THE BACTERICIDAL AND PHOTOCHEMICAL PROPERTIES OF IRRADIATED COD LIVER OIL AND AN OZONIDE OF OLIVE OIL

FRANKLIN A. STEVENS

Department of Medicine, College of Physicians and Surgeons, Columbia University and the Presbyterian Hospital, New York City

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The bactericidal and photochemical properties of oils exposed to ultraviolet light have been studied by many investigators. Both these effects were, at first, attributed to invisible light rays but recent experimentation has shown that bacteria are killed and photographic films are fogged by vapor from the oils (references in bibliography). Following the observation of Proctor and Milas (1931) that germicidal peroxides were formed by the exposure of certain substances to ultraviolet light, Harris, Bunker and Milas (1932) studied the peroxide content and the bactericidal effect of vapors from various irradiated oils. They concluded that the germicidal effect of the emanations from these irradiated oils was due to substances of a gaseous nature composed partly of volatile peroxides. Recent studies show that bactericidal peroxides occur in irradiated cod liver oil and in some irradiated essential oils (Stevens, 1936 a and b). In the following experiments the bactericidal properties of irradiated cod liver oil have been further investigated, the germicidal effects of an ozonide of olive oil have been studied and the bactericidal and photochemical properties of the vapors from these oils have been correlated.

BACTERICIDAL EMULSIONS OF ULTRAVIOLET-IRRADIATED COD LIVER OIL

In previous experiments it was found that peroxides were the only bactericidal substances in emulsions of irradiated cod liver

oil (Stevens 1936 a). These peroxides adhered to bacteria suspended in the emulsions and their bactericidal action was continued after the bacteria were removed from the emulsions and resuspended in dilute reducing solutions. No attempt was made to study the effect on a variety of bacteria or to determine the optimum period of irradiation. In the following experiments the bactericidal properties of emulsions of cod liver oil irradiated for 3, 6, 9, 12 and 15 hours have been determined. The germicidal effect of the emulsions on the common upper respiratory bacteria has been investigated. Emulsions made with salt solution have also been compared with emulsions made with a buffered phosphate mixture. This comparison was necessary because the emulsions made in salt solution were quite acid.

Experiments I and II. The bactericidal properties of emulsions of cod liver oil irradiated with ultraviolet light

A commercial sample of cod liver oil was distributed among several large Petri dishes to a depth of 0.5 cm. These dishes were placed under a mercury-quartz-arc at a distance of 20 inches. At the end of 3, 6, 9, 12 and 15 hours two samples of 4 cc. each were removed and placed in sterile bottles. To one of these samples, 16 cc. of sterile salt solution were added. To the second were added 16 cc. of sterile buffered phosphate mixture (KH_2PO_4, Na_2HPO_4, M/15, pH 7.4). The bottles were covered with rubber caps, shaken one hour in a machine, then inverted and allowed to stand overnight in the cold to permit the unemulsified oil to separate.

Three strains of hemolytic streptococcus (273, C 203, and NY V) and two strains of *Staphylococcus aureus* (S and E) were grown for 18 hours in proteose peptone broth. Nine cubic centimeters of each of these cultures were used to test the bactericidal effect of oil irradiated for 3, 6, 9, 12 and 15 hours and emulsified either in salt solution or in phosphate mixture. Nine cubic centimeters of each culture were centrifuged and the bacterial sediment was suspended in 3 cc. of the respective emulsions aspirated through the rubber cap without disturbing the supernatant layer of oil. The suspensions were cultured at hourly intervals on blood agar plates. The control cultures in emulsions of normal oil have been

TABLE 1

The bactericidal properties of emulsions of irradiated cod liver oil

IRRADIATION	STRAIN				
	273 (H. S.)	N. Y. V. (H. S.)	C 203 (H. S.)	Staphylococcus S	Staphylococcus E
Emulsified in salt solution					
hours					
3	D 22 hr.	D 22 hr.	D 22 hr.	L 72 hr.	L 72 hr.
6	S 6 D 22	D 22	S 6 D 22	L 72	S 48 D 56
9	S 5 D 6	S 5 D 6	S 5 D 6	D 30	S 22 D 30
12	S 5 D 22	S 4 D 5	S 4 D 5	D 30	
15	D 4	D 4	D 4		
Emulsified in phosphate solution, M/15, pH 7.4					
3	D 22	D 22	D 22	L 46	L 46
6	S 6 D 8	S 8 D 22	S 8 D 22	D 22	D 30
9	S 6 D 7	S 6 D 7	S 5 D 8	D 22	D 22
12	S 5 D 7	S 5 D 6	S 5 D 6	D 22	S 5 D 8

TABLE 2

The bactericidal effects of emulsions of cod liver oil on upper respiratory tract bacteria

	STRAIN							
	Pneumococcus I	Pneumococcus II	Pneumococcus III	Pneumococcus bacillus	*Streptococcus viridans*	*Staphylococcus aureus*	*Micrococcus catarrhalis*	Hemolytic streptococci
Normal oil emulsified in phosphate mixture	D 30 hr.	D 30 hr.	S 22 hr. D 30 hr.	L 78 hr.	L 30 hr.	L 78 hr.	D 60 hr.	L 48 hr.
Irradiated oil emulsified in salt solution	D 1	D 1	S 1 D 2	S 1 D 2	S 2 D 3	D 4	D 2	S 5 D 8
Irradiated oil emulsified in phosphate mixture	S 2 D 3	D 3	D 3	D 2	S 4 D 5	S 7 D 8	D 4	S 5 D 7

omitted. These were all living at the end of 48 hours. The results of these cultures are to be found in table 1. In this table L indicates an abundant growth, S scant growth, and D no growth.

In experiment II, the bactericidal properties of emulsions of oil irradiated for 12 hours were studied. The common upper respiratory pathogenic bacteria were used in these experiments (table 2).

THE BACTERICIDAL PROPERTIES OF AN OZONIDE OF OLIVE OIL

While irradiation renders most fish oils bactericidal by increasing the peroxide content of the oils, most vegetable oils are

TABLE 3

The bactericidal properties of an ozonide of olive oil

	BACTERIUM					
	Pneumococcus	Friedlander bacillus	Hemolytic streptococci	*Micrococcus catarrhalis*	*Streptococcus viridans*	*Staphylococcus aureus*
Number of bacteria	25[9]	50[9]	80[9]	50[9]	80[9]	110[9]
Salt solution emulsion O = 0.005 gram per 100	S 3 hr. D 4	D 18 hr.	D 18 hr.	S 4 hr. D 8	D 4 hr.	L 30 hr.*
Ozonide						
O = 0.03 gram per 100	D 3 hr.	L 7 hr.*	D 4 hr.	D 3 hr.	L 7 hr.*	L 24 hr.*
O = 0.06 gram per 100	D 2 hr.	D 7 hr.	D 3 hr.	D 10 min.	D 4 hr.	D 5 hr.
O = 0.13 gram per 100	D 2 hr.	D 4 hr.	D 1 hr.	D 4 min.	D 30 min.	D 5 hr.
O = 0.26 gram per 100	D 20 min.	D 1 min.	D 1 min.	D 1 min.	D 15 min.	D 4½ hr.
O = 0.52 gram per 100	D 2 min.	D 1 min.	D 1 min.	D 1 min.	D 3 min.	D 3½ hr.
Emulsion in serum	D 6 hr.	S 6 hr. D 20 hr.	D 6 hr.	D 6 hr.	S 7 hr. D 20 hr.	D 40 hr.

* Culture discontinued.

not so affected by this treatment. Irradiation has little or no effect on olive oil but by ozonization of the oil oxygen can be added to the double bond. Harada (1934) prepared ozonides of olive oil and found them fungicidal. In the following experiment the bactericidal effects of an ozonide of olive oil have been investigated (experiment III). The effect of suspending bacteria in emulsions of ozonide in salt solution and in diluted serum have been studied. The bacteria were also suspended in the ozonide diluted in mineral oil so that it contained varying percentages of available oxygen.

Experiment III. The bactericidal properties of olive oil ozonide

A sample of ozonide of olive oil 24 months old was used in these experiments. This oil originally contained 1.3 grams per cent of available oxygen, of which it had lost approximately 30 per cent. A few cubic centimeters of this oil were diluted with mineral oil so that the mixture contained 0.3 gram per cent of oxygen. One part of the mixture was shaken for one hour in a shaking machine with isotonic phosphate mixture (pH 7.4). The rubber-capped flask containing the emulsion was then inverted in the cold overnight. Cultures of pneumococcus, Friedlander bacillus, hemolytic streptococcus, *Micrococcus catarrhalis*, *Streptococcus viridans* and *Staphylococcus aureus* were grown eighteen hours. Six cubic centimeters of these cultures were centrifuged and the sediment was suspended in 2 cc. of the emulsion withdrawn through the cap of the inverted flask. Cultures of these suspensions were made at frequent intervals on blood agar plates.

In the second part of the experiment the sediment from 6 cc. of culture was suspended in 2 cc. of mixtures of ozonide of olive oil with mineral oil containing varying percentages of available oxygen. At intervals these suspensions were cultured in broth. After incubation these broth cultures were inoculated on plates.

In the third part of the experiment ozonide diluted to contain 0.5 gram per cent of available oxygen was shaken with rabbit serum, diluted twice with salt solution. The bactericidal effect of the resulting emulsion was studied.

THE CORRELATION OF THE FOGGING AND BACTERICIDAL EFFECTS OF THE VAPOR FROM IRRADIATED COD LIVER OIL AND AN OZONIDE OF OLIVE OIL

The emanation from irradiated oils which fogs photographic plates is evidently of a gaseous nature because, while it can penetrate films of aceto-cellulose (Schmidt, 1908), glass, metal or quartz interposed between the oil and the plates abolish the effect. The vapor arising from irradiated oils contains both peroxides and aldehydes (Stevens, 1935). In previous experiments the peroxides in irradiated oils were shown to be the only bactericidal substances formed as a result of irradiation. Chemi-

cals which destroyed the peroxides rendered the irradiated oils non-bactericidal. In the following experiment irradiated oils and ozonides of olive oil have been treated with both organic and inorganic chemicals to reduce the peroxides and ozonides. The vapor from these irradiated oils no longer fogged plates.

Experiment IV. The photochemical effects of vapor from irradiated cod liver oil, mineral oil, and an ozonide of olive oil

Chemicals destructive to peroxides were added directly to the oils in this experiment and the fogging effect of the vapor from the treated oils was studied. Normal mineral oil which did not fog plates was used as a diluent and as a vehicle to test the fogging effect of the chemicals used. Five sets of tests were done. The first was a control set of tests in mineral oil. To each of three 5 cc. lots of normal oil were added 1 gram of cysteine, cystine, and alanine respectively. One-half gram of powdered KI and a few drops of glacial acetic acid were added to a fourth sample. The KI and acetic acid added to a fifth tube were neutralized by adding 0.5 gram of $CaCO_3$ a few hours previous to testing the vapor; 0.5 gram of I was added to a sixth tube. After a few days 1 cc. samples of each of these treated oils were placed in small dishes with ground glass lips and photographic plates were inverted over the dishes. The plates were developed after 24 hours. In a second series of tests the chemicals mentioned above were mixed with cod liver oil which had been irradiated for 12 hours. These mixtures were shaken frequently for a week. In a third series the cod liver oil was allowed to stand in contact with the chemicals for two weeks before the photochemical qualities of its vapor were tested. A fourth series of tests was done with ozonide of olive oil diluted with mineral oil so as to contain 0.1 gram per 100 of available oxygen. The ozonide was allowed to stand for one week before testing. In a fifth series irradiated mineral oil was used. The effect of adding these chemicals to the oils has been charted in table 4 in which the degrees of blackening caused by the vapor from the treated oils have been indicated by + signs.

In the previous experiment (experiment IV) the fogging effect

of the vapor from irradiated mineral oil and cod liver oil was affected only by the addition of cysteine and of acetic acid and potassium iodide which abolished the effect after a few days' contact with the oils. While the potassium iodide and acetic acid destroyed the fogging properties of the vapor from the cod liver oil within a week, the oil required two weeks in contact with cysteine before photographic plates were not affected. The initial blackening effect of the cod liver oil was much more intense and the concentration of peroxides, much greater than in the mineral oil, probably accounting for the greater length of time necessary for the completion of the reducing effect of the cysteine. While neither cystine, alanine, nor iodine affected

TABLE 4

The photochemical effects of vapor from irradiated oils and ozonide of olive oil

VARIETY OF OIL	IRRADIATION	DURATION OF CONTACT	UNTREATED OIL	OIL AND CYSTEINE	OIL AND CYSTINE	OIL AND ALANINE	OIL, KI, AND ACETIC ACID	OIL, KI, ACETIC AND $CaCO_3$	OIL AND IODINE
	hours	*days*							
Mineral oil.....		7	−	−	−	−	±	−	−
Mineral oil.....	8	7	+	−	+	+	±	−	+
Cod liver oil.....	12	7	++++	++	++++	++++	±	−	++++
Cod liver oil.....	12	14	++++	−	++++	++++	±	−	++++
Ozonide of olive oil...		7	++++	−	++	+++	±	−	+++

the plates or interfered with the photochemical action of the vapor from the mineral oil or cod liver oil, they definitely affected the ozonide, suggesting that it was a more active oxidizing agent than the irradiated oils. Various degrees of blackening of the plates were obtained by the addition of cysteine, cystine and alanine to the ozonide so that it has been possible to compare different degrees of fogging with the bactericidal effect on *Staphylococcus aureus*. One cubic centimeter samples of the ozonide which had been treated with these amino acids were placed in dishes similar to those used in testing the effect of the vapor on photographic plates. One cubic centimeter of nutrient agar was poured into a second set. This agar when cooled was sprayed

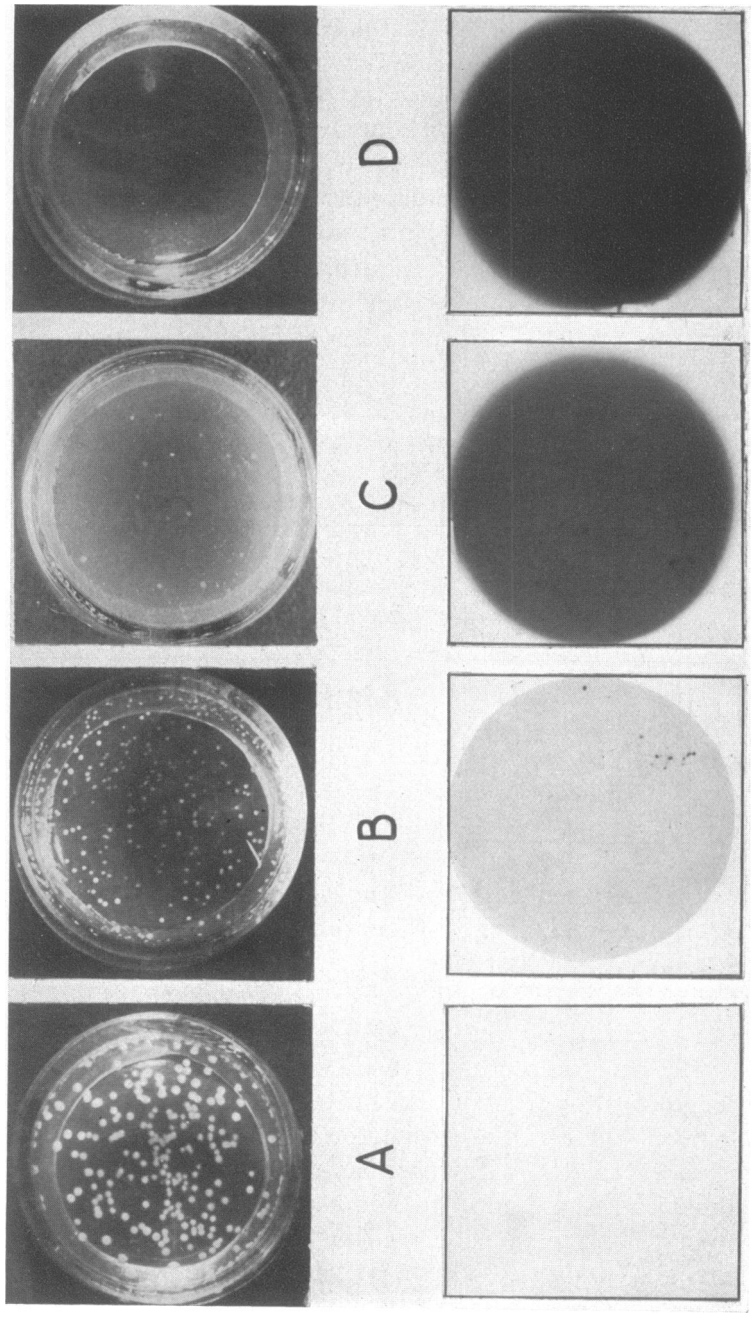

FIG. 1. COMPARISON OF FOGGING AND BACTERICIDAL EFFECTS OF VAPOR FROM OZONIDES OF OLIVE OIL

A, in contact with cysteine; *B*, in contact with cystine; *C*, in contact with alanine; *D*, ozonide in mineral oil; (0.1 gram per 100 available O).

with a culture of *Staphylococcus aureus* diluted so that the colonies would be discrete. These seeded agar dishes were inverted over the dishes of oil and the junction sealed with tape. After twenty-four hours' incubation the colonies developing on these dishes were photographed and compared with the degree of blackening obtained with similar samples of oil. The photographs of the seeded plates and the degrees of blackening obtained with the corresponding samples of oil have been reproduced in figure 1. A comparison of the fogging and bactericidal effects obtained in this experiment shows that they are quantitatively related. The rate of growth of the bacteria is also affected, since the colonies on the plates exposed to vapor from oils which fogged the plates more intensely are smaller. That the growth rate of bacteria might be affected by suspending them for a short time in emulsions of irradiated cod liver oil in salt solution was observed in some of the previous experiments. In experiments I and II where growth was scant the majority of the colonies were small. Permanent dissociation of a stable M pneumococcus or Friedlander bacillus to an intermediate MS variant has also occurred under these conditions.

SUMMARY AND CONCLUSIONS

In the experiments described the fogging and bactericidal effects of the vapor from irradiated cod liver oil and from ozonide of olive oil have been found to be due to substances liberating active oxygen. Ozonides of olive oil, emulsions of ozonide in salt solution and in serum, and the vapor from ozonides are all germicidal. Bacteria are not only killed by the oxygen liberated from these oils but their growth rate may be retarded or dissociation may occur if the bacteria are subjected to sublethal doses.

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