A STUDY OF THE NATURE, GROWTH AND CONTROL OF BACTERIA IN CUTTING COMPOUNDS

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Heavy bacterial growth in oil-water emulsions used as lubricants and cooling agents in the cutting and grinding of metals has been the cause of much trouble and expense to industries using large amounts of cutting compounds. This is due (1) to development of foul or sour odors objectionable to the workers, (2) to interference with the emulsification of the mixture, and (3) to dermatitis and skin infections which are often attributed to it.

The oil dermatitis of workers in contact with cutting compounds, according to McConnell (1933), makes its first appearance as comedones in the orifices of the hair follicles. These small nodules become surrounded by reddish macular eruptions from one to several millimeters in diameter, and develop into papules. McConnell (1922) found that of 2060 workers coming in contact with the oil, 557 (27 per cent) suffered from oil dermatitis. Tower (1938) on the other hand states that in large industrial plants in New England the incidence is only from 0.1 to 2.7 per cent.

There have been a number of theories advanced to explain the dermatitis. It is a widely held view, particularly among the workers themselves, that it is due to infection. The compound in circulating through a shop has ample opportunity to become contaminated by pathogenic organisms as it flows over the hands of workers and collects in pans around the machines into which the men frequently spit, and in which they sometimes stand. On the other hand, there is ordinarily no apparent inflammation at the site of the primary lesion such as would be expected if it were due to infection. Pustules sometimes form, but not as a

rule, and their presence is almost certainly due in the majority of cases to secondary infection. Kendall (1920) was unable to find any bacteria in smears taken from primary lesions under aseptic conditions. One of the corollaries of the infection theory is that the bacteria enter the skin through microscopic abrasions made by numerous minute steel chips present in the circulating fluid. However, McConnell (1922) states that in plants where germicides were employed to kill the bacteria or where magnets were used to remove the steel particles there was no decrease in oil dermatitis. He does not, however, state whether the germicides remained effective over a period of time.

Another theory advanced to explain oil dermatitis is that it is due to chemical irritants such as free fatty acids and unsaturated hydrocarbons contained within the oil. The absence of inflammation at the site of the primary lesion makes this improbable, and the occurrence of the dermatitis in equal degree even when entirely different types of oil are used for emulsions is also against it. Some investigators have considered the dermatitis allergic, and some of the cases may very well be, but negative patch tests show that most of them are not. Furthermore, the eruptions are not characteristic of allergic skin reactions.

The most probable explanation is that the oil tends to clog the ducts of the sebaceous glands in the hair follicles, leading to mechanical congestion and irritation. The presence of steel particles, dirt, foreign types of bacteria, and possibly acids or other irritating products of bacterial decomposition may aggravate the trouble. McConnell (1933) found that there were fewer cases of oil dermatitis among workers with oily skins than among those with dry skins, and advances the theory that the ducts of sebaceous glands, and hair follicles, are more likely to be clogged by extraneous matter in dry-skinned than in oily-skinned individuals. Secondary infections probably develop only when the papules are broken by scratching or squeezing, and the germs then involved might be acquired from many sources other than the cutting compounds.

Even though the evidence is against the bacteria in cutting compounds being the direct cause of oil dermatitis, they may play a part in the process by producing irritating decomposition

products. These products, whether actually irritating to the skin or not, are highly objectionable because of their unpleasant odors. It is hard to convince shop workers that their skin troubles are not due to infection from the cutting compound when the latter is obviously spoiled. Moreover the stability of the emulsion is interfered with as the result of bacterial decomposition. For these reasons it becomes necessary to discard the compounds and replace them at fairly frequent intervals, entailing considerable expense.

The present study was made to get some more accurate information than has hitherto been available with respect to the nature and source of the bacteria present in cutting compounds, and if possible to find some feasible method of controlling them. We wish to extend our sincere thanks to the Hughes Tool Company of Houston, Texas, and particularly to their Mr. Henry Woods, Mr. Gorham Woods and Mr. R. W. White for interest and cooperation in the work, for supply of material, and for practical tests in their shops.

BACTERIOLOGICAL STUDY OF CUTTING COMPOUNDS

The cutting compounds studied consisted of mixtures of one part of emulsifying or "soluble" oil with 7 or 8 parts of water to produce a milky emulsion known as "pigeon's milk." Two types of emulsifying oils were used in this work; one was a sulphonated lard oil, whereas the other consisted of petroleum products, the exact nature of which could not be ascertained. These cutting compounds were in use in the machine shops of the Hughes Tool Company. About 500 gallons are kept in continuous circulation in each shop, being pumped from, and returned to, concrete pits. The compound runs from a hose in a steady stream upon the surface of the metal that is being cut, then splashes into a pan surrounding the machine, and drains back to the tank, falling through the air for a distance of about six feet after leaving the return pipe. This continual aeration and circulation causes evaporation and loss of the compound, and more oil or water is added every day or so to maintain the correct proportions of oil and water in the emulsion.

The pits are cleaned out every six to eight weeks and fresh

compound put in, but the pipes and the pans around the machines are not cleaned out, so that the fresh lot of compound is immediately contaminated by the old.

A sample of compound was obtained on the average of once a week from one of the shops for a period of five months. The number of organisms per ml. in many of these samples was determined by serial dilution, usually to 1:100,000 in distilled water, and plating on nutrient agar. Dilutions of less than 1:1,000 were unsatisfactory even when fewer bacteria were present, because of the milky appearance of the agar and consequent difficulty in counting colonies.

Over the entire period of the investigation one kind of organism was present almost to the exclusion of all others. In fact only one other type of organism was found, and this at infrequent

TABLE 1

HOURS IN USE	BACTERIA PER ML.
2	14,480,000
24	27,400,000
52	31,400,000
96	37,800,000
200	25,000,000

intervals. This persistent state of a practically pure culture of one species of bacterium was unexpected under conditions where there was ample opportunity for contamination from many different sources. Cutting compound obtained from another plant some distance away was found to contain the same organism in practically pure culture.

These organisms grow prolifically in the cutting compound. The count on the material circulating in the machines was seldom lower than 15,000,000 per ml., and usually it was around 25,000,000 per ml. Counts in excess of 50,000,000 were not uncommon.

The bacterial count goes up very rapidly either in freshly made compound or in that which has been sterilized by heat. Table 1 will demonstrate the rapidity of the increase of the bacterial

population in freshly made emulsion after circulation in the plant.

IDENTIFICATION OF THE ORGANISMS

The organism found so abundantly in the oil-water emulsion is a short motile rod which stains negatively by Gram's method. It is not acid-fast and no capsules or endospores are present. It grows well either at room temperature (usually between 25 and 30°C.) or at 37.5°C.

When grown on agar this organism appears to be almost coccoid in shape, measuring about 0.8μ by 0.5μ. There is little variation in size in the organisms in an agar colony. A 24-hour old broth culture exhibits moderate turbidity with a slight amount of yellowish viscid sediment. No pellicle or ring is found and no color is produced in the broth. After three hours' growth in the broth at 37.5°C., the organisms increase in length to about 1.5μ. They are found singly and in pairs. Repeated subcultures in nutrient broth cause most of the bacteria to increase to two or three times their original length, and a few short chains are found. Some of the larger rods exhibit marked bipolar staining. No fermentation was observed in broth to which 2 per cent of various sugars was added; glucose, lactose, sucrose, galactose, xylose, mannitol, salicin and glycerol were thus tested. Stab cultures in agar with various sugars added gave the same result; growth extended only about two-thirds the length of the stab. The organism seems to be a strict aerobe, and its growth is favored by bubbling air through cultures, and by the aeration received by cutting compounds during circulation in machine shops.

Indole is not formed; nitrates are reduced to nitrites. Starch is hydrolyzed. Gelatin is not liquefied after six weeks. No change is produced in litmus milk. Good growth occurs on potato slants.

The surface agar colonies after 24 hours are 1 to 2 mm. in diameter. After about five days, if the colonies are not crowded on the plate, they measure about 5 mm. in diameter. These colonies have an opaque creamy appearance, and when viewed by transmitted light present a marked fluorescence. The edges

are smooth in young colonies, but become slightly erose in some of the older colonies. Most colonies are smooth and raised with shining convex surfaces, but when plated directly from the emulsion, some of the colonies show a slightly raised ring halfway between the center and edge of the colony.

The deep agar colonies are buff-colored and lens shaped, measuring from 1 to 1.5 mm. in length and about 0.5 mm. across. These colonies are not fluorescent. If the plates are crowded with colonies, these deep agar colonies are very small, being about 0.25 mm. in length, and they do not assume the buff color, but remain an opaque gray shade.

The growth on an agar slant shows erose edges but a smooth raised surface. It is fluorescent but none of the fluorescence is imparted to the medium.

The colonies on the gelatin plate are similar to those on agar plates except for size, not exceeding 1 mm. in diameter. They have the same characteristic fluorescence, which is not imparted to the medium.

This bacterium belongs to the order Eubacteriales, family Bacteriaceae, tribe Chromobacteriae. Except for the fact that the fluorescent quality of the colonies is not imparted to any of the artificial media used, it conforms with the characteristics of the genus *Pseudomonas*, members of which are abundant in soil.

Sohngen (1913) describes certain pseudomonads which are able to utilize hydrocarbons in their metabolism. Gray and Thornton (1928) found that *Pseudomonas boreopolis* was able to utilize aromatic compounds such as naphthalene. The species here described differs from the latter species in its failure to liquefy gelatin with production of red pigment, and to produce acid from glucose, and it does not conform in all its characters with any other known species of the genus. It is therefore considered a new species, for which the name *Pseudomonas oleovorans* is proposed.

SOURCE OF FOOD

The oils used in the cutting compounds are not generally thought of as favorable media for bacterial growth. The bac-

teria present are usually attributed to germs washed off the hands of workers, or derived from their sputum or other extraneous sources. Food for multiplication is thought to be supplied by remnants of lunch, etc., which are occasionally found in the pits containing oil. However, the excessively high counts on the material circulating in the machines are comparable with those in stale and soured milk, and make it evident that the cutting compound itself serves as a source of food for the growth and multiplication of the bacteria found in it. Furthermore, the continued presence of a single type of organism of a species not commonly found as a contaminant from human hands, sputum or excreta, or as a common inhabitant of foodstuffs, renders the hypothesis of miscellaneous pollution and nutrition from outside sources entirely untenable.

Experiments were made to see if the bacterium isolated from cutting compound was able to grow in sterile cutting compound which had never been used in the shop.

Using the soluble oil which, so far as we were aware, contained no vegetable or lard base, an emulsion of one part oil and eight parts water was made. The resulting emulsion was sterilized in the autoclave, then inoculated with a water suspension of the bacterium that had been grown on agar. To 10 ml. amounts of material 1 ml. of a bacterial suspension containing 22,500 bacteria per ml. was added, the resulting mixture containing approximately 2045 bacteria per ml. Counts made four days later showed that the bacterial population had reached 3,075,000 bacteria per ml. In another instance in which 1,000 organisms were inoculated into 10 ml. amounts of sterile laboratory-made cutting compound, the bacteria increased in number from the original 100 per ml. to 11,550,000 per ml. in three days and to 22,600,000 per ml. after five days. Using oil that had a sulphonated lard base to make up the emulsion, similar experiments were tried. Inoculating larger amounts (100 ml.) so that each ml. contained 5 or 6 bacteria per ml., the count at the end of 5 days averaged five million per ml. on the four samples so treated.

Cutting compound that had been circulating in the shops was sterilized in the autoclave and reinoculated with a water suspension containing 300 bacteria per ml., so that the emulsion in the

flasks contained 3 bacteria per ml. After 72 hours incubation the count averaged 3,000,000 bacterial per ml. in two samples. The oil that had been used in the emulsion had a sulphonated lard base.

In another instance 10 ml. of sterilized cutting compound that had previously been circulating in the shops was inoculated with 300,000 bacteria (30,000 per ml.); after 48 hours incubation the count showed the presence of 50 million bacteria per ml. The oil used in making this emulsion was from petroleum products and did not contain lard oil so far as we are aware.

From these experiments it is clear that the bacteria do not require any food supply other than the emulsion itself. Furthermore, the composition of the oil, that is, whether it is a soluble oil containing animal or vegetable fat or a purely mineral oil, seems to make no difference in the number of bacteria which develop in it. The pH of the laboratory-prepared material and shop material was the same, measuring 9.4 in both instances as determined by the Wolf apparatus.

Since the bacteria were able to utilize the oil as a source of food material an effort was made to determine what was in the oil that could be used by these organisms. It was thought possible that there might be some water-soluble substance in the oil that could be utilized as a source of food.

Various methods were tried for breaking the emulsion in order to obtain the water fraction free from oil, and this was satisfactorily accomplished by addition of enough $CaCl_2$ or NaCl to give a 6 to 7 per cent solution. Tests for protein, fat, and carbohydrate were negative on the water portion obtained from the emulsion made up with soluble oil containing no lard oil. From the water fraction obtained from the emulsion made with oil having a sulphonated lard oil as a base, a slight amount of saponification was secured.

These tests proving unsatisfactory, it was assumed that the bacteria were obtaining their food supply from the compounds in the oil which they were able to utilize when the oil was emulsified. Petroleum contains a variety of aliphatic and cyclic compounds, as saturated and unsaturated hydrocarbons, some cresol, naph-

thenic acids, benzene, etc., some of which might be used by bacteria as food. The naphthenic acids present serve as emulsifying agents, since soaps are unsatisfactory for technical reasons.

It was thought possible that the naphthenic acids might form at least one important food source for the bacteria. To obtain some light on this, a 1 per cent solution of crude naphthenic acids in Locke's Basal Solution was inoculated with the bacteria isolated from the cutting compound. These bacteria did not grow in the medium unless furnished with a source of nitrogen. When 0.1 per cent potassium nitrate was added to the medium the bacteria were able to grow. The bacteria did not grow in a medium containing only carbohydrate as a carbon source, proving that they were able to use the crude naphthenic acids as a source of carbon.

Nitrogen is probably supplied by the petroleum in the nitrogenous bases present in it. Presence of these bases could not be demonstrated in the oil used in these experiments due to inability to concentrate the oil sufficiently.

The organisms have rather a long lag phase and grow less abundantly when inoculated into emulsions made up in the laboratory. Only in a few instances were the counts in the laboratory comparable to the counts obtained on plant material, probably due to the continual aeration while circulating in the plant.

CONTROL

Many attempts to suppress the growth of organisms in cutting compounds have been made. In our own laboratory we have tested the effectiveness of various proprietary coal tar disinfectants from time to time in the past. When added to cutting compounds in the laboratory they were effective, counts after 10 to 24 hours showing that 99 to 100 per cent of the bacteria formerly present had been killed. Yet when the same concentrations of these disinfectants were used in the material circulating in machine shops they were found to be ineffective. After 24 to 48 hours the bacterial count was from 5,000,000 to 10,000,000 per ml. in spite of the fact that the odor of the disinfectant

was still very noticeable. All of the coal tar disinfectants tested are soluble in oil, and it seemed probable that these compounds had entered the oil phase of the emulsion and were no longer available for the destruction of bacteria living in the water phase. The use of these disinfectants failed to prevent the development of a bad odor and blue color in the cutting compounds.

In the hope of finding some chemical that could be used to advantage in keeping down the numbers of bacteria in the cutting compounds without otherwise damaging them we tried out a number of water-soluble substances.

Since aeration had been found to be beneficial to the organisms, the effect of bubbling CO_2 into cultures was tried. Three hours of such treatment resulted in a reduction of about 90 per cent in the numbers of bacteria present, even when air was also bubbled through.

Chlorine applied as bubbled gas or as chlorine water was effective in destroying the bacteria immediately, but was impractical because it caused the emulsion to break after a few hours. Iodine added at the rate of one part in thirty of a 5 per cent alcoholic solution killed all the bacteria present, but on reinoculation the bacteria grew in a normal manner. It was evident, therefore, that the iodine must have been taken up by some of the organic compounds in the oil, perhaps the free fatty acids or unsaturated hydrocarbons.

Acriflavine was tried, since it is known to have a certain predilection for gram-negative organisms and for staphylococci. Since it acts as a skin irritant except in high dilution, only concentrations of 0.08 and 0.15 per cent were tried. Although these concentrations effected a very great reduction in the number of bacteria, they failed to sterilize. Boric acid even in such large amounts as 1 part of a saturated solution to 4 parts of emulsion was ineffective. Copper sulphate could not be used because it broke the emulsion at once.

In contrast to these unsatisfactory results, our experiments with resorcinol indicated that this was a highly effective chemical for disinfection of the cutting compounds. In a preliminary experiment 10 ml. of a 20 per cent aqueous solution of resorcinol

was added to a 200 ml. sample of cutting compound, giving a concentration of about 1 per cent; the sample contained about 24,000,000 bacteria per ml. Counts made 3, 5, and 24 days later were uniformly negative, while the controls gave counts of from 10,000,000 to 20,000,000 organisms. In another experiment 1 per cent of resorcinol was added to an old sample of compound which had developed the characteristic blue color and bad odor, and contained 8,500,000 bacteria per ml. Counts after 24 days showed no bacteria present, while the untreated material gave a count of 6,000,000. The bad odor disappeared after the material was treated with resorcinol and did not develop again. In another experiment the emulsion, after addi-

TABLE 2

BACTERIA PER ML.	TREATED (300 ML.)	UNTREATED (300 ML.)
Initial	24,300,000	24,300,000
After 1 day	0	38,500,000
Reinoculated with 219,000 bacteria per ml.		
After 3 days	0	31,400,000
After 5 days	0	37,100,000
After 8 days	0	26,000,000

tion of 1 per cent resorcinol, was aerated and reinoculated after 24 hours. The results are shown in table 2.

In addition to the reinoculation after 24 hours, the material was also contaminated by sputum on the fourth day, and was left uncovered throughout the experiment. By the eighth day considerable evaporation had occurred, so this experiment was discontinued.

The results of these experiments indicated that resorcinol in a concentration of 1 per cent is effective in sterilization of cutting compounds, and that it does not lose its effectiveness in the course of a few hours or days as is the case with coal tar disinfectants. In our laboratory experiments, resorcinol had no injurious effects on the emulsion, and it could not be harmful to machines with which the treated emulsions might come in con-

tact. One per cent resorcinol was not found to have any injurious effect on the skin, and it is easily applied in aqueous solution. Although more expensive than most disinfectants hitherto used, if it proved as effective under practical conditions as in the laboratory protection of the oil from decomposition and consequent need of replacement at frequent intervals would more than justify its use.

The uses of resorcinol as a disinfectant for cutting compounds has been tried out under practical conditions in the shops of the Hughes Tool Company. The company reports that in practice a 1 per cent solution of resorcinol proved to be a powerful germicide, and that much weaker solutions have markedly retarding effects on bacterial growth. The surface tension of an emulsion containing 1 per cent resorcinol was found to be 2 or 3 dynes/cm. greater than the same emulsion without the resorcinol, but this was not sufficient to affect the wetting properties.

The testing of the emulsion for the percentage of oil present was found at first to be more difficult with resorcinol in it, due to the fact that the oil layer does not separate well and contains bubbles when acid is added. This difficulty was overcome, however, by adding a few ml. of 2.5 per cent potassium permanganate to oxidize the resorcinol, and adding a few drops of alcohol to break the bubbles.

A method of determining the approximate amount of resorcinol present was developed by treating with ferric iron and comparing the blue color produced with that of standards containing known concentrations of the resorcinol. Experience showed that about 50 pounds of resorcinol per month to a 500-gallon pit was sufficient to keep the bacterial count low; even 15 pounds per month was enough to keep the emulsion from spoiling, although the bacterial count would sometimes run up to 20,000,000 to 30,000,000 per ml. Sometimes emulsions containing more than 0.25 per cent resorcinol turned a muddy red color. The reason for this has not been ascertained; we did not encounter it in our laboratory experiments.

In order to get sufficient reduction of bacteria by the use of

coal tar disinfectants to prevent spoiling, larger amounts must be used, and at more frequent intervals, and the men complain of the odor. The cost of the resorcinol is about $35.00 per month if a low bacterial count is maintained, but only $12.00 per month for enough to prevent spoiling. Although this is higher than the $5.00 to $7.00 cost of coal tar disinfectants it is a very small item compared with the cost of the cutting oil, and fully justified by the less frequent changing of the emsulsion that is required.

SUMMARY

Cutting compounds circulating in machine shops have very high bacterial counts which are seldom less than 15,000,000 per ml. and may exceed 50,000,000 per ml.

One species of bacterium, *Pseudomonas oleovorans* n. sp., was found to be present almost to the exclusion of all others. The principal morphological and physiological characteristics of this species are described.

This organism grows rapidly in the cutting compounds; its growth is favored by the aeration to which the circulating fluid is exposed.

The organism lives on some normal constituent of the cutting compound, probably on the naphthenic acids which act as emulsifying agents. It will grow in a medium consisting of Locke's Basal Solution with 1 per cent of crude naphthenic acids and 0.1 per cent of potassium nitrate.

Coal tar disinfectants, although immediately destructive to bacteria in the cutting compounds, quickly become ineffective, apparently because taken up by the oil phase of the emulsion.

Various water-soluble substances were tried as disinfectants, but many could not be used because of unfavorable effects upon the emulsion. Resorcinol proved to be an effective and usable disinfectant; 1 per cent was sufficient for permanent sterilization under laboratory conditions, and smaller amounts were sufficient to prevent spoilage of the compound. Under practical conditions in machine shops as little as 15 pounds per month for a 500-gallon tank was found sufficient to prevent spoilage.

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