# EFFECTS OF IODIZED OILS ON BACTERIA

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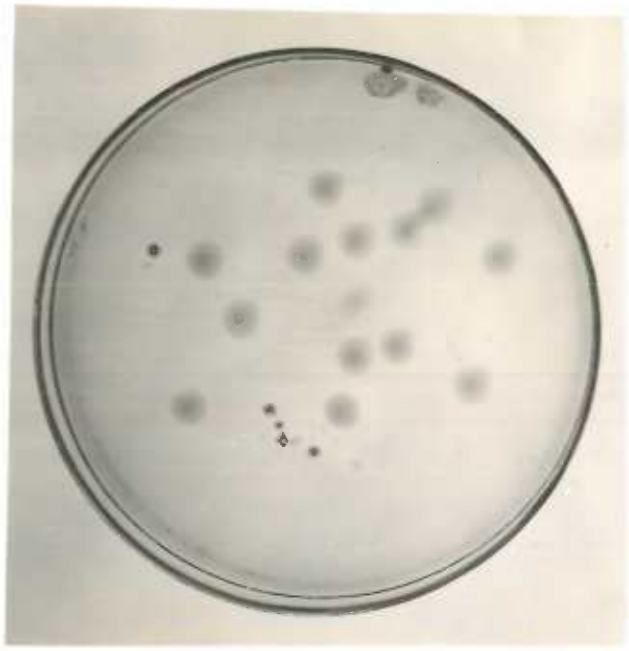

PLATE I

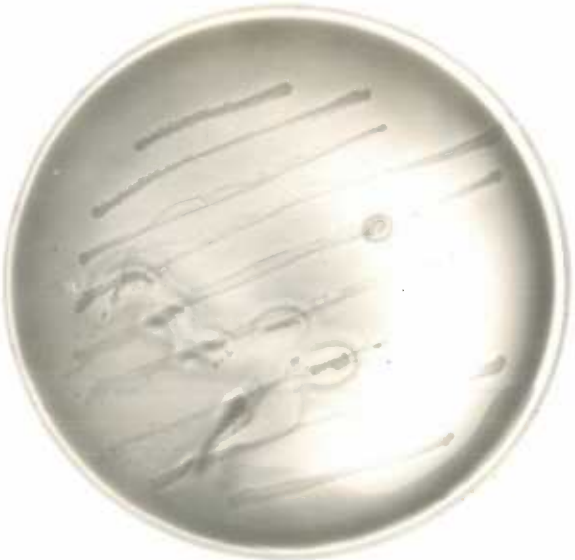

PLATE II

# EFFECTS OF IODIZED OILS ON BACTERIA

Refinements in diagnosis by means of the Roentgen Ray have been numerous in the comparatively few years that this means of study of human pathology has been in clinical use. To the uninitiated the X-ray is a magic power which in the hands of the master will divine the seat and fundamental character of any illness to which the human race falls heir.

The limitations and pitfalls of interpretation are only too well known, however, to those who have had even a limited experience in using the X-ray as an aid in diagnosis. An X-ray picture may only represent changes in density, or, in other words, differences in resistance to the passage of the ray through various tissues. The more nearly the density of one tissue is to that from which a differentiation is desired the greater the difficulty for the roentgenologist becomes.

The limitations of the natural differences in density of the various soft tissues of the body has been so great that roentgenologists have found it of extreme value to introduce various contrast media into the lumina of many organs or into the real or potential spaces surrounding them for the purpose of increasing, or decreasing, densities in order to obtain contrast.

Numerous examples might be cited of the ingenious modes which have been pursued to determine these ends, but these do not lie within the scope of this paper. We will here limit our consideration to the use of iodized oils as contrast media in the various anatomical regions in which they have been employed, with particular attention to effects on bacteria of these oils.

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A number of iodized oils of similar nature have been used for this purpose, among them, Iodumbrin by the Danes, Iodipin by the Germans, and Lipiodol by the French. We have confined ourselves to the latter two oils.

Lipiodol-Lafay was introduced into France about 1902 as a therapeutic agent, according to Forestier (a), by Dr. Lafay. The former states that it was at that time used intramuscularly for prolonged iodine medication in asthma, goitre, and chronic rheumatism. In 1921 Sicard and Forestier, impressed by the opacity of this oil in the tissues, commenced experiments by injection into the epidural space (b). They later used the oil in the subarachnoid space and bronchial cavities (c).

Lipiodol-Lafay has been recently admitted to "New and Non-Official Remedies." It is said to be an addition product of poppyseed oil containing 39 to 41 percent of iodine (0.54 gm. of iodine per cc.) in organic combination. It is manufactured by Andre Guebert & Company, Paris, and is distributed by E. Fougera and Company in this country. It is a thick viscid oily liquid, having an alliaceous odor and an oleaginous taste, insoluble in water. On exposure to air and sunlight it decomposes, turning a dark brown color. Specific gravity at 20 degrees Centigrade is from 1.340 to 1.350. It is said to contain no free iodine and repeated tests on samples purchased in the local market gave no evidence of free idoine by the starch paste test, by treatment with sodium thiesulfate, or on contact with carbon bisulfide.

NOTE
Letters in the text refer below to numbers in bibliography.
(a) 59a. (b) 184. (c) 186.

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Bettman is quoted to the effect that Iodipin was produced by Winternitz in 1897, the latter using it to demonstrate the impossibility of subcutaneous fat nourishing for human beings. The deposit of this iodized fat in the subcutaneous tissues is said, however, soon to have led to its use as a therapeutic agent in those diseases in which protracted iodine therapy was indicated. Fritsch is said to have first discovered the advantages of Iodipin as a contrast medium in roentgenography.

Iodipin 40 percent (d) which is marketed by Merck & Company, was used in our experiments. According to "New and Non-Official Remedies" it consists of an addition product of sesame oil containing 39 to 41 percent of iodine in organic combination. It is an oily liquid, insoluble in water, having an oleaginous taste. Its color is brown to black, depending on the thickness of the layer examined. Specific gravity at 15 degrees Centigrade is from 1.370 to 1.372. It is said to contain no free iodine, but in a number of freshly opened stock bottles purchased on the local market we found free iodine by the starch paste test, i.e. production of a blue color; it was almost entirely decolorized by solution of sodium thiosulphate indicating absorption of free iodine; and carbon bisulfide received a deep violet color in contact with the substance, denoting presence of free iodine. Many observers speak of the presence of free iodine in iodized oils exposed to air and light, but it will be noted that these samples tested were from freshly opened stock bottles.

The pioneer work of Sicard and Forestier was followed by a widespread use of iodized oils as a contrast media for the

(d) 90.

exploration of almost every real or potential space in the body in a wide variety of normal and pathological conditions. We can consider the clinical aspects of their use only in the briefest sort of fashion as a background in stimulating our experimental work regarding the effect of these oils on bacteria. In attempting this problem, however, it was necessary to review a tremendous number of clinical reports in an effort to become acquainted with any experimental work relating to this problem.

In so far as the use of these oils in the central nervous system is concerned, there is little mention of any action on bacteria in these anatomical spaces by the iodized oils. Devé, however, has devoted several articles (e) to reporting experimental work to show that the observations of Mourge-Moline and Lapeyre, indicating that the combined action of Lipiodol and rœntgen ray exposure had resulted in a hydaticidal effect, led to erroneous conclusions. Devé concludes that Lipiodol has little, if any, parasiticidal action on the hydatids.

Among the writers who advocate the use of Lipiodol in the exploration of the central nervous system, after the method of Sicard and Forestier, may be mentioned, Mixter, Reese, Sargent, Rowley, Purves-Stewart, Mackeddie, Paraf, Laplane, Viner, Peiper and Klose, and Brouwer and Denk. (f)

The following writers feel that Lipiodol should be used only with greatest caution and have noticed various types of unsatisfactory results from its use, viz.: Vincent, Sharp and Peterson, Saethre, Lindblom, Krause, Ayer and Mixter, Sachs, Maclaire, Bailey,

(e) 55, 56, 220, 221. (f) 132, 158, 172, 255, 253, 244, 245, 246, 195, 210, 148, 147, 133, 123, 94, 58, 34, 53.

and others.(g) Among the unsatisfactory results were scar formation with increase of symptoms, errors in localization, and an aseptic meningitis; which, however, many of the observers feel to be only a transient reaction and that it clears up with no permanent pathology. Some fear foreign body phenomena as the oil is only slowly absorbed from the tissues. Forestier (h) agrees that the oil remains in the central nervous system up to two years or even longer, but that the oil is completely absorbed and never forms an abscess or tumor.

The need for some method of diagnosis more satisfactory than those in use previous to the use of iodized oils is emphasized by a statement by Frazier and Spiller (i) that in a series of spinal cord tumors reported in 1922 the final diagnosis was not established until an average of two and three fifths years had elapsed from the date of the initial symptom.

The use of iodized oils for intra-tracheal injection and lung mapping has apparently extended even more widely and many supporters have flooded the literature with reports of cases in which iodized oils were used as an aid in diagnosis. Sergent and Cottenot (j), and Armand-Delille were among the pioneers in France (k). The following report satisfactory results in various types of lung pathology: Fiessinger and Olivier, Kovats, Landes, Svejcar,& Dreuschuch, Tapie, Vinson & Lemon, Burrell & Melville, Guy & Elder, Singer, Archibald & Brown, Ballon, Voorsanger & Firestone, Hedblom, Larson, Abramovitch, Brown, Head, Matson, Pirie, and many others. (l).

(g).209,177,178,171,111,105,13,170,118,15. (h) 59a. (i) 225.
(j) 175 (k) 11, 12, 10. (l). 222,237,238,265,266,269,39,78,
197,89,18,19,81,106,1,35,82,153.

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It appears that the great majority are agreed that the field where iodized oils find their greatest usefulness is in cases of bronchiectasis. Many cases are reported in the literature of bronchiectasis having been diagnosed as tuberculosis previous to oil injection. Interspersed among these favorable reports are notes of warning. Archibald and Brown (m) devote an entire paper to discussion of the dangers of iodized oil in the tracheo-bronchial system, in which this subject is well covered. O'Donovan (n) reports a case in which death followed the use of Lipiodol intratracheally. His case was, however, complicated by multiple staphyloccic abscesses previous to the injection. Schröter (o) considers the method not without danger and advises against its use except as a last resort. Lichtwitz (p) reports a tuberculous patient who had a sudden flare-up after a small Lipiodol injection. A few other fatalities were reported but, in all, patients were in rather grave conditions previous to being subjected to the injection. Some report cases of acute iodism following injection, but it appears that this happens rarely unless a fairly large amount of the oil reaches the stomach and passes on to the alkaline intestinal juices. If aspirated from the stomach before it passes on this usually may be avoided. As to the use of these oils in tuberculous cases, much difference of opinion exists concerning its use in the chronic cases. The general opinion as to acute cases is well expressed by Armand-Delille & Moncrieff (q) when they state: "Cases of frank tuberculosis should not be submitted to this method for the congestive action of iodine may produce

(m) 9. (n) 144 (o) 181. (p) 109 (q) 12.

harmful effects, and even in doubtful cases an ordinary radiograph may clearly indicate a diagnosis." --- A few, however, believe that even acute tuberculosis is benefitted.

Many report a marked improvement in clinical symptoms following the injection of iodized oil into the lungs, especially in cases of bronchiectasis. Among those who have noted marked clinical improvement are: Pirie, Forestier, Armand-Delille, Miller, Allen, White, Iglauer, Pritchard, Knauer, Furstenberg, Leroux, Chandler, Ballon, Gilston, and others (r). Ochsner stresses this point. He believes that free iodine is given off slowly, which acts directly on the organisms. He reports one case where bacterial count on the patient's sputum before the intraduction of the Lipiodol showed 4,200,000 organisms per cc. and that two days after the injection the sputum contained only 200,000 per cc.(s)

These oils have been widely used also in diagnosis of uterine conditions. Heuser (t) in attempting to use the material to produce abortion found this unsuccessful, but found that he had a method of diagnosis. Forestier, in discussing a paper of Stein & Ahrens (u), states that Lipiodol may cause miscarriage in pregnancy. Carreras believes it is safe to use Lipiodol even in the presence of inflammation in or about adnexa.(v) Green & Pendergrass have had uniform success in the uterus and tubes and feel that there is a "suggestion of some therapeutic value". (w) Mathieu reports a series of eighty cases in which excellent results were obtained.(x) Haselhorst and Odenthal, however, call attention to four cases of severe reaction following hysterography. (y).

(r)153,64,65,10,127,5,270,233,152,102,70,224,44,21,11. (s) 143
(t) 85 (u) 264 (v) 41 (w) 74 (x) 125 (y) 79

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Cotte and Bertrand (z) have had no ill effects in their own cases, but report three cases of localized peritonitis and four deaths following exploration of the tubes. Collargol was used in most of these cases, however.

Of the many other uses to which these oils have been applied only a few need be mentioned. Neuswanger (aa) reports very satisfactory results in animal experiments on dogs and in a small series of patients by injecting the ureters and kidney pelves, concluding that Lipiodol 40% appears to offer a pyelographic medium which is superior to those in use at the present time with regard to toxicity and degree of opacity to the roentgen ray.

Of more striking interest is the work on visualization of the blood vascular system. Sicard and Forestier (ab) in 1923 studied reactions in dogs with intra-vascular injections and report one injection into the femoral vein of the human. No complications were noted and no difficulty was experienced with oil embolism. Harvier and Lemaire (ac) in 1927 used Lipiodal in 14 cases with satisfactory results and no serious complications except in one case, which developed gangrene eight days after the injection, but in which they feel that there is no certainty that the oil was responsible for it. Large amounts were used intravenously in dogs by Neuswanger (ad), who found areas of congestion and hemorrhage in the lungs without any permanent anatomical changes, the animal showing no symptoms

(z)50, (aa)138, (ab)189, (ac)230, (ad)138

of irritation or toxicity over a period of two months. Carnett and Greenbaum (ae) also report a very satisfactory series and show some interesting plates of their findings in various types of pathology. Sicard and Forestier (af) state that there is no danger that the Lipiodol will block the collateral branches because it goes easily through the peripheral capillary network.

In roentgenography of the paranasal sinuses iodized oil has found many supporters. Goodyear (ag) found it of value in those cases difficult to diagnose by ordinary methods. In a series of 34 cases he found the procedure simple and the iodized oil without irritation to the nasal membranes. "In fact,"he says, "it had considerable medical value." MacCready (ah) found it satisfactory roentgenologically, and with no untoward side effects, but feels that it is removed from the sinuses too rapidly to be of any considerable therapeutic effect.

Keith (ai) comments on previous injections of the parotid gland, and states that with no untoward results he has been able to inject even the finer ramifications of the ducts of the parotid gland. Many writers speak of the value of these oils in outlining fistulous tracts, abscesses, empyema, and similar conditions. Lawson (aj) reports that the use of as much as 50 gms. is without untoward effects. Brams (ak) reports the value of iodized oils in pilonidal cysts. Dew (al) found it of extreme value in outlining a ruptured hydatid cyst when other methods had failed.

Visualization of the eustachian tube is found to be

(ae)40, (af)260, (ag)72, (ah)121, (ai)236, (aj)107, (ak)33, (al)57.

satisfactory by Spielberg (am) in a preliminary report outlining his technique.

Many other clinical applications might be cited in which these oils have found a place in diagnosis or treatment, but we will content ourselves with the report of only one additional use which Sicard and Forestier have made of these oils. Forestier (an) states that they have taken advantage of the analgesic property of Lipiodol and utilized it for local injection in the treatment of sciatica, lumbago, periarthritis and arthritis.

The literature is replete with statements regarding the antiseptic value of iodized oils. (Sicard and Forestier make the statement repeatedly in the literature that Lipiodol is antiseptic (ao). Beard (ap) states in regard to Lipiodol that the opaque and antiseptic qualities make it very effective for examining various cavities by X-ray. Knauer (aq),also speaking of Lipiodol, makes the following statement, "It is a mild antiseptic and liberates slowly but continuously iodine." Ochsner (ar), who was quoted previously regarding the reduction of bacterial counts in sputum after Lipiodol injections in cases of bronchiectasis, says, "Concerning the therapeutic action of the iodized oils it is safe to say they do have an antiseptic action. This is shown both clinically and bacteriologically. Rubin and Bendick (as) in their paper on uterotubal roentgenography with Lipiodol remark, "It is aseptic and antiseptic.")

(am)263, (an)59a, (ao)64,65, (ap)23, (aq)102, (ar)143, (as)166

11. EFFECTS OF IODIZED OILS ON BACTERIA

We have been unable to find in the literature on iodized oils any statements regarding experimental work to prove that iodized oils have an antiseptic value. The statements supporting its antiseptic qualities have apparently gone unchallenged until in August, 1926, when Neuswanger (at) made the statement that iodized oil 40% has no marked bactericidal or bacteriostatic properties. In support of this statement he says that agar plates upon which iodized oil was smeared were inoculated with bacillus coli, staphylococcus aureus, and streptococcus hemolyticus. Upon examining these plates he states it was found that the organisms were growing in close proximity to the globules of oil, and no inhibitory action was observed. He noted also , however, that quantities of iodized oil exposed to the air for several months showed no evidence of decomposition and no colonies of bacteria or fungi were observed.

Archibald and Brown (au), citing the work of Neuswanger, conducted two additional experiments to test the bactericidal power of iodized oils. We will quote these experiments verbatim:

> "EXPERIMENT 1.--Three cubic centimeters of Lipiodol was mixed with an equal quantity of thick tenacious sputum taken from a patient with bronchiectasis, by drawing the material back and forth into a small glass syringe, until a mixture was obtained resembling a lather, the oil being in a fine state of subdivision and the sputum in very intimate contact with it. This was kept at room temperature for forty-eight hours and then streaked on various culture mediums and incubated. A luxuriant growth was obtained, including pseudodiphtheroids, pneumococci, staphylococci and Micrococcus catarrhalis.
>
> "EXPERIMENT 2.--Equal quantities of sputum and Lipiodol were placed without mixing in a glass container, where they separated into layers. This was kept at room temperature for eighteen days, by which time the sputum had become decomposed and was found to be thoroughly mixed with the oil, not

(at)138, (au)9.

even fine globules of oil being discernible. This was then plated and incubated, and here likewise a profuse growth of organisms, including pseudodiphtheroids, short chain streptococci, and Staphylococcus aureus, were obtained."

These authors conclude from these experiments that no trust can be placed in any presumed antiseptic action of Lipiodol.

In view of these diametrically opposed views, we felt that some quantitative work to determine more accurately the effects of these oils on bacteria would be of value.

The following experiments were therefore attempted in an effort to contribute toward a solution of this problem.

EXPERIMENT 1.

One loop full of a 24 hour growth of Hemolytic staphylococcus aureus was placed in 1 cc. of Iodipin in a 5 cc. test-tube, stirred, and agitated for 30 minutes at top speed in a shaking machine in an attempt to get a grossly appearing homogeneous suspension.

A similar preparation was made substituting Bacillus coli communis.

It was found to be impossible to break up the clumps of organisms in the heavy oil with this technique. However, a loop full of the contents of each of these tubes was inoculated into tubes of infusion broth. A loop full of each was also inoculated for a pour plate into liquid agar at 45 degrees centigrade. The remaining contents of each tube were similarly plated on agar.

In all of these tubes and plates, luxuriant growth resulted. Repetition of this experiment gave similar results.

We were not at all satisfied with this technique, however,

feeling that the organisms within the clumps were protected from the action of the oil. We therefore sought some means of procuring a fine suspension of the organisms in the iodized oils. As Iodipin is an addition product of sesame oil and Lipiodol an addition product of poppy seed oil, we selected these lighter oils to attempt to produce a finer suspension. Accordingly, we attempted the following experiment.

EXPERIMENT 2

One loop full of 24 hour growth of Hemolytic staphylococcus aureus was placed in 1 cc. of poppy seed oil in a 5 cc. test-tube, stirred, and agitated for 30 minutes at top speed in a shaking machine. This was repeated with the same organism in sesame oil and with Bacillus coli communis similarly in both oils. We were able to get a more nearly homogeneous suspension than we had previously been able to do with the heavier iodized oils, but clumps were still macroscopic. Two loops full of the poppy oil suspension were inoculated into 1 cc. of Lipiodol in the case of both organisms, and similarly two loops of the sesame oil suspensions into 1 cc. of Iodipin. These latter mixtures were agitated in a shaking machine and inoculations were made from them into infusion broth and pour plates were made, in agar, with the balance.

Repetitions of this experiment, with minor modifications as to time and quantity, were made and widely varying results were obtained; even when exactly the same technique was used, luxuriant growth being present in some cases and none in others. We felt that the clumping of the organisms, though not so apparent as in Experiment 1, was the factor in the irregularity of these results.

EXPERIMENT 3.

In experimenting with these oils, we found that we could get what appeared to be a fairly homogeneous suspension of the organisms in the lighter oils (poppy seed and sesame), by inoculating into them a loop full of heavy broth culture of the organisms.

A number of experiments were undertaken on the basis of this sort of a mixture of which the following will serve as an example.

One loop full of 48 hour growth of Hemolytic staphylococcus aureus was inoculated into 1 cc. each of poppy seed oil and sesame oil. These were shaken at irregular intervals for periods of thirty minutes to one hour over a period of twenty eight hours. Two loops full of poppy mixtures were inoculated into each of four tubes, containing respectively, 1 cc. pure Lipiodol, 1 cc. mixture 50% Lipiodol and 50% poppy oil, 1 cc. mixture 25% Lipiodol and 75% poppy oil, and 1 cc. mixture 10% Lipiodol and 90% poppy oil. Similarly two loops full of sesame mixture were inoculated into each of four tubes, containing respectively, 1 cc. pure Iodipin, 1 cc. 50% Iodipin and 50% sesame oil, 1 cc. 25% Iodipin and 75% sesame oil, and 1 cc 10% Iodipin and 90% sesame oil. These eight tubes were shaken in an automatic shaker for a period of fifty minutes and two loops full of each were inoculated into tubes of infusion broth. The remaining portions of oil mixtures in these tubes were poured into liquid agar at 45 degrees and plated, respectively. Inoculations were made from the original poppy oil mixture and sesame oil mixture into tubes of infusion broth and the balance of each mixture was plated in liquid agar. In the infusion broth cultures from the first eight above no growth was

EXPERIMENT #3 Continued.

obtained and none of the plates showed growth except the one in which one cc. of Iodipin had been used. The broth cultures and the pour plates from the original mixtures were all positive.

Had it not been for the fact that the suspension in the Iodipin showed growth in this, one might feel that there was rather solid evidence of bactericidal action from the various concentrations of the iodized oils. Similar discordant results attended other experiments of this type and we often had sterile cultures resulting when we inoculated into the poppy oil, for example, from a suspension in poppy oil and had growth from an inoculation into Lipiodol, the two being plated out with exactly the same technique. This made us feel that there was a strong probability that the mechanical factor of the oil surrounding the much more finely divided suspension in our dilutions was the primary factor rather than the bactericidal properties of the oils themselves. An experiment, previously carried out, in our attempts at working out a satisfactory technique might be cited in this regard.

EXPERIMENT #4

Bacterial growth from the surface of a slant with a 24 hour growth of Bacillus coli communis was suspended in 4cc. of sterile water. A similar suspension of Staphylococcus aureus was prepared.

The following tubes were arranged:

Tube #1 contained 1cc. poppy plus 2 loops suspension of Staph.

EXPERIMENT #4 Continued.

Tube #2 contained 1 cc. Lipiodol plus 2 loops of suspension Staph.
" #3 " 1cc. poppy " 2 " " " B. coli.
" #4 " 1 cc. Lipiodol " 2 " " " "
" #5 " 1 CC. poppy " 2 " " growth(slant)Staph.
" #6 " 1 cc. Lipiodol " 2 " " " " "
" #7 " 1 cc. poppy " 2 " " " " B. coli.
" #8 " 1 cc. Lipiodol " 2 " " " " "

These tubes were shaken for one half hour in the shaking machine, allowed to stand over night and again 24 hours later shaken for one half hour.

Two loops full were inoculated from each of these tubes into eight tubes of infusion broth, numbered as the tubes from which the inoculations were made.

The remainder of each suspension was poured onto an agar plate numbered as the tube.

Broth cultures and plates from tubes 1, 2, 3, and 4 were sterile. Tubes and plates 5, 6, 7, and 8 were found to have profuse growth.

As further evidence that ~~that~~ the factors determining whether growth will take place or not in the presence of these oils are largely mechanical and on a basis of contact with the media or lack of contact because of the fineness of the suspended particles surrounded by oil the following experiment is presented. It is simply selected as one of a number which we feel show, largely through their lack of uniformity of results, that the bactericidal powers of these iodized oils have little to do with the question of growth in their presence.

## EXPERIMENT #5

One loop full of a 24 hour growth of Bacillus coli communis on an agar slant was suspended in approximately one half cc. of infusion broth. Two loops of this suspension were inoculated into 1 cc. of poppy oil. A similar preparation of Staphylococcus aureus was made. These were shaken for fifteen minutes in the shaking machine. Inoculations were made from each of these into pure lipiodol and pure poppy oil and mixtures of the two. These preparations were shaken for stated periods of time and tested for sterility or better, possibly, to say, for the ability of the organisms contained in them to produce growth, by allowing one drop of the final mixture , with aseptic precautions to fall into a tube of infusion broth and the remainder to drop from a pipette onto the surface of an agar plate. Results of procedure in tabular form were as follows.

Bacillus coli communis

| Poppy oil | 10 min. exp. Broth | 0 | Plate | 0 | 15 min. exp. Broth | 0 | Plate | 0 | 30 min. ex. Broth | 0 | Plate | 0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10%lip.90%p.o. | " | 0 | " | 0 | " | 0 | " | 0 | " | 0 | " | 0 |
| 25%lip.75%p.o. | " | 0 | " | 0 | " | 0 | " | 0 | " | 0 | " | 0 |
| 50%lip.50%p.o. | " | Ø | " | 0 | " | 0 | " | 0 | " | 0 | " | 0 |
| Lipiodol | " | 0 | " | 0 | " | 0 | " | 0 | " | 0 | " | 0 |

Staphylococcus aureus

| Poppy oil | 10 min. exp. Broth | POS | Plate | POS | 15 min. exp. Broth | POS | Plate | 0 | 30 min. exp. Broth | 0 | Plate | 0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10%lip.90%p.o. | " | POS | " | POS | " | POS | " | POS | " | 0 | " | 0 |
| 25%lip.75%p.o. | " | 0 | " | 0 | " | POS | " | POS | " | 0 | " | Ø |
| 50%lip.50%p.o. | " | 0 | " | 0 | " | 0 | " | POS 2 colonies | " | 0 | " | 0 |
| Lipiodol | " | 0 | " | 0 | " | 0 | | PØS 1 colony | " | 0 | " | 0 |

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In conducting experiments of this type ~~and~~ additional point in favor of the mechanical action of these oils in preventing bacterial growth was noted. If, in blowing the oil suspension from the pipette, in inoculating the agar plate, the oil was spread into fine droplets, growth often occurred in the fine droplets and not in the large globules of oil suspension ~~on~~ the plate. The pipettes were, of course, cotton stoppered and, we believe, the possibility of contamination is very remote. This, we think, is very probably due to the organisms in the smaller droplets being thrown into contact with the media while those in the larger drops were completely surrounded by oil . This phenomena is well shown in plate 1 of the frontispiece.

This led us to conduct a simple experiment which we feel is very conclusive.

EXPERIMENT #6

Agar plates were streaked with a 24 hour growth of Bacillus coli in infusion broth. Cultures of Staphylococcus aureus were similarly streaked. Immediately after streaking these plates some of each type were covered in portions with Lipiodol, others with Iodipin, some with poppy oil and still others with sesame oil. In all of these plates growth took place under the oil upon incubation of the plates at 37 degrees C. Both organisms grew well under Lipiodol, in fact, some of the plates suggested a stimulation of the growth. Plate II of the frontispiece shows one of the ~~the~~ plates with the Staphylococcus growing under Lipiodol. It will be observed that the colonies under the oil appear more thriving than those exposed to the air. This, we believe, however, is not a result of stimulation from the oil, but simply due to the action of the oil in the prevention of evaporation of the moisture in the plate

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Iodipin exerted a definite inhibitory action on the growth of both organisms but, as stated before growth took place. The inhibition appears to us in this case to have been due to the small amount of free iodine which our tests showed to be present in the samples of Iodipin which we used. The poppy oil seemed to exert an inhibitory action on both organisms more particularly on the colon bacillus. No inhibition was noted in the case of the sesame oil on either organism. These results have been confirmed on these organisms by several repetitions of this experiment. Similar results were found using Micrococcus Catarrhalis, also on a streptococcus hemolyticus.

In reporting these results on "in vitro" experiments with these iodized oils we wish to call attention to the fact that there is a possibility of bactericidal compounds being formed in the tissues of the body. Roger , Binet, Verne and Sicard, Fabre and Forestier have contributed some valuable reports on their studies on fat digestion (av) in the various tissues of the body. In a later paper we hope to report some experimental work regarding bactericidal properties of iodized oils "in vivo".

## CONCLUSIONS

1. Survey of the literature on iodized oils reveals a widespread use of these oils in diagnosis and therapy.

2. Certain investigators have issued warnings regarding their use, but many writers consider iodized oils antiseptic.

3. Experiments "in vitro" do not indicate any bactericidal power.

4. Possibility of bactericidal power "in vivo" is suggested as worthy of further investigation.

(av) 29, 30, 146, 185, 160, 161, 162.

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