THE BACTERICIDAL PROPERTIES OF ULTRAVIOLET IRRADIATED PETROLATUM

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In a preliminary paper from this laboratory (Ross, 1932) it was shown that an ultraviolet irradiated petrolatum-lanolin mixture had marked bactericidal power for *Staphylococcus aureus* while the same mixture without irradiation possessed no such quality. *B. pyocyaneus* exhibited considerable resistance to the destructive effect of the irradiated mixture. Several investigators have reported the acquisition by the fatty oils of bactericidal properties upon being irradiated. In this paper we desire to report our investigation of the comparative effect upon a number of bacterial species of ultraviolet irradiated and non-irradiated petrolatum without the admixture of the fatty oils. Some previous reports on this subject appear in the literature. Eising (1931) in discussing the therapeutic use of irradiated petrolatum, states that its effect is "dependent upon a strong bactericidal action upon the ordinary organisms of wound infection together with a potent stimulus to the processes of healing." He carried out no direct *in vitro* experiments, however, to demonstrate bactericidal action but assumed such from the observation that when petrolatum was applied directly to the infected wound "the discharge changed from a purulent to a serous one and the bacterial count diminished rapidly." Thompson and Sheard (1931) reported that a number of oils, including petrolatum and lighter mineral oils, became bactericidal after irradiation from an ultraviolet source.

THE BACTERICIDAL ACTIVITY OF IRRADIATED PETROLATUM

In our tests, old laboratory strains of the bacteria were mixed directly with the material the bactericidal power of which it was

desired to determine. The physical properties and presumably the chemical composition of samples of petrolatum obtained at random from drug houses vary greatly. In this problem we were interested only in the nature and diversity of the killing power of the irradiated oil and made no attempt therefore to discover the raw product which would yield the most bactericidal end substance. Preliminary tests indicated that a preparation having a melting point of from 40 to 45°C. gave somewhat more satisfactory results than samples melting at higher temperatures. The lower melting material also presented fewer difficulties in handling. We therefore procured a large quantity at one time and used the same sample in all our tests.

Heat-sterilized petrolatum was delivered to ordinary 100 mm. Petri dishes in 15 cc. amounts. The dishes were then placed under a Burdick quartz tube lamp at a distance of 12 inches with covers removed. The lamp developed a temperature of about 56°C. in the petrolatum which therefore remained melted during irradiation. The white petrolatum changed in color under the influence of the ultraviolet rays. In four hours it became lemon yellow. Longer periods of exposure produced a deep butter-yellow color. Experiments with products irradiated under the same conditions for varying lengths of time seemed to indicate that the maximum bactericidal power was obtained with our set-up in about four hours. All of the detailed results discussed in this paper were carried out with material irradiated for that length of time.

Obtaining an even distribution of the organisms in the petrolatum offered some difficulties but it was found that a quite uniform mixture could be obtained in the following way: Fifteen-cubic-centimeter portions of petrolatum in Petri dishes were warmed until melted and subsequently maintained at a temperature of 45°C. One-half cubic centimeter amounts of twenty-four hour broth cultures of the organisms were added from a pipette and stirred into the petrolatum with a platinum wire. Stirring continued until the petrolatum had cooled and solidified. The plates were then covered and placed in the incubator at 37°C. At intervals rather large portions of the material were removed

with a loop and melted off into tubes of broth which had been previously warmed to approximately 45°C. The broth tubes were incubated for forty-eight hours and the presence or absence of growth noted. The kind of medium used for the growth of

TABLE 1

Comparative bactericidal tests with four-hour ultraviolet irradiated and non-irradiated petrolatum

ORGANISM	GROWTH AFTER CONTACT FOR VARYING PERIODS WITH IRRADIATED PETROLATUM (HOURS)										GROWTH AFTER CONTACT FOR VARYING PERIODS WITH NON-IRRADIATED PETROLATUM (HOURS)									
	2	3	4	5	12	13	24	48	72	168	2	3	4	5	12	13	24	48	72	168
Staph. aureus No. 1	+	+	−	−	−	−	−				+	+	+	+	+	+	+			
Staph. aureus No. 0	+	+	+	+	+	−	−				+	+	+	+	+	+	+			
Strep. viridans	+	+	+	−	−	−	−				+	+	+	+	+	+	+			
Strep. non-hemolyticus (Clawson's strain)	+	+					−				+	+	+	+	+	+	+			
Strep. hemolyticus	+	−	−	−	−	−	−				+	+	+	+	+	+	+			
C. diphtheriae	+	−	−	−	−	−	−				+	+	+	+	+	+	+			
C. Hofmanni	−	−	−	−	−	−	−				−	−	−	−	−	−	−			
Bact. coli	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bact. typhosum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bact. paratyposum A	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+		
Bact. paratyphosum B	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+		
Bact. dysenteriae, Flexner	+	+	+	+	+	+	+				+	+	+	+	+	+	+			
Bact. mucosus-capsulatus	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	
B. pyocyaneus No. 199a	+	+	+	+	+	+	−	−			+	+	+	+	+	+	+	+		
B. pyocyaneus No. 195	+	+	+	+	+	+	+	−	−		+	+	+	+	+	+		+	+	
Br. abortus	−	−	−	−	−	−	−				−	−	−	−	−	−	−			
Myco. tuberculosis (Novy)	+	+	+	+	+	+	+	−	−		+	+	+	+	+	+	+	+		
Myco. smegmatis	+	+	+	+			−	−			+	+	+	+	+	+	+	+		
N. catarrhalis	+						−				+	+	+	+	+	+	+			
Cl. Welchii	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	
Cl. tetani	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	
B. anthracis	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	
B. subtilis	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	

the organisms both before and after mixing them with the petrolatum varied with the organism used. Meat-extract broth was used for those organisms which multiply readily in that medium. Meat infusion was used for the streptococci and diphtheroids. For the tubercle bacillus Lubenau's coagulated egg medium was

employed to produce the culture, which was then suspended in broth before being mixed with the petrolatum. After exposure it was again inoculated to Lubenau's medium to test for viability. Incubation of these latter cultures was continued for two weeks before any test was called negative.

In table 1 are given the results of these tests. It will be seen that the only organisms we failed to recover from the non-irradiated petrolatum after quite long periods of exposure were the diphtheroid, *C. Hofmanni*, and *Brucella abortus*. It would appear that these organisms are killed by the raw product. Of the organisms which survived long contact with the non-irradiated oil but were killed by the four hour irradiated product, the diphtheria bacillus and a strain of *Streptococcus hemolyticus* succumbed in the shortest time. Live organisms were proven in these cases after two hours but not after three hours. The spore-forming aerobic and anaerobic organisms proved to be insusceptible to the product, surviving the maximum period tested, seventy-two hours. Perhaps the most striking feature observed in these results is the insusceptibility of the Gram-negative intestinal group of bacteria. All of these strains survived the maximum period of exposure tested, which was in no case less than twenty-four hours and in the cases of *Bact. coli* and *Bact. typhosus* as high as one hundred sixty-eight hours. Of the two strains of *B. pyocyaneus* one was killed in twenty-four hours and the other survived that period but was not viable at the end of forty-eight hours. The tubercle bacillus tested was the non-pathogenic, rapidly-growing Novy strain. It was not recovered after forty-eight hours admixture with the irradiated petrolatum though positive cultures were obtained at the end of twenty-four hours. The strain of the smegma bacillus used could not be cultured after twenty-four hours' exposure.

We were unable to devise any accurate quantitative method of testing the killing power of the petrolatum. A rather crude method was attempted with the colon bacillus in order to determine whether this organism was wholly insusceptible to the bactericidal power of the irradiated petrolatum or whether failure to procure sterilization was due to the extraordinary resist-

ance of some individuals only in the cultures. This test consisted in mixing the organisms with the petrolatum in the manner already described, then at intervals removing a portion of the material roughly measured with a very small aluminum spoon and melting it off into 10 cc. of warm broth. After thorough mixing measured portions of this broth were plated in duplicate. The colonies developing on the plates were counted after forty-eight hours' incubation. A number of these tests were carried out and while the figures obtained show clearly that the method was only very roughly quantitative, the differences obtained between the irradiated and non-irradiated samples were in general no greater than those between two tests on the same sample at different times and indicated, we believe, that *Bact. coli* remains unaffected by the irradiated petrolatum for the duration of the test, twenty-four hours.

EXPERIMENTS ON THE PROPERTIES OF THE GERMICIDAL AGENT IN IRRADIATED PETROLATUM

There seem to have been three hypotheses advanced to account for the bactericidal effect of ultraviolet irradiated oils. Wrenn (1927) obtained killing of *Staphylococcus aureus* inoculated upon the surface of agar plates when the latter were exposed at some distance to dishes containing various irradiated fatty oils. He believed the killing effect to be due to emanations of radiant energy stored in the oils during irradiation. Eising (1931) in his first paper on the bactericidal properties of irradiated petrolatum expressed his belief also that these properties were resident in secondary rays emanating from the irradiated material. In his later papers (1932, 1933), however, he repudiates this belief in favor of the supposition that a chemical non-gaseous substance, possibly of the nature of the sterols, is the responsible agent. Harris, Bunker and Milas (1932) carried out experiments similar to Wrenn's, chiefly with the vegetable oils, and concluded that the killing effect exerted at a distance by the irradiated products was due to volatile chemical compounds and not to secondary emanations of radiant energy. With mineral oils they obtained no killing by their method.

The reasoning leading to the radiant energy hypothesis has been considerably affected by the fact that both vegetable and mineral oils when irradiated acquire, along with their bactericidal powers, the property of affecting photographic plates exposed to them in the dark room. Eising (1931) and French (1932) have published clear-cut stencil designs obtained both by direct exposure of plates to irradiated petrolatum and by exposure through an x-ray film to which the photographic plates were hermetically sealed. Harris, Bunker and Milas (1932) believe that they have shown this effect, at least so far as the vegetable oils is concerned, to be due to the presence of peroxidic compounds given off from the irradiated products in vapor form. They believe these peroxidic vapors are likewise responsible for the bactericidal effects of the oils. It appears, however, from a personal communication from these authors cited by Eising (1932) that they have been unable to detect any peroxidic oxygen given off by irradiated petrolatum though they have obtained fogging of photographic plates even when the latter were protected by x-ray film screens. To accept a gaseous emanation as the direct cause of the fogging of the plates would necessitate the assumption that such a gas could pass through the x-ray film. This has been suggested by Eising (1932) as probably occurring. The latter author (1933) seems to incline to the opinion, however, that the effect upon photographic plates and the bactericidal action of irradiated petrolatum are due to different constituents of these preparations.

The results of our experiments upon the problem of the nature of the germicidal agent in ultraviolet irradiated petrolatum are by no means conclusive, but we believe they throw some light upon the subject.

We were unable, as were Thompson and Sheard (1931), and Harris, Bunker and Milas (1932), to obtain killing by exposure of the organisms to irradiated petrolatum without actual contact between the organisms and the oil. Using the method of Harris, Bunker and Milas, surface streaks on agar plates were made of *Staphylococcus aureus* and placed uncovered over dishes of the irradiated petrolatum. Growth invariably occurred on these

plates. Later, in order to rule out possible protective effects by the medium, drops of a water suspension of the organism were placed on sterile glass slides which were then inverted over the petrolatum at distances no greater than 1 or 2 mm. The dishes containing these tests were placed in a moist chamber to prevent drying. Some were held at room temperature, some at body temperature, for periods up to forty-eight hours. None showed dependable evidence of being affected by the petrolatum. When subcultured after exposure they always showed abundant growth. Drops of a water suspension dried upon slides and exposed in the same way likewise remained viable for twenty-four- and forty-eight-hour periods.

A somewhat different experiment intended to test the killing power of the irradiated petrolatum when not in contact with the exposed organisms was carried out as follows:

A Petri dish was partly filled with the irradiated petrolatum and warmed until just melted. By means of a syringe or a capillary pipette droplets of a water suspension of *Staphylococcus aureus* were injected into it. The plate was then quickly cooled. By exercising care it was possible to obtain droplets of the suspension imprisoned in the solidified oil ranging from 3 or 4 mm. in diameter to a size that was barely visible. By covering the surface with another layer of the petrolatum these droplets would remain stationery indefinitely. The plates were placed in the incubator at 37°C. and at the end of twenty-four hours the individual droplets were cut out with a sterile wire and placed in broth warm enough to melt the petrolatum. The tubes were incubated for forty-eight hours and growth noted. Accurate measurements of the droplets were not attempted but a number of tests indicated that in the irradiated petrolatum the smaller ones were invariably sterilized while the larger ones were not. We would estimate the maximum size of the droplets which were rendered sterile to be not in excess of 1 mm. in diameter. In the non-irradiated material none of the droplets were sterilized.

The results of this last experiment were at first interpreted as favoring the hypothesis that the bactericidal power lay in the action of secondary radiant energy emanating from the irradiated

petrolatum, the reasoning being that the amounts of this hypothetical energy available for each organism in the smaller droplets would be many times greater than that impinging upon them when exposed from a plane surface. The position of many of the organisms in the larger droplets would, of course, approach that of those exposed to the radiant plane and hence it did not seem inconsistent with this hypothesis that the larger droplets were not sterilized while the smaller ones were.

Direct experiment, however, to detect possible radiant energy emanations yielded negative results. We obtained, as have others, definite fogging of photographic plates when the latter were exposed directly to the irradiated petrolatum, but when the films were screened behind ordinary glass, quartz glass or cellophane, no fogging could be detected. The last two substances permit the passage from the ultraviolet lamp of the rays which confer the bactericidal powers upon the petrolatum as was shown by the fact that petrolatum irradiated through them assumed the characteristic yellow color and became bactericidal for *Staphylococcus aureus*.

From these experiments it seems probable that the effect upon photographic plates exposed to irradiated petrolatum is due to gaseous emanations from this material. So far, however, there seems to be no evidence that such emanations are in any degree germicidal. It would appear then that the germicidal agent must be sought in some non-volatile chemical substance formed in the petrolatum under the influence of the rays from the ultraviolet lamp.

That a chemical reaction takes place in the petrolatum during irradiation would be assumed from the change in color. This assumption is supported by the results of the following experiment which further suggests that the change involves oxidation. That such oxidative change is correlated with both the appearance of the yellow color and the attainment of bactericidal power appears also from this experiment.

Two quartz glass tubes were sterilized and petrolatum from the same sample used throughout these tests was placed in each. One tube was filled completely full and corked tightly with a cork

stopper which was sealed in with sealing wax. No air remained in the tube. The other tube was only half filled and was stoppered with a cork having a portion removed to permit free access of air. These two tubes were placed on their sides under the ultraviolet lamp and irradiated for four hours. The tube from which air was excluded underwent no change in color under the influence of the rays but the other assumed the same lemon yellow color always acquired by the petrolatum irradiated in the open Petri dishes. Upon subsequent exposure to air the first tube retained its original white appearance. Bactericidal tests on the two samples showed that the material irradiated in the absence of air had acquired no bactericidal powers for *Staph. aureus* while the sample irradiated through the quartz glass in the presence of air sterilized a culture of *Staph. aureus* in six hours when mixed with it in the manner described above. In a second test the same results were obtained except that seven hours were required for the petrolatum irradiated in the open tube to sterilize the culture.

Long ago Roux (1887) showed that anthrax spores would not germinate in a medium that had been exposed for several hours to direct sunlight. Later investigations by Burnet (1925) indicated that this was due to the presence in the medium of hydrogen peroxide formed by the oxidation of water under the influence of the ultraviolet rays. We shook up melted irradiated petrolatum repeatedly with warm water and tested the water for the presence of hydrogen peroxide. None was found. The addition of the water to equal quantities of broth also failed to render the latter inhibitive to the growth of staphylococci. Extraction of the petrolatum with warm broth in a similar manner added nothing to the broth that would inhibit the growth of staphylococci. Apparently the bactericidal agent in this material is not extractable with water or with broth. This would seem to rule out compounds such as peroxides, acids, bases and even ozone. The latter compound would likewise be ruled out by the fact that the irradiated petrolatum retained its bactericidal power for *Staphylococcus aureus* after having been heated to temperatures of 50 to 60°C. for several hours.

We also attempted to wash out any volatile bactericidal sub-

stance from the irradiated petrolatum by allowing air to bubble slowly through a flask of the substance (which was melted and kept at a temperature of 60°C.), and subsequently to bubble through a flask of broth. After twelve hours of this washing the broth remained without any growth-inhibiting effect on *Staphylococcus aureus* and the irradiated petrolatum appeared to have lost none of its bactericidal power. In a somewhat similar experiment water was distilled from a mixture of irradiated petrolatum and water, the distilling flask being constantly agitated to facilitate mixing. The distillate was tested for bactericidal effect on *Staphylococcus aureus* and was found negative.

We next attempted to remove the bactericidal substance by extraction with alcohol, ether and petroleum ether. These tests were on the whole unsatisfactory but the results with alcohol are worth recording. When melted irradiated petrolatum is shaken up with warm alcohol and then allowed to stand, two layers form. The lower layer is yellow and appears little different from the irradiated petrolatum alone. The upper is only slightly tinged with yellow and when evaporated on a water bath, leaves a very small residue. This is brownish in color and on cooling has the appearance of a heavy oil rather than a semi-solid material like petrolatum. Both the extracted material (the lower layer) and the brownish residue, when freed from alcohol as completely as possible by evaporation, were bactericidal for *Staphylococcus aureus*.[1] Since small amounts of alcohol may have remained in both specimens, however, we hesitate to infer that the bactericidal substance is alcohol-soluble.

SUMMARY AND CONCLUSIONS

The experiments reported in this paper show that when white petrolatum is irradiated for four hours from a source of ultraviolet light it changes to a lemon yellow color and acquires the property of slowly sterilizing suspensions of certain bacteria, when intimately mixed with them. The bactericidal effect thus

[1] By a special method several ounces of the alcohol extractive were obtained and used clinically by Dr. Arthur Jones who believed it to be therapeutically more potent than the ordinary irradiated product.

demonstrated appears to be selective in the sense that it acts upon certain vegetative forms of bacteria only. Spores are not destroyed and certain Gram-negative intestinal organisms seem to be likewise wholly unaffected.

Evidence is brought out to indicate that the germicidal agent in this ultraviolet irradiated petrolatum is a non-volatile chemical substance rather than a gaseous or radiant energy emanation and that this substance is formed as the result of an oxidative process. It is further shown that this chemical agent is not extractable with water or with ordinary nutrient broth and that it cannot be carried out of the melted material with a stream of air or distilled out with steam. There is some indication that the active bactericidal agent is extractable with alcohol, but we do not feel that our experiments are conclusive on this point.

It seems to us a reasonable conclusion from the above facts that irradiated petrolatum contains a very weakly germicidal substance of such low solubility and slow diffusibility that it can be depended upon to kill susceptible bacteria only when the organisms and the petrolatum are brought into very intimate contact. To expect this substance therefore to exert any effective bactericidal action upon organisms contained in pus or exudates or embedded in tissue, conditions which would certainly prevent the necessary contact, is illogical. It is much more logical to suppose that any action which irradiated petrolatum may show in freeing infected wounds of their organisms is due to a stimulating effect upon the local tissue defenses rather than to direct bactericidal action.

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