUSIONS

1. It has been shown that the vapors of certain oils inhibit or kill bacterial smears on nutrient agar.

2. Certain oils exhibit this property without irradiation with ultraviolet light, whereas others show it only after such treatment.

3. As shown elsewhere this germicidal action has been demonstrated as being due presumably to volatile substances containing peroxidic-oxygen, the formation of which is accelerated by ultraviolet irradiation.

4. *Bacillus subtilis* and *Clostridium sporogenes*, as was expected, were more resistant to this treatment.

5. In general, animal oils are more active in this respect than vegetable oils.

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