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**How Oil Kills Anopheline Larvae**

**BY**

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## INTRODUCTION.

IN 1892, when L. O. Howard <sup>(1)</sup> first suggested putting oil on water as a method of killing mosquito larvae, the idea underlying the suggestion was to make a uniform film of oil which would act like a complete sheet of ice and prevent the larvae reaching the air to breathe. The idea that a complete film is essential still persists; for example, W. E. Hardenburg <sup>(2)</sup> writes in 1922:—

“The oil must be thin enough to spread and form a film, since, unless there is a uniform film over the surface, many of the larvae will not be suffocated.”

But, since oil often kills all the larvae in a pool even when it does not form a complete film, its method of action is more complicated than that of an inert layer preventing access to the surface. Le Prince and Orenstein <sup>(3)</sup> writing in 1916, to describe their experience in Panama, note that “larvae die shortly after coming in contact with oil,” and discuss the possible causes of the larvicidal properties of oil. Since then much work has been done on the way in which oil acts as a larvicide, and the theories that have been put forward to account for its action may be summarised under the following four headings:—

- (1) Mechanical prevention of access to air.
- (2) Blocking of spiracles or breathing tubes.
- (3) Reduction of surface tension so that the larvae can no longer rest at the surface.
- (4) Toxic action of the oil either directly by contact, or indirectly through the water.

While testing the effect of some oiling of open earth pools, which had been done as a precaution against malaria during the building of a railway, I saw a striking example of the effect of incomplete films. As the pools were being continually altered by the engineering operations small quantities of oil had been applied each day with a mop instead of, as in the usual method, applying larger amounts at weekly intervals. The oil had spread so badly that the engineer who also inspected the area condemned the work at once, and yet not a single larva was found in the oiled area, although in similar pools and cuttings just outside the area many larvae were found. These field observations were recorded in the Federated Malay States Malaria Bureau Reports for 1919 <sup>(4)</sup> and the way in which oil acts was taken up as a subject for more detailed study by laboratory methods. The

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(1) Howard, L. O. : An Experiment against Mosquitoes. *Insect Life*, 1892, Vol. V., pp. 12-14, 109-110 and 199.

(2) Hardenburg, W. E. : Mosquito Eradication. New York, 1922, p. 160.

(3) Le Prince, A. J., and Orenstein, A. J. : Mosquito Control in Panama. New York, 1916, pp. 151-172.

(4) Hacker, H. P. : F. M. S. Malaria Bureau Reports, 1919, Vol. I., pp. 55-60.

problems for investigation were (a) whether the method of oiling adopted had any advantage over the usual method in which the oil is exposed to the action of wind and water for a week or more ; and (b) the reason why the oil in this area had not spread.

The first chapter deals with the appearances seen in larvae killed by contact with oil. The second describes some results caused by differential wetting, whereby water is unable to wet certain parts of the larva which can be wetted by oil or soap solution.

Attempts to devise strictly comparable experiments on the effect of films failed because the spread of any given oil varied in each experiment, and therefore emulsions were prepared and studied as a method of getting oils into a more uniform state for comparison. Chapter III describes these experiments on emulsions, and Chapter IV deals with the effect of vapours.

Chapter V discusses the conditions that govern the spread of oil and is based mainly on the work of Hardy and Langmuir referred to therein.

Spreading of oil, wetting, and surface tension must all depend ultimately on chemical constitution, as the work of Hardy suggests, and each of these groups of facts can be studied independently. In this paper the adsorption theory of film formation, advocated by Langmuir, has been used instead of attempting to apply surface tension data to the problem.

Chapter VI consists of a discussion of the relationship between the spreading power and the toxicity of oils, based on the experiments described in the two previous chapters.

Each chapter ends with a summary of its results and the general conclusions which can be made are gathered together at the end of the paper.

In most of the experiments described the temperature is an important factor, and particularly is this true of the experiments on vapours, and on the spread of oil. But the one advantage of working in the Malay States is the uniformity of temperature and moisture throughout the day and throughout the year. There is neither dry season nor a cold season. The temperature in Kuala Lumpur never reaches 38° C. (100° F.) nor falls below 21° C. (70° F.). This regularity throughout the year is shown by the following meteorological records from the F.M.S. Museum Department for the ten years, 1913-1922 :—

*Monthly mean Maximum.*

Highest in March	...	...	...	90·9° F. (32·7° C.).
Lowest in January	...	...	...	87·5° F.

*Monthly mean Minimum.*

Highest in May, July and August	...	...	...	73·7° F.
Lowest in February	...	...	...	72·2° F. (22·3° C.)

In the laboratory and during the day the conditions were even more uniform, and all the experiments were done between 26° C. and 31° C., and the majority at 28° C ± 1. This variation of temperature can have no effect on the results of the rough methods adopted.

The paper deals primarily with anophelines, and culicines are mentioned only when they were noticed to differ markedly from anophelines.

In comparing the effects of various substances certain precautions were taken to keep the larvae used for the tests as uniform as possible. Also the same methods

were used throughout to detect how long the larvae lived after exposure to the substances. These points will be described here in order to avoid repeating them whenever the experiments are described.

*A. vagus*, the species most readily obtained, was used, the larvae were caught fresh each day, and only those about 4 mm. long were used. The measurement was done by placing the slide containing the larva after identification over squared paper ruled in millimetres, and those obviously larger and smaller than this standard were rejected. Any larva showing much infestation with Infusoria was also rejected, so that the condition of the breathing tubes after death could be examined readily.

The length of survival after exposure was counted from the moment the larva was removed from the action of oil until it no longer reacted to stimulus. For this work all the staff of the Malaria Bureau who could write were impressed, including the clerk and the messenger. They were trained by trial experiments until they could obtain uniform results, and a uniform nomenclature was used such as: "moves," "dives," "bites" or "bites tail" for the movement to remove adherent oil from its spiracle, "bends" for the peculiar side movement of the head, being the terms used for independent movements; and "reacts" and "slight" being the terms used for movements elicited by stimuli.

These major movements only were counted as evidence of life since if the slight jerking movements, or jactitation, were taken into account most irregular results were obtained, depending largely on the sight or opinion of the observer. Only occasional notes of striking occurrences were made while the larva was freely moving and obviously alive, but nearing the end notes were jotted down at quarter-minute intervals, which were shouted out by a man in charge of a stop-watch. When an early death was anticipated only one larva was allotted to each man, but if the life was expected from preliminary experiments to last over an hour, two or even three were entrusted to one observer. During the night one man at a time had charge of some 10 to 20 survivors and the controls to watch. The watching was continued for some time after complete loss of reaction, to make sure there was no return of reactivity. In recording the results seconds will be recorded in times up to 1 hour, but the results must be regarded as possibly being 30 seconds too long. Up to 5 hours the time recorded may be 1 minute too long, and above that the error may be half an hour. Every attempt was made to make the interval between the last reaction and complete loss of reactivity as small as possible, and this limit of error may fairly be applied to all the experiments recorded. The work was done under my personal supervision, and most of the larvae were examined microscopically after death. The night watching was done efficiently, as I lived on the premises, and often checked the results.

I wish to express my gratitude to the whole staff for the steady work they did in collecting and sorting out the material, and for their keenness in the difficult process of watching the larvae. In particular I must mention Mr. P. D. Rajamoney, Laboratory Assistant. Without the scrupulous care for cleanliness and experimental detail that he displayed, the work could not have been done.

The main disadvantage of working in the Tropics is the difficulty of getting literature, but this was minimised by the Librarians of the Tropical Diseases Bureau, London, and the Rockefeller Institute, New York, who kindly sent out annotated lists and even duplicates of original articles on the subject.

# CHAPTER I.

## THE APPEARANCES SEEN IN LARVAE KILLED BY CONTACT WITH OIL.

PRELIMINARY experiments confirmed the fact mentioned in the Introduction that mere contact with oil is sufficient to kill larvae, and one of these may be quoted.

Five larvae of *A. vagus* were placed in contact with a freshly prepared film of kerosene. After about half a minute they were taken out and washed free from contaminating oil by being passed consecutively through three large pie-dishes containing fresh water. They were then placed in small dishes and watched to note how long they survived.

As a control experiment larvae were subjected to all the manipulations of the experiment except the exposure to oil, and lived satisfactorily.

The larvae that had been exposed to oil died as follows :—

A larva exposed for	33	seconds	died in	4	minutes	50	seconds
" " "	30	" "	" "	6	" "	30	" "
" " "	30	" "	" "	6	" "	35	" "
" " "	34	" "	" "	8	" "	—	" "
" " "	31	" "	" "	12	" "	10	" "
Average time of contact			...	31.6		seconds	
Average length of survival			...	7		minutes 37 seconds	

When the larvae killed by contact with oil were examined microscopically they were found to contain oil in their breathing tubes, and it is the appearance presented by this oil that is the first subject for discussion.

The normal appearance of the breathing tubes is fully described in textbooks (<sup>1</sup>), and only the essential features need be mentioned. They are a pair of tubes extending from the penultimate segment of the abdomen to the thorax, where they break up into fine branches which extend into the head. They communicate with the exterior by only one pair of openings, the spiracles, on the back of the penultimate segment. In each segment and in the thorax they give off lateral branches to supply the muscles and other organs with air.

(<sup>1</sup>) Howard, L. O., Dyar, H. G., and Knab, F. *The Mosquitos of North and Central America and the West Indies*, 1912, Vol. I., p. 89, which quotes :—

Raschke, E. Walther. *Die Larve von Culex nemorosus*. Ein Beitrage zur Kenntniss der Insecten Anatomie und Histologie. *Archiv Naturgesch.*, Vol. LIII., pp. 133-163.

Giles, G. M. *A Handbook of Gnats or Mosquitoes*, 1902, pp. 48-51.

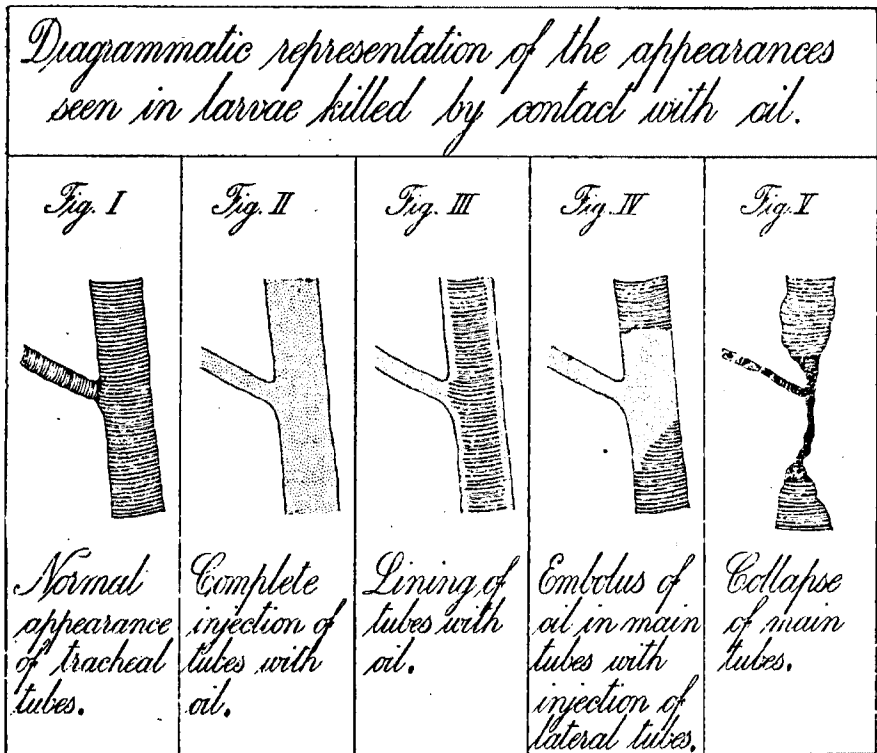
Patton, W. S., and Cragg, F. W. *Medical Entomology*, 1913, pp. 200-201.

## 6 APPEARANCES IN LARVAE KILLED BY CONTACT WITH OIL

In a normal, freshly caught larva the main tubes are almost opaque, owing to the air that they contain, and show the minute transverse striations seen in fig. I and at B in photograph 4. The tube is fixed at each septum between the segments and sags outwards a little in the middle of the segment, but the outline is perfectly clean cut and uniformly curved.

### COMPLETE INJECTION OF TUBES.

If such a larva is placed in kerosene or crude oil the tubes become translucent, because the oil enters the tubes and completely fills them. The striation on the tubes is lost and the course of the tubes can only be made out by careful focussing. The small tubes are rendered perhaps a little more evident owing to their glistening turgid condition, but they also lose their natural opacity. This effect is shown diagrammatically in fig. II and actually at E in photograph 4.



An example of Fig. I is seen at B in photograph 4.

" " " II is seen at E in photograph 4.

" " " III is seen in photograph 3.

" " " IV is seen in photograph 4.

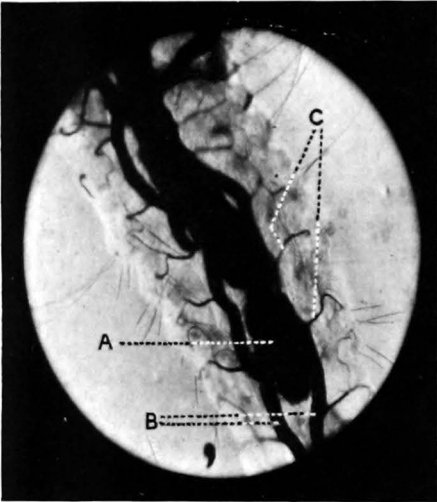
The oil can be seen entering the tubes, and the progressive injection of the tubes can be watched under the microscope. A larva is immobilised by placing a cover-glass over its head and thorax, leaving its tail exposed. If now a drop of kerosene be applied to the tail the oil can be seen moving in a jerky manner along the tubes, and successive portions of the tube become translucent. Any wriggling



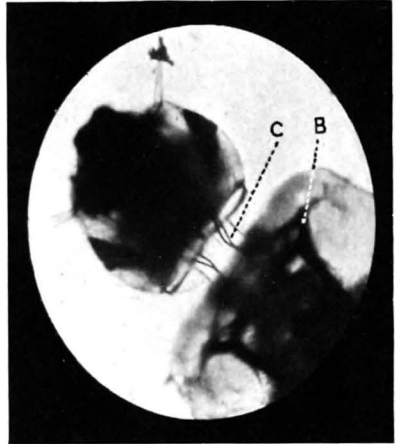
PHOTOGRAPHS ILLUSTRATING OIL IN THE BREATHING TUBES.

In Photographs 1, 2, and 3, coconut oil stained red by Sudan III was used.  
In Photograph 4 the oil was kerosene.

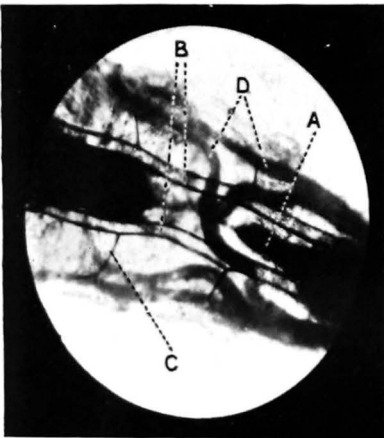
- A = food material in alimentary canal.
- B = main breathing tubes.
- C = branches of main tubes.



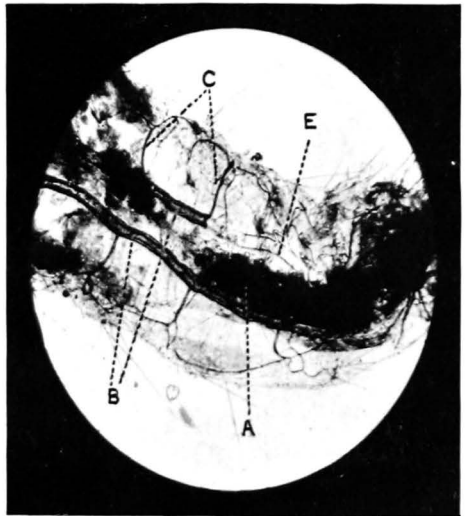
No. 1.—Complete injection of breathing tubes, with the lateral branches also injected.



No. 2.—Complete injection showing oil in the minute branches to the head.



No. 3.—Lining of the tubes. The lateral tubes are completely injected. The objects marked **D** are Malpighian tubes.



No. 4.—Embolus of oil rendering part of main tube and the lateral tube transparent. At **B** and **C** normal tubes are seen.

movement of the larva causes the oil to move more rapidly. When the oil reaches the point at which a branch tube is given off the branch is injected so quickly that the eye can hardly follow the progress of the oil.

Various methods were tried to demonstrate the distribution of oil in the tubes.

Suspensions of lamp-black and of carmine in kerosene failed because the particles were filtered out at the spiracle and the oil alone entered. Oildag (1) however, which is a proprietary lubricant, claimed to be a colloidal solution of graphite, entered the tubes, carrying the particles of graphite with it. Brunswick black, when diluted with a little xylene, also entered the tubes and stained them brown.

Another method tried was to inject the larva with olive oil and then to stain the oil in the tubes with osmic acid, since olive oil previously stained with osmic acid did not carry in the black particles of osmium oxide. But the osmic acid did not penetrate the cuticle of the larva until each segment had been either pricked with a needle, or broken by pressure on the cover-glass. This spoilt the larva as a microscopic specimen, but parts could be found in which the finest tubes were stained a deep black. The staining with osmic acid did not occur in larvae which had not been injected with oil, a point of some importance in the future discussion (see p. 9.)

The best method was found to be that used in preparing the photographs, namely, to stain a vegetable oil with Sudan III before using it for injection. Vegetable oils were chosen in preference to kerosene because Sudan III is only slightly soluble in mineral oils, but stains a vegetable oil a deep red. The oil is stained by shaking up a slight excess of the Sudan III in the oil and warming gently. Too much heat should not be applied, neither should the stained solution be kept long. In either case small red needle-shaped crystals separate out which do not enter the tube. Photograph 1 shows the abdominal segments of a larva. Its breathing tubes are distended with this red oil which also extends into the lateral branches. Photograph 2 shows complete injection of the main tubes, B, in the thorax, and also of the small branches, C, to the head.

Other small branches which have been found injected are the group of vessels which are distributed to the hind end of the intestine, and the small branch which runs back towards the thin-walled last segment and tracheal gills.

#### LINING OF THE BREATHING TUBES.

Figure III and photograph 3 show an appearance which is sometimes seen in a living larva that has not received an amount of oil sufficient to completely fill the tubes. The large vessels are lined by a layer of oil which in optical section gives the appearance shown in the photograph. The branch tubes, however, are completely filled. Occasionally an irregular or incomplete injection occurs; part of the tube is completely injected and the rest shows only this lining of the wall.

Lining also occurs about an hour after a fully injected larva has died, some of the oil is extruded and clings as a small drop to the spiracle. This happens so regularly that it is impossible to make a permanent preparation of a fully injected

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(1) Prepared and given by E. G. Acheson, Ltd., 40, Wood Street, Westminster.  
Memorandum on Solid Lubricants, Dept. Sci. and Indust. Res., Bull. No. 4, 1920.

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larva. That this is not due to the pressure of the cover-glass forcing out the oil is shown by the fact that it occurs even when the larva is left in water. It is probably due to some post-mortem change driving the air back into the tubes from which it had been expelled by the oil. What happens to the air when the oil enters the tubes is a difficult question. If a small terrestrial insect be placed in oil the air can be seen leaving some of the spiracles while the oil is entering at others. The breathing tubes of anopheline larvae, however, have only one pair of spiracles and when the oil enters it must displace the air into the muscles and other organs of the body. Sometimes a minute bubble of air is liberated at the moment that the oil touches the spiracle, but no escape of air can be detected with the microscope while the oil is moving along the tube. As a rule no bubble of air can be demonstrated if the larva is squashed flat under a cover-glass after injecting it with oil; only on rare occasions can any free air be seen. But it can be shown that there is still air in the tissues of the larva by reducing the atmospheric pressure and so causing the air to expand. An injected larva sinks in water, and if the pressure is reduced by 15 cm. of mercury a small bubble of air forms at the spiracle,<sup>(1)</sup> the larva becomes lighter than the water, and floats to the surface. The air must be partly dissolved in the plasma and partly held in intimate contact with the muscle fibres and organs of the body. After-death changes in the plasma making the air less soluble and also post-mortem contraction of the muscles, explain the expulsion of the oil.

If a larva is killed in formalin vapour the muscles are contracted and putrefactive changes are decreased, and in this way a permanent preparation showing injection of the tubes can, but still with difficulty, be made.

### EMBOLISM OF TUBES.

Sometimes only a small drop or embolus of oil is seen in a tube. This usually occurs in larvae exposed to an emulsion of oil, and the embolus is probably a globule of oil that has entered the tube, but it also occurs after momentary contact with a film of oil. It is most often seen opposite the origin of a lateral branch, and then the branch is full of oil as shown in Figure IV and Photograph 4. The embolus is not stationary, but moves from place to place in the tube with the movements of the larva, filling any lateral tube against which it stops. This causes an appearance that is sometimes seen, namely, oil in the lateral tubes and in the branches to the head, but none in the main tubes. Sometimes the embolus only blocks the spiracle and end of the main tube.

### COLLAPSE OF TUBES.

This appearance, shown diagrammatically in Figure V, usually accompanies embolism, but may occur independently in larvae that show no obvious oil in the tubes. The normal uniformly curved appearance of the tubes is lost and the wall becomes wrinkled and irregular. In places the tube is reduced to a dark thread and the cavity of the tube is obliterated by the sticking together of its inner surface. A given area of collapse may be watched and observed to recover entirely, and this distinguishes this appearance from ecdysis of the lining membrane.

These appearances explain why larvae are killed by contact with oil. Imme-

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(<sup>1</sup>) The water in this experiment must be boiled previously in order to free it from dissolved air.

diately after contact with oil the larva can be seen to "bite its tail" as if it were trying to remove the oil which has entered its breathing tubes. The oil in the main tubes must interfere with the tracheal method of respiration; and the injection of the group of vessels to the intestine, and of the branch to the tracheal gills, must interfere with the intestinal and gill method of respiration respectively. Also a number of larvae were found killed with emboli in the vessels to the head without other evidence of oil in their tubes, and this suggests that the presence of oil in the neighbourhood of the nervous ganglia may cause death. There is also the effect of poisonous substances in the oils to be considered. Some larvae injected with olive oil stained with Sudan III were found alive on the following day. They were quite lively and the tubes were still full of red oil, but they ultimately died before pupation. Olive oil is not poisonous and there is a marked contrast between its action and the action of the short contact with kerosene described in the experiment at the beginning of this chapter. In considering the toxic action of oils it should be remarked that many of the larvae showed red-stained oil in the stomach, in addition to the oil in the breathing tubes. In 1902 Ross (1) suggested that oil entered the breathing tubes and caused suffocation of the larvae, but the first experimental proof that I can find is a paper by Takatsuki (2) in 1917. In 1918 Freeborn and Atsatt (3) gave a very clear account of the entry of oils into breathing tubes and used Sudan III for staining kerosene.

In his first paper Takatsuki suggested that there might be some "fat-like substance in the membrane, which repels water, but briskly combines with petroleum." The breathing tubes show no evidence of fat. Osmic acid did not stain either the large or small tubes when applied as described on p. 7, unless olive oil was present. Sudan III did not stain the tubes, but only adhered to the membrane when it was dissolved in oil. When treated with carbol-fuchsin and hydrochloric acid the tube was not acid-fast, although parts of the cuticle retained the stain. Antiformin first dissolves the softer tissues, and the resulting gas fills the tubes and makes them prominent, but the tubes do not resist the action of antiformin, and ultimately dissolve. We may conclude, therefore, that there is no fatty or waxy substance in the tube, and Takatsuki in his second paper, 1920, attributes the entry of oil to a specific chemical affinity of petroleum for the lining membrane of the tubes.\*

Oil is also able to wet the palmate hairs which resist wetting by water but not so successfully as the spiracles. In larvae killed by oil a globule of oil is often seen clasped by the leaflets of the palmate hairs.

In natural conditions larvae are not drowned by water entering their breathing tubes, but in the laboratory they sometimes show collapse, lining and emboli of

(1) Ross, R. *Mosquito Brigades*, 1902, p. 34.

(2) Takatsuki, A. *An Essential Property of Petroleum for Mosquito Control*. *Kyoto Igaku Zasshi*, 1917, Vol. XIV., p. 84.

Takatsuki, A. *Die echte Wirkungsweise des Petroleums auf die Insekten*. *Kyoto Igaku Zasshi*, 1920, Vol. XVII., p. 67.

(3) Freeborn, S. B., and Atsatt, R. F. *The Effects of Petroleum Oils on Mosquito Larvae*. *Journ. Econ. Entom.*, 1918, Vol. XI., pp. 299-308.

(4) Shozo Toda also referred to this specific affinity in a paper at the Pan-Pacific Conference, 1923. An abstract appears in *Med. Journ. Australia*, 1923, 10th year, p. 341.

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their tubes, even when they have not been exposed to oil. This partly explains the difficulty in rearing larvae, and later it will be shown that laboratory water can increase in wetting power (see p. 33).

The entry of oil depends upon the power of wetting, as can be shown by the following simple experiment: if a capillary tube be lined with wax, water is not able to enter, but paraffin oils do so readily. The pure hydrocarbons mentioned on p. 35 are able to enter the breathing tubes of larvae, and therefore it is not the impurities in crude oil and kerosene which have the affinity for the breathing tube which Takatsuki describes, but it is the hydrocarbon part of the oil.

It is not yet understood why certain liquids wet certain substances. The preparation of some oil paints furnishes a good example of differential wetting. The powder is made into a paste with water, and this paste is ground up with oil. The oil has a greater attraction for the powder than has the water, and takes the place of the water which flows away. The subject of differential wetting is ably discussed by Nuttall (<sup>4</sup>), and in the following chapter some of its effects during the aquatic life of the anopheline will be described.

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(<sup>4</sup>) Nuttall, W. H. Industrial Applications of Wetting Power. British Association. Fifth Report on Colloid Chemistry, 1923, pp. 39-47.

Other examples of differential wetting are described by W. Clayton. The Theory of Emulsions and Emulsification, London, 1923, p. 135.

## CHAPTER II.

### SOME EFFECTS OF DIFFERENTIAL WETTING.

THE spiracle has been shown to resist wetting by water. The palmate hairs do so to a less extent, but the rest of the larva is completely wetted. These facts have an effect on the life-history of the larva, and on the mode of action of oil.

The spiracular apparatus has been redescribed in detail by S. R. Christophers<sup>(1)</sup> with excellent figures showing the homologies that exist between the anopheline and culicine structures.

Most of the effects to be described depend on the depression that occurs round dry objects floating on the surface and the heaping up of water that takes place around wet objects: this unevenness of water surface can be seen around a floating larva. These deformations of surface produce an unstable equilibrium in the water which results in neighbouring particles converging or diverging. Two dry objects converge; two wet objects converge; a wet and a dry diverge<sup>(2)</sup>. If a dry match is placed in water near a floating piece of dry wax, the wax converges, or is "attracted," to the match. If a wet match be used, the wax diverges, or is "repelled," from the match. In each case by moving the match the wax can be "dragged" or "pushed" all over the water, just as a floating needle can be moved with a magnet. But these movements are obviously not due to attraction or repulsion, although the terms will be used for the sake of brevity.

We are now able to discuss the effects due to the dryness of the spiracle. The larvae are heavier than water, and sink after death. Batches of 10 larvae were placed in salt solutions of different specific gravities. All the *A. vagus* sank in a specific gravity of 1.036, all floated in one of 1.060; and only three out of thirty were heavier than 1.052. All the *A. hyrcanus* larvae were heavier than 1.018 and lighter than 1.036. The larvae therefore are only able to float on the surface owing to the dryness of their spiracles, just as a waxed needle can float on the water.

The horizontal position of the anopheline larva is mainly due to the flat quadrangular surface of the spiracular apparatus, but the palmate hairs also assist. Some anopheline larvae have no palmate hairs, and the palmate hairs of larvae kept in the laboratory often become wet and therefore useless. But these larvae also lie horizontally; the only difference being that the small depressions of the water caused by the contact of dry palmate hairs do not occur. A 0.1 per cent. certainly,

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(1) Christophers, S. R. The Development and Structure of the Terminal Abdominal Segments and Hypopygium of the Mosquito, with Observations on the Homologies of the Terminal Segments of the Larva. Ind. Journ. of Med. Res., 1922, Vol. X., pp. 530-572.

(2) Watson, W. A Textbook of Physics, 1907, pp. 180-186.

and even a 0.01 per cent. solution<sup>(1)</sup> of sodium oleate is able to wet the spiracle, and a very interesting state of affairs is seen. The larvae are able to stay at the surface by muscular movements only, but are unable to rest there. They ultimately die exhausted with their tubes full of soap solution. The pupae, however, are lighter than water and float when dead. Pupae of both *A. vagus* and *A. hyrcanus* floated in a solution of alcohol of specific gravity 0.958, but sank in one of 0.944. Soap solution has the same specific gravity as water, pupae lie inert at its surface, and die from wetting of their spiracles. Pupae die more quickly than larvae owing to the absence of the protective mechanism that the larvae have, as a result of which they are able to close their spiracles into a cavity containing air. During the struggles of the larvae the air can be seen to leave these cavities, when it is displaced by the soap solution. To use soap as a larvicide would be an expensive method, since sufficient must be added to precipitate the calcium and magnesium salts in the water before the soap can act. In some parts of the tropics, however, a crude soap might be made on the spot, and the method might be useful. An interesting observation bearing on this point was made on a series of standpipes. Larvae were found in the waste water from these, but not in the waste from one which was constantly used for the washing of clothes.

Differential wetting also explains the position that anopheline larvae assume against the side of a dish. They rest, as shown in photograph A, at right angles to the edge of the water, disposed radially with their tails outwards and their heads towards the centre. This depends on the fact that the hind end of the segment bearing the spiracles and the tail hairs are wet, and therefore "attracted" to the edge. The spiracles are dry and therefore "repelled" from the edge, forcing the larva out into this characteristic position. If the side of the dish is kept dry by lining it with paraffin wax the larvae lie parallel to it, owing to the dry spiracle being "attracted." Photograph B shows the same features by using a wet and a dry microscopic slide.

Differential wetting also explains the geometric patterns, stars, triangles and other figures, that the eggs make when floating on water. Giles<sup>(2)</sup> regards this as "accidental and of no moment," but he quotes Nuttall and Shipley's<sup>(3)</sup> beautiful observation that the sharp or tail end of the egg is drawn up on to a wet edge, leaving the blunt head end to point downwards, so that the "larva emerges into the water and not into the air." This depends on the tail end being more wetted relatively to the head end. If the eggs are drawn out of the water on a wet slide most of the eggs will present the tail end uppermost, but if a dry slide be used the head end is uppermost. The geometrical figures occur because the dry ends are "attracted" to one another and the wet ends are "attracted" to the wetter parts of the eggs. If the eggs are wetted by immersion in soap solution they still float on water, but they cling together in masses and no longer show the geometrical patterns.

These results of differential wetting are important in the life history of the

(1) These percentages are only approximate because the amount of moisture in sodium oleate is variable.

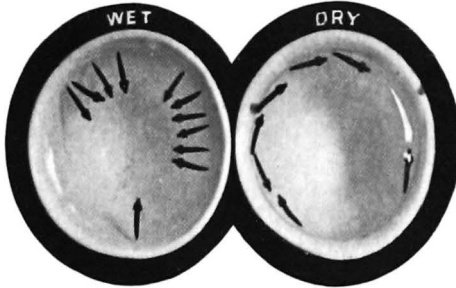
(2) Giles, G. M. Gnats or Mosquitoes, 1902, Second Edition, p. 125-6.

(3) Nuttall, G. H. F. and Shipley, A. E. On the Structure and Biology of *Anopheles (maculipennis)*. Journ. of Hygiene, 1901, Vol. I., p. 49.

### THE POSTURE OF ANOPHELINE LARVAE ON THE SURFACE OF WATER.

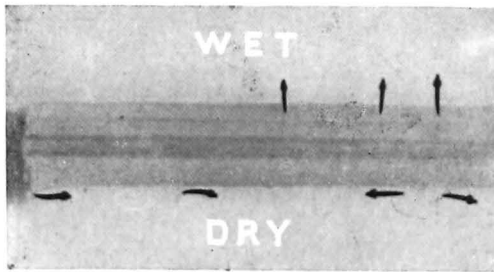
The photographs are about natural size and were taken with the camera pointed vertically down towards the surface of the water. In Photograph B the two microscopic slides are resting against each other with their upper edges projecting slightly from the water.

PHOTOGRAPH A.



Two dishes showing position assumed by anopheline larvae at wet and dry edges.

PHOTOGRAPH B.



Showing larvae at right angles to a wet slide and parallel to a dry one.



larva. The horizontal position enables the larva to escape the notice of fish. Experiments have shown that culicine larvae, hanging down into the water as they do, are eaten by fish more readily than anophelines. The "attraction" to the edge is a further assistance in escaping from fish; and the position due to differential wetting puts the head of the larva into the best position for obtaining food, and also the tail hairs in the best position for grappling with their hooklets for support. These hooklets were first described in 1920 by Iyengar <sup>(1)</sup> for certain species in India, and Lamborn <sup>(2)</sup> independently described them for most of the species in the Malay States. In a later paper on Indian species Iyengar criticises a remark in Lamborn's paper, and says the position at right angles to the side of a dish does not depend on the action of the caudal hooklets, because it occurs when they have been cut off. The position has been seen to depend on the dryness of the spiracle and the wetness of the tail. It occurs with dead larvae and can be imitated by a small wet splinter of wood with a bead of paraffin to represent the spiracle. Such a model will move to the edge and take up a position at right angles to it; if disturbed with a needle it will return to its original position. It is interesting to note that such an apparently vital and purposeful movement is due wholly to physical forces.

The results of differential wetting also explain the observation of Thibault <sup>(3)</sup> that a dry vegetable powder can be used to kill larvae. He says that the larvae die after contact with the powder, even when immediately removed to another dish, but that the powder loses its effectiveness as soon as it is thoroughly wet. This is probably due to the "attraction" between dry spiracle and dry powder. I have not had the same results with polyporous spores, pollen from rushes, and talc powder, but probably these powders became wet too quickly. Paraformaldehyde, <sup>(4)</sup> however, collects round the spiracles, and permanent preparations have been made showing the powder still adhering to the spiracles.

Differential wetting also explains why a complete film of oil was not necessary in the case mentioned in the Introduction. Sooner or later the larvae will come near to the edge of the oil film. Their spiracles will be "drawn" into the dry oil, contact will take place, and the larvae will be killed in the way described in the first chapter.

Certain differences presented by culicines may now be described. They are much more difficult to inject with oil because their breathing tubes open into a

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<sup>(1)</sup> Iyengar, M. O. T. Preliminary Report of a Malaria Survey of Calcutta and Environs. Ind. Journ. Med. Res., Special Ind. Sci. Cong. Numb., 1920, p. 15-16.

Iyengar, M. O. T. A note on Grappling Tail-hooks in Anopheline Larvae. Ind. Journ. Med. Res., 1922, Vol. IX., p. 630-633.

<sup>(2)</sup> Lamborn, W. A. The Nature and Function of the Caudal Tufts of Malayan Anopheline Larvae. Bull. Entom. Res., 1921, Vol. XII., p. 91-97.

<sup>(3)</sup> Thibault, J. K. Vegetable Powder as a Larvicide in the Fight against Mosquitoes. Journ. Amer. Med. Assoc., 1918, Vol. LXX., pp. 1215-6.

<sup>(4)</sup> Roubaud suggests the use of this substance, and says it has a specific effect on anopheline larvae.

Roubaud, E. Emploi du trioxyméthylène en poudre pour la destruction des larves d'Anophèles. C. R. Acad. Sci., 1920, Vol. CLXX., pp. 1521-2.

cavity in the long syphon.<sup>(1)</sup> This cavity becomes full of oil, but the pressure of air in the tubes is able to keep it out. If the front part of the larva be crushed or cut across, however, the oil enters readily and permanent preparations have been made in this way showing the tubes full of oil.

The structures at the tip of the syphon are, as Christophers points out, homologous with the spiracular apparatus of anophelines, but the distribution of wet and dry areas are more symmetrical and there is no projection of the wet hind end of the larva as there is in the anopheline. As a result of this the culicine larvae are neither "attracted" nor "repelled" by a wet edge and lie scattered over the surface of water in a dish. Neither are they "attracted" to each other or to a dry piece of paraffin wax floating on the surface. The wetness of the tip of the syphon can be increased by placing the larvae in a 0.01 per cent. solution of sodium oleate, and when this is done they are "attracted" to the wet side of the dish like anopheline larvae.

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(<sup>1</sup>) See p. 539 of the paper by Christophers, quoted on p. 11.

### CHAPTER III.

## THE EFFECT OF WATER ON THE KILLING POWER OF OIL.

EMULSIONS were prepared by shaking a small quantity of oil in a large volume of water, and they were studied as a means of comparing the action on larvae of different oils under uniform conditions.

The scheme of the experiments was to prepare an emulsion of given strength, to expose larvae to its action, and then to wash them free from any adhering oil and to observe how long they survived in clean water. The preparation of the emulsion of course varied in different experiments, but the technique for exposing larvae to its action was the same in most experiments, and the method of washing and observing the length of life was the same in all. These common parts of the experiments will be described at once for the whole series, to save repetition.

*Method of Immersing the Larvae.*—Perforated spoons were made out of wire gauze. These were placed on small porcelain dishes containing water so that the bowl of the spoon was below the surface. The measured larvae used in the test were placed in the spoon. The emulsion was shaken and a sample was poured out into a similar dish. The spoon containing the larvae was taken off the water and put on the emulsion. After an interval of about half a minute the spoon was lifted off and the larvae were transferred to water for washing. After each experiment the spoons were freed from oil by passing them through a flame.

*Technique for Washing.*—Four pie dishes, each containing about 30 oz. of water, were used. The larvae, after exposure to the emulsion, were transferred from one dish to the next by similar wire gauze spoons. One of these was made for each dish and they were cleaned by passing them through a flame after each experiment.

The fourth dish was divided into two parts by a piece of mosquito gauze, and in one half control larvae were kept. These controls were 10 larvae that had been passed through the first three dishes. They were left in the fourth dish throughout the experiment to show that no oil had reached that dish during the washing of the experimental larvae. The first three dishes were emptied and filled with clean water between each experiment.

*Method of Observing the Length of Survival.*—For observing how long the larvae lived they were removed from the fourth dish and placed in small porcelain cups, holding about  $\frac{1}{2}$  oz. of water. The length of life was determined by the method described in the Introduction.

A second series of 10 control larvae was put up in similar cups to show that the manipulations of the experiment did not kill the larvae. These larvae lived satis-

factorily, but in the conditions of the experiment any larvae living for 600 minutes (10 hours) may be regarded as not showing any effect of contact with the oil.

The first experiments showed a marked difference between the effect of an emulsion when it was first prepared and its effect when tested subsequently. This was thought at the time to be a demonstration that oil lost activity rapidly in contact with water and, therefore, to bear on one of the questions put forward in the Introduction. Many experiments were done on these lines before it was discovered that the loss of activity was not due directly to contact of the oil with water. Some of these experiments will be quoted to show how regular the loss of activity was in certain cases. It must be emphasised that these are selected experiments, many of the results are not so regular and some showed marked discrepancies. These aberrant results led to the discovery of the true explanation of the loss of activity, and this will be discussed after the experiments have been described.

EXPERIMENT A.

*Technique.*—A 0.5 per cent. emulsion of kerosene was prepared by mixing 2.5 c.c. of kerosene with 500 c.c. of water in a large stoppered bottle. The capacity of the bottle was about 1,000 c.c. and its internal diameter about 8.5 cm. The emulsion was shaken vigorously before each test.

*Results.*—The following tables show the results obtained in two such experiments, but many similar ones could be quoted :—

Age of emulsion.	Length of exposure of emulsion.	Length of survival in fresh water.
2 min.	35 sec.	2 min.
35 "	35 "	31 "
76 "	40 "	59 "
125 "	40 "	78 "
270 "	38 "	81 "

Age of emulsion.	Length of exposure of emulsion.	Length of survival in fresh water.
Freshly prepared	35 sec.	6 min.
126 min.	50 "	92 "
315 "	40 "	Lived for 4 days
1,419 "	40 "	" " 4 "
1,554 "	40 "	" " 4 "

*Deduction.*—Each time the emulsion was tested it showed a progressive decrease in activity. In the second experiment the emulsion became quite inactive.

EXPERIMENT B.

A similar experiment was done with crude oil emulsions.

*Technique.*—500 c.c. of a 0.5 per cent. emulsion was made in the same way in large bottle, and three larvae were exposed at each test.

## EFFECT OF WATER ON THE KILLING POWER OF OIL 17

*Observations.*—The following table shows the time the larvae survived contact with the emulsion when freshly prepared, and after 154 minutes:—

Freshly prepared emulsion.	Emulsion 154 minutes old.
13 min. 30 sec.	22 min. 15 sec.
46 " 0 "	405 "
50 " 30 "	405 "
Average life = 36 min. 40 sec.	Average life = 277 min. 25 sec.

*Deduction.*—The emulsion is more active when freshly prepared.

### EXPERIMENT C.

The effect of the age of crude oil emulsions was shown by a different method.

*Technique.*—2.5 c.c. of crude oil was placed in 500 c.c. in two stoppered museum jars of an internal diameter of 10 cm. One jar was shaken for 1 minute to produce a freshly prepared emulsion, and the other was shaken 9 times for 5 minutes with 10-minute intervals. The activity of the emulsions was compared by exposing 10 larvae for 30 seconds in a wire spoon.

*Observations.*—The length of survival of the larvae in fresh water is shown below:—

Freshly prepared emulsion.	Well shaken emulsion.
6 min. 15 sec.	11 min. 45 sec.
9 " 15 "	12 " 15 "
9 " 15 "	22 " 30 "
16 " 15 "	27 " 30 "
22 " 15 "	61 "
45 " 30 "	70 "
47 " 45 "	75 "
73 "	131 "
360 "	3,515 "
360 "	9,126 "
Average life = 95 min.	Average life = 1,305 min.

*Deduction.*—Each individual larva of the series exposed to well shaken emulsion survived longer than the corresponding one in the series exposed to freshly prepared emulsion. Two of the larvae exposed to well shaken emulsion survived for a very long time. The well shaken emulsion is therefore less active than the freshly prepared.

These experiments can now be summarised and discussed. They certainly show that the emulsions were most active when first prepared, and were less active at successive examinations. It was ultimately decided, however, that this was not due directly to the effect of water on the oil, but to the fact that a perfect emulsion was without effect on the larvae. The freshly prepared emulsion was active because some oil remained as a film, and the more perfect the emulsion was made

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with each successive shaking the less active it became. The reasons for this decision may be summarised as follows:—

(1) Sometimes the results of the experiments were by no means as regular as those quoted above. This might be explained as accidental entry of oil globules from the emulsion into the breathing tubes, but it is more probably due to irregular contact with residual film on the surface.

(2) The effects were not obtained when the emulsion was 1 per cent. or stronger, probably because there was excess of oil which could not go into the state of emulsion.

(3) When the strength of the emulsion was reduced to 0.1 per cent. the emulsion was inactive, even when freshly prepared by shaking for 15 seconds. This amount of oil formed a perfect emulsion and no excess separated as film.

(4) If the emulsion was kept standing till next day it regained activity, but became inactive again on shaking. This can be explained by part of the oil separating from the emulsion to form a film.

(5) The amount of emulsion prepared had an effect on the results. 100 c.c. was prepared instead of 500 c.c. in Experiments A and B, and the emulsion was inactive from the first shaking, 5 larvae living over 2,000 minutes. This was probably due to the smaller amount of oil which separated as a film being insufficient to kill larvae.

These reasons show that the loss of activity was not due directly to contact of the oil with water, as was thought when the experiments were planned, but that it was due to a gradual conversion of film into emulsion and that a perfect emulsion does not kill larvae. The experiments do not conflict with those, like Bacot's (1) which find that the action of oil emulsions is better than that of oil films, or with those, like Darling's (2), which show that toxic substances kill larvae very actively when in the state of emulsion. Those experiments deal with larvae left in contact with emulsions, while these are merely concerned with the effect of momentary contact with oil, or in other words, the power of oil to enter the breathing tubes. The globules of oil in an emulsion appear to be wet and therefore unable to enter the spiracles.

When, however, a demonstration that a perfect emulsion was harmless to larvae was projected, another difficulty was met with. It was readily proved that milk did not kill by contact and perfect emulsions of crude oil and kerosene were prepared by shaking them up with a dilute gum tragacanth. Control experiments showed that larvae were able to live satisfactorily in a gum of about 0.25 gm. to 100 c.c. of water, and this was used for making the emulsion. This emulsion did not kill larvae except in rare "accidental" cases, but to complete the experiment by centrifuging out the oil and proving it to be active was quite impossible. The oil separated out as a dull opaque yellow film and the exceptional deaths that it caused were obviously due to gross contact. The gum had wetted the oil and the oil had lost the power of exerting the "attraction" of a dry substance described in Chapter II. This was proved by the fact that larvae could lie at right angles to it instead of being "drawn in" by their spiracles.

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(1) Bacot, A. W. Experiments dealing with the Destruction of *Stegomyia fasciata*. Yellow Fever Committee (West Africa), 1916, Vol. III., pp. 153-155.

(2) Darling, S. T. Studies in Relation to Malaria. Isthmian Canal Commission, 1910, Second Edition, pp. 30-81.

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A similar thing happens when oil is left in contact with water for a long time. If a thick film of crude oil is made in a tall glass jar the lower surface becomes in time a yellowish layer of wet or emulsified oil. And sometimes larvae can be seen lying at right angles to the free edge of a film that has been a long time on water. Experiments have shown that films become less active when they are left on the surface of water. A museum jar containing about 1,000 c.c. and measuring 10.5 cm. in diameter was taken. The surface of the water was divided into two halves by a strip of cork. On one half a film was made by adding 0.5 c.c. of oil and the other half was left free from oil. Larvae were placed under the film from the clean water with a curved pipette. After contact with the oil for half a minute the larvae were pushed below the film with a needle and removed through the clean surface with the pipette. They were washed free from adherent oil and their length of survival was determined.

Experiments with kerosene are inconclusive since loss of activity might be due to evaporation. Crude oil, however, evaporates slowly and later, on page 27, it will be shown that it loses no vapour capable of killing larvae. The following results were obtained with films of different ages:—

Age of film.	Life of larvae.
Freshly prepared.	40 min. and 41 min.
1 day old.	68 " " 160 "
8 days old.	1,312 " "
11 " "	462 " " 6,215 "

A progressive loss of activity occurs with the ageing of the film, and it has been seen that wetting of oil by water has this effect.

Allied to this subject is the question whether water is able to dissolve out substances which are toxic to larvae. This would also be a reason for films of oil losing their power of killing larvae when left exposed on the surface of water. Attempts were made to extract such materials from crude oil, and the following experiments may be quoted:—

### EXPERIMENT D.

*Technique.*—Equal volumes of crude oil and water were shaken together for two hours. The mixture was allowed to stand for seventy-two hours and the lower watery layer was syphoned off, filtered and placed in a stoppered bottle. The extract was clear, colourless, and had a very faint smell.

*Observations.*—The length of life of ten larvae in this solution in stoppered jars was as follows:—

- One died in 1,332 minutes.
- One died in 1,507 minutes.
- Six lived for three days at least.
- Two pupated and emerged.

Ten controls lived in the stoppered jars for three days.

*Deduction.*—The solution was inactive, or only very slightly active, eight out of ten of the larvae not being affected by it.

## EXPERIMENT E.

*Technique.*—20 c.c. of water and 60 c.c. of crude oil were mixed together in a stoppered bottle and allowed to stand for two months. The watery layer was syphoned out and filtered through two layers of filter paper. A deep yellow solution, quite clear and with a faint smell, was obtained.

*Results.*—The length of life of ten larvae in open porcelain cups containing this solution was as follows:—

- One died after three days.
- Six lived for six days.
- Three pupated and emerged.

*Deduction.*—The solution was not toxic and larvae were able to pupate and emerge from it.

Experiments D and E are only examples quoted to show the nature of the evidence. No proof was obtained that any toxic material could be dissolved out of the oil. But the small quantity of water that is found at the bottom of almost every drum of crude oil was proved to be toxic. This water has been in contact with the oil from its origin and consists of a dark brown solution of tarry substances from the oil.

## EXPERIMENT F.

*Technique.*—From the 65-gallon drum of crude oil in the laboratory about 20 oz. of this material was obtained. This was filtered through two wetted filter papers to free it from oil globules, and a very dark brown solution was obtained. Two watch-glasses were filled with this solution and 5 larvae were placed in each.

*Observations.*—The following are the times at which the larvae lost their reaction to stimuli. Before complete loss of reaction was attained a difficulty in staying at the top was noted, but the larvae were kept at the surface as far as possible. The loss of reaction was observed to occur very suddenly, the larva remaining active up to a few minutes before its complete loss of reaction.

First watch-glass.	Second watch-glass.
116 min.	89 min.
119 "	91 "
129 "	156 "
131 "	250 "
133 "	272 "

Average life of 10 larvae, 148 minutes.

*Deduction.*—The solution is obviously toxic to larvae.

## EXPERIMENT G.

*Technique.*—A large quantity of the watery extractives similar to that used in Experiment F was obtained from the crude oil tanks of the Electric Light Department. The drums of crude oil were emptied into big tanks and a solution of extractives from a large quantity of oil was readily obtained from the bottom of these tanks.

A sample of this material was tested for toxicity by placing 10 c.c. of it in each



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of 10 small porcelain cups. One larva was placed in each cup and the time taken to cause complete loss of reactivity was noted.

A second sample of 100 c.c. was evaporated almost to dryness on a water bath, made up to 100 c.c. again, and tested on another series of 10 larvae in the same way as the first sample. This was to determine whether the toxic material was volatile at the temperature of boiling water.

A third sample of 100 c.c. was vigorously boiled over an open flame for half an hour. It was prevented from complete evaporation by adding distilled water from time to time. This experiment was intended to determine whether the toxic substance was volatile enough to be boiled off. The temperature never rose to 105° C., and was usually about 103° C.

*Observation.*—The following table shows the time 10 larvae took to lose their reaction to stimulus :—

Filtered extract.	Filtered extract evaporated down on water bath, and then made up to original strength.	Filtered extract boiled for half an hour in evaporating dish over open flame, with addition of water.
190 min.	175 min.	334 min.
210 "	220 "	363 "
279 "	230 "	372 "
301 "	311 "	383 "
337 "	322 "	397 "
346 "	331 "	414 "
355 "	351 "	480 "
370 "	355 "	493 "
399 "	356 "	499 "
509 "	456 "	574 "
Average = 329.6 min.	Average = 310.7 min.	Average = 430.9 min.

Fifteen of the larvae were removed from the solution after complete loss of activity and placed in fresh water to ascertain whether any recovery would occur. Some of them recovered, and the observations on these larvae are recorded in Experiment X on page 30.

*Deduction.*—The toxicity of the sample obtained from the Electric Light Department was not so great as the sample obtained from the drum in the laboratory.

The toxic substance was not sufficiently volatile to be driven off by a slow evaporation on a water bath, but the sample treated by vigorous boiling showed a slight loss of toxicity. The fact that some of the larvae recovered after complete loss of reactivity when placed in fresh water will be discussed on pages 59-60, but it proves that death was not due to globules of oil which had escaped filtration, but to some poisonous material.

EXPERIMENT H.

The fact that the poisonous substance is organic in nature is proved by incinerating the dry residue obtained by complete evaporation of the extractives.

No toxic action was shown by the solution obtained by macerating the ash in water.

The results of this chapter may be summarised as follows :—

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(1) An emulsion of oil does not kill by contact because the oil globules cannot wet the spiracles of larvae. If an emulsion kills in this way it is due to a film formed by an excess of oil on its surface.

(2) Water is able to wet a film of crude oil after it has been on the surface for some time. Such oil does not "attract" the spiracle of the larvae in the way that dry oil does. This is a cause of oil losing activity when exposed on the surface of water.

(3) Water is unable to extract by ordinary means sufficient toxic material from crude oil to kill larvae. The solution of toxic material cannot therefore be an explanation of the action of incomplete films of oil, nor can it be the cause of oil losing its power of killing larvae.

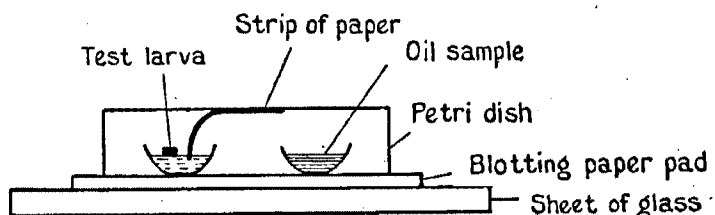
(4) The dark tarry solution of extractives which is found at the bottom of crude oil drums is, however, toxic to larvae, and the mode of its action will be discussed later.

## CHAPTER IV.

### THE EFFECT OF OIL VAPOURS.

SEN (1) describes a toxic effect on larvae of the vapours of various substances including benzene and kerosene. The loss of this volatile toxic part of the oil might cause a loss of activity when the oil is exposed on the surface of water, and the following series of experiments was done to determine the extent to which this occurs.

A part of the technique of the experiments is common to the whole series, and this will be described first. An air-tight chamber was made by inverting a large petri dish over a piece of blotting-paper resting on a flat pane of glass. A petri dish which rested evenly all round its edge was chosen so that it could be moved slightly from side to side without allowing the vapour to escape or air to enter. In some of the experiments a ring of vaseline was placed round the edge.



The test larva was introduced into this chamber in a watch-glass containing about 2 c.c. of water. A triangular piece of stiff paper was gummed by its base to the inner surface of the petri dish, and bent so that its point dipped into the water, as indicated in the diagram. By moving the petri dish laterally the point could be made to disturb the larva, and in this way the moment at which the larva lost the power of reacting to stimuli was detected.

At a given moment the second watch glass containing a sample of the substance to be tested was introduced, and the time taken to cause loss of reactivity was noted. The effect of saturating the air with vapour is being tested and therefore there should always be an excess of substance left at the end of the experiment, but the actual amount of substance is immaterial. The uniformity of temperature conditions has been mentioned in the Introduction.

The death of the larva in these experiments may not be due directly to the vapour, but to a film formed by the vapour condensing on the surface of the water. Sen realised this possibility in his experiments, and wrote :—

(1) Sen, S. K. Observations on Respiration of Culicidae. Ind. Journ. Med. Res., 1914-15, Vol. II., p. 696.

“ Apart from the presence of vapour there is no apparent reason why the larvae in scented phials should not have lived as long as those in the control, as no definite surface-film was formed under the conditions of the experiments.”

To exclude definitely the possibility that the water had become harmful to larvae either by condensation of a film on its surface or by the absorption of toxic vapours, control experiments were done. The larva was removed from the water and replaced by a fresh larva. The watch glass was quickly covered with a small petri dish to prevent the evaporation of any evanescent film of oil. This was done 5 times after experiments with kerosene and 6 times after petrol had been used. The time taken over the manipulation varied from 15 seconds to 60 seconds, but all the larvae lived satisfactorily for at least 20 hours. This shows that the water had not become harmful to larvae, and that the effects of the experiments were due entirely to vapour.

#### EXPERIMENT I.

*Technique.*—In the first series of tests, 1 c.c. of kerosene was carefully measured out by means of a pipette into the watch-glass. It was, therefore, thoroughly exposed during the manipulations necessary for measurement to evaporation of its more volatile contents. The activity of the vapour in this series varied greatly. In case this might depend on the varying amounts of volatile substances lost during the measurement, a second series of tests was done in which a sample of 1 c.c. of kerosene was poured out straight from the bottle.

*Observations.*—The following table shows the time taken by the larvae to lose reaction to stimuli.

Life of 5 larvae exposed to vapour of 1 c.c. of kerosene exposed by the manipulation of measurement to evaporation.	Life of 10 larvae exposed to vapour from about 1 c.c. of kerosene poured direct from the bottle.
32 min.	20 min.
58 "	21 "
179 "	30 "
213 "	33 "
756 "	41 "
	45 "
	52 "
	66 "
	72 "
	77 "

*Deduction.*—The results with kerosene not exposed to evaporation by the manipulations necessary for accurate measurement are more regular, and the action of the vapour is more rapid than with kerosene exposed to evaporation.

#### EXPERIMENT II.

The fact that the vapour of the sample of kerosene which was accurately measured out was less active than the kerosene poured directly from a bottle led to an investigation whether kerosene allowed to evaporate at room temperature showed the same loss of activity.

*Technique.*—A sample of kerosene was tested, and then 100 c.c. of it was exposed to free evaporation at room temperature by pouring it from a graduated cylinder into an open dish and back again till its volume was reduced to 70 c.c. The action of the vapour was again tested, and then the evaporated kerosene was left in a stoppered bottle for two days and tested once more.

*Observations.*—The following table shows the length of time larvae remained reactive to stimuli in the vapours.

Length of life in larvae exposed to vapours of kerosene.		
Kerosene—original sample.	Kerosene evaporated at room temperature from 100 c.c. to 70 c.c.	Evaporated kerosene kept in a stoppered bottle for 2 days.
39 min. 35 sec.	837 min.	48 min. 55 sec.
47 " 20 " *	1,146 " "	107 " "
49 " 40 " *	4,952 " †	115 " "
Average = 45 min. 32 sec.	Average of the first two results = 991 min.	Average = 90 min. 18 sec.
<p>* This observation replaces one of 6.301 minutes, which was discovered to be due to a fold in the paper allowing the escape of vapour and the entry of air.</p> <p>† No leakage of the apparatus was detected in this experiment, but its result is excluded from the average.</p>		

*Deduction.*—The residue left after evaporation was less active than the original sample. It became more active after being kept in a corked bottle for two days.

EXPERIMENT III.

The results of the previous experiment were so remarkable that it was repeated with another sample of kerosene, and with 5 larvae in each test.

The following results were obtained :—

Kerosene original sample.	Kerosene evaporated from 100 c.c. to 70 c.c.	Evaporated kerosene kept in stoppered bottle for two days.	Evaporated kerosene kept in a stoppered bottle for 20 days.
81 min.	492 min.	482 min.	325 min.
132 " "	507 " "	872 " "	341 " "
172 " "	602 " "	872 " "	352 " "
187 " "	1,132 " "	873 " "	358 " "
257 " "	1,313 " "	5,683 " *	428 " "
Average = 166 min.	Average = 809 min.	Average of first four = 775 min.*	Average = 361 min.
<p>* This result is evidently due to some error in technique, probably leaking of vapour, but the fault was not discovered at the time. The observation is not included in the average which is given for the first four larvae only.</p>			

## THE EFFECT OF OIL VAPOURS

*Deduction.*—The vapour of this sample was not so active as that used in the previous experiment. The same loss in activity occurred after evaporation. The vapour had not regained activity after 2 days as in the last experiment, but when examined after 20 days the vapour was more active.<sup>(1)</sup>

## EXPERIMENT IV.

*Technique.*—The toxicity of petrol vapour was then tested in a similar way to that used by kerosene in Experiment I.

*Observations.*—Time taken by petrol vapour to cause loss of reaction :—

Petrol, 1 c.c. measured by pipette.	Petrol, about 1 c.c. poured straight from bottle.
7 min. 45 sec.	5 min. 30 sec.
30 " 0 "	6 " 15 "
36 " 0 "	7 " 30 "
38 " 30 "	7 " 30 "
43 " 0 "	10 " 0 "
54 " 30 "	11 " 0 "
	12 " 0 "
	12 " 30 "
	12 " 30 "
	14 " 30 "
	16 " 0 "
	17 " 0 "
Average for 5 larvae = 34 min. 57 sec.	Average for 10 larvae = 11 min. 1 sec.

*Deduction.*—Petrol vapour is much more active than kerosene. There is the same difference between the sample exposed to evaporation and the sample poured direct from the bottle as shown by kerosene in Experiment I.

## EXPERIMENT V.

This similarity of the results of petrol and kerosene led to testing whether evaporated petrol lost activity and regained it in the same way as kerosene.

*Technique.*—A sample of petrol was tested. 300 c.c. of the sample was rapidly evaporated until its volume was reduced to 200 c.c. The vapour was again tested and the residue was stored in a stoppered bottle and re-tested after 2 and once more after 21 days.

Unmeasured samples of roughly 1 c.c. were used throughout.

*Observations.*—Time taken by petrol vapour to cause loss of reaction :—

(1) In the light of later knowledge we may suspect that the bottle in which this sample was kept was fuller than the bottle used in the previous experiment. See experiments described on pages 51-57 and Conclusion No. 10.

Original sample.	Evaporated sample.	Evaporated sample kept for 2 days.	Evaporated sample kept for 21 days.
6 min. 25 sec.	13 min. 5 sec.	8 min. 25 sec.	5 min. 40 sec.
8 " 50 "	15 " 50 "	9 " 10 "	5 " 50 "
9 " 45 "	18 " 15 "	9 " 30 "	7 " 30 "
12 " 30 "	27 " 45 "	10 " 35 "	9 " 30 "
12 " 40 "	28 " 30 "	13 " 55 "	10 " 45 "
13 " 55 "			
14 " 50 "			
15 " 55 "			
16 " 10 "			
21 " 40 "			
Average = 13 min. 16 sec.	Average = 20 min. 41 sec.	Average = 10 min. 19 sec.	Average = 7 min. 51 sec.

*Deduction.*—The evaporated petrol was markedly less active than the fresh sample. The vapour regains activity when kept, just as kerosene does (see Experiments II and III). In this instance the petrol was more active after keeping than the original sample.

#### EXPERIMENT VI.

The effect of crude oil vapour was then tested.

*Technique.*—Two samples were tested. One was taken direct from the bottle and the other was heated to 100° C. to get rid of any very volatile vapour. Five tests were done with each sample.

*Observation.*—No deaths occurred up to 3,000 minutes (50 hours) when the experiment was stopped. The experiment was repeated with a fresh sample of crude oil on 6 more larvae, and no deaths occurred up to 2,000 minutes.

*Deduction.*—The vapour of crude oil is inactive.

#### EXPERIMENT VII.

Since the vapour of crude oil was inactive the fractional distillates of the sample were tested. Lieut.-Col. S. W. Bunker, D.S.O., kindly prepared these distillates. The distillation was done *in vacuo* and therefore the temperature at which they distilled cannot be given. The first three distillates, each of 10 per cent., were clear limpid fluids, the 4th, 5th and 6th fractions of 10 per cent. were yellowish-brown clear liquids, and the 40 per cent. residue was an opaque dark brown liquid resembling crude oil.

*Technique.*—As for Experiment I. Each fraction was tested on 5 larvae.

*Observations.*—The results are shown below:—

Fraction tested.	Life of 5 larvae.					Average life.
	1st	2nd	3rd	4th	5th	
	Min.	Min.	Min.	Min.	Min.	Min.
1st, 10 per cent. ...	38	43	44	49	50	45
2nd, 10 per cent. ...	540	600*	720*	1,418	2,670	1,543
3rd, 10 per cent. ...	684	944	1,306	2,005	2,919	1,572
4th, 10 per cent. ...	788	878	1,456	2,439	2,598	1,632
5th, 10 per cent. ...	2,260	2,499*	5,319	5,933	6,168	4,920
6th, 10 per cent. ...	2,495	4,475	5,014	5,470	6,165	4,724
Residue, 40 per cent. ...	1,063*	7,123	9,103	10,783	1,3573	10,145

\* Pupated (not counted for average life).

*Deduction.*—The first 10 per cent. is active, about equivalent to the kerosene used in Experiment I; the 2nd, 3rd and 4th fractions are slightly active and all of about the same value; the rest of the oil can be regarded as giving no vapour or an inert vapour.

The active 10 per cent. does not appear to leave the crude oil as vapour at ordinary temperatures (see Experiment VI).

#### EXPERIMENT VIII.

Sen, in the paper already referred to on p. 23, says with reference to kerosene:—

“I have reason to believe that the odour of this hydrocarbon exerts a direct anæsthetic (?) effect which may largely contribute to the larva's death.”

And this experiment was done to test the truth of this statement by seeing whether the larvae that had lost their power of reaction could recover when taken away from the vapour.

*Technique.*—Forty-four larvae were taken from Experiments I, II and III, after they had completely lost their reactivity. Twenty-five of these were placed on the surface of water in small porcelain cups with their spiracles exposed to the air. The remaining 19 were placed in clean water in separate cups, but completely immersed at the bottom of the cup. The larvae were then watched to see whether they would recover.

*Observations.*—The following tables show how long after loss of reactivity they were removed from the vapour, how long they took to recover, and how long they were observed to be alive afterwards.

Larvae placed on surface of water with the spiracles exposed to air.		
Length of exposure to vapour after complete loss of reactivity.	Time taken to recover reactivity.	Time lived after recovery.
5 min. 0 sec.	2 min. 15 sec.	5,760 min.
6 " 0 "	0 " 30 "	4,624 " *
4 " 30 "	1 " 30 "	1,413 " *
3 " 0 "	6 " 30 "	2,962 "
6 " 30 "	9 " 0 "	4,207 "
3 " 30 "	3 " 30 "	5,605 "
2 " 30 "	3 " 0 "	5,619 "
6 " 30 "	13 " 0 "	5,601 "
5 " 0 "	4 " 0 "	7,423 "
5 " 0 "	2 " 0 "	4,024 "
6 " 0 "	4 " 0 "	3,318 " †
5 " 0 "	2 " 30 "	8,955 "
5 " 0 "	3 " 30 "	8,627 "
7 " 0 "	6 " 30 "	7,634 "
6 " 0 "	3 " 30 "	5,407 "
5 " 0 "	4 " 0 "	7,804 "
7 " 0 "	4 " 0 "	8,647 "
5 " 0 "	3 " 30 "	13,009 "
6 " 30 "	8 " 0 "	293 "
6 " 0 "	5 " 0 "	10,114 "
6 " 0 "	2 " 0 "	5,139 "
5 " 0 "	3 " 0 "	7,395 "
5 " 30 "	Died	—
7 " 0 "	"	—
5 " 0 "	"	—

\* Pupated and emerged.  
† Pupated and died.



Larvae totally immersed in water.		
Length of exposure to vapour after complete loss of reactivity.	Time taken to recover reactivity.	Time lived after recovery.
5 min. 30 sec.	Died	—
4 " 30 "	"	—
7 " 0 "	"	—
8 " 0 "	"	—
6 " 0 "	"	—
5 " 0 "	"	—
6 " 0 "	"	—
6 " 30 "	"	—
5 " 0 "	"	—
5 " 0 "	"	—
6 " 0 "	"	—
6 " 0 "	"	—
5 " 0 "	"	—
6 " 0 "	"	—
5 " 0 "	"	—
5 " 0 "	"	—
6 " 0 "	"	—
6 " 0 "	"	—
6 " 30 "	3 min.	4,460 min.

*Deduction.*—Most of the larvae placed with their spiracles in contact with air recovered, and even in water away from direct contact with air, 1 out of 19 recovered. Provided the larvae can breathe, therefore, they usually recover.

EXPERIMENT IX.

A similar experiment was done with larvae that had lost reactivity from the effects of petrol vapour.

*Technique.*—Eighteen larvae derived from Experiments IV and V were used. Nine were placed on the surface of water and 9 were submerged.

*Observations.*—The following results were observed:—

Larvae placed on the surface of water with the spiracles exposed to air		
Time exposed to action of vapour after complete loss of reaction.	Time taken to recover movement.	Length of life after recovery.
5 min. 30 sec.	1 min. 30 sec.	2,065 min.
6 " 30 "	7 " 30 "	350 "
6 " 30 "	1 " 45 "	361 "
5 " 30 "	8 " 30 "	338 "
3 " 0 "	7 " 0 "	88 "
3 " 30 "	2 " 0 "	342 " *
5 " 15 "	5 " 0 "	2,767 "
5 " 0 "	3 " 0 "	2,768 "
4 " 45 "	3 " 15 "	2,770 "

\* Pupated and died.

The 9 larvae totally immersed in clean water all died. The times that they were exposed to the vapour after complete loss of irritability were 3, 5, 5, 5, 6, 6, 7, 7, 7 minutes respectively.

*Deduction.*—The action of petrol vapour is similar to that of kerosene. All the larvae exposed to air recovered and all those immersed in water died.

#### EXPERIMENT X.

A similar experiment was done on larvae which had been subjected to the action of the watery extract of crude oil used in Experiment G, page 20.

*Technique.*—Eight larvae were placed with their spiracles exposed to air and 7 were submerged.

*Observations.*—The following results were observed :—

Larvae placed at the surface of water with the spiracles exposed to air.	
Length of exposure to solution after complete loss of reactivity.	Time taken to recover movement.
1 min.	2 min.
5 "	2 "
6 "	3 "
6 "	3 "
6 "	8 "
6 "	10 "
6 "	12 "
6 "	Died

The 7 larvae submerged in water all died.

*Deductions.*—Of 8 larvae exposed to air all recovered except 1; all of those submerged died. Therefore the action of this watery extract of crude oil is similar to that of the vapours of kerosene and crude oil. The importance of this experiment is that the substance producing the effect is not volatile at 100° C.

The results can now be summarised as follows :—

(1) The vapour of kerosene and petrol can kill larvae and petrol vapour is more active than that of kerosene.

(2) The vapour first given off by these substances is more active than the less volatile fractions. When the sample is kept in a bottle the vapour regains activity, and may become even more active than that of the original sample.

(3) The vapour from crude oil did not kill larvae, but the first ten per cent. distillate from the crude oil gave off an active vapour.

(4) The larvae were able to recover from the effect of the vapours when left with their spiracles exposed to air. But they died, as a rule, when kept under water.

(5) Larvae were similarly able to recover from the effects of the substances extracted from crude oil by water. These substances were not volatile, since the solution can be heated to 100° C. without altering its effects on larvae.

An obvious result of these facts is that oil exposed to evaporation on the surface of water will lose part of its effect on larvae, and this is another reason in favour of using small quantities of fresh oil at short intervals.

But the main interest of the results lies in the similarity between the activity of the vapours and the spreading power of oils. The facts will be used as the basis for discussion after experiments on the spreading power of oils have been described in the next chapter.

## CHAPTER V.

### SOME CONDITIONS AFFECTING THE SPREAD OF OIL ON WATER.

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#### Va. THE EFFECT OF INVISIBLE FILMS.

During the experimental work that has just been described, a great difficulty was experienced in producing uniform films from the same amount of oil dropped on the same amount of water in the same sized glass petri dishes. Both crude oil and kerosene produced all variations in extent of spread. The result of any one drop could not be prophesied. It might form a lens-shaped blob over one millimetre thick, a thick film of varying but limited extent, or an extremely thin film extending to the edge of the dish. It was impossible, therefore, to devise suitable tests for the effect on larvae of different oils in the state of film. The discordant opinions of practical men (<sup>1</sup>) about the relative spreading power of oils probably arise in this way, since in one experiment kerosene might spread better than crude oil, while in another the opposite result might be obtained.

All attempts to produce uniform results by using distilled water and salt solutions instead of tap-water failed. But when studying the effects of wet and dry dishes on the position assumed by anopheline larvae, as described on page 12, certain conditions were found to produce constant results. When the dishes were lined with wet blotting paper the oil always formed a blob, and when they were

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(<sup>1</sup>) The popular opinion is that the lighter kerosenes spread better than the crude oils, but some authors state the reverse; for instance, Fisher states that kerosenes do not spread as well as crude oil, and Sollman finds that the spreading power of kerosene is improved by adding asphalt.

Fisher, H. C. Report of the Health Department of the Panama Canal for the year 1921.

Sollman, T. The Spreading Power of Coal Oils. Journ. Amer. Med. Assoc., 1918, Vol. LXXI., p. 1553.

lined with paraffin wax it spread into a thin film. Apparently the condition of the side of the dish had an effect on the spread of oil just as it controls the position of anopheline larvae: and it seemed that a wet dish might prevent the spread of oil by "repelling" (1) the dry edge of the oil film, that a dry dish might promote spread by "attracting" the dry oil, and that patches of wet and dry surfaces on the side of the dish might explain the intermediate degrees of spread. But this idea was proved to be wrong by finding that the oil spreads when the side is made wet by chemical cleansing (2). The possibility that this inactivation of the water might be due to the dissipation of free energy on its surface during the work done in wetting the paper was then considered and disproved.

Finally the absence of spread in dishes lined with blotting paper was attributed to an invisible film of greasy substances derived from the paper or from the fingers during the preparation of the dish. A complete invisible film prevents the spread of crude oil or kerosene; an incomplete invisible film allows the oil to spread only in the unoccupied area; and absence of the film allows the oil to spread over the whole surface. This explains all the variations in extent of spread just described.

The formation of invisible films is a well-known fact (3) and is usually demonstrated by their effect on light dust particles floating on water. If lycopodium or talc powder, for instance, be sprinkled on the surface of clean water, and some part of that surface be touched with a finger or with some substance like oleic acid, the particles will be swept away by the spreading of an invisible film from the source of contamination. Moreover, the movements of particles of thymol on the surface of clean water can be stopped at once by contaminating the surface in the same way.

Leak (4) refers to the fact that soapy and greasy substances prevent the spread of crude oil, but the effect of invisible films is not sufficiently known among field workers engaged in oiling.

The term invisible as applied to the films in question is a literally true one, and the effect of invisible films differs markedly from the effect of the thinnest visible film. The importance of the invisible film and the impossibility of determining its presence by sight justify a description of the following methods for demonstrating its presence.

For the purpose of the experiments the film may be prepared by contaminating

(1) See Chapter II.

(2) Various methods of cleaning were used, such as potassium bichromate and sulphuric acid, a strong caustic soda solution, xylol and alcohol, followed in each case by thorough rinsing. The dishes were kept under water until they were required, and only the outside of the dish was handled. Perhaps the most convincing method of cleansing was the use of a platinum dish heated in a blow-pipe and allowed to cool.

(3) Tomlinson described a "film from camphor which is not visible." C. Tomlinson. *Experimental Essays*, I, 1863, p. 35.

Their effects were studied by Lord Rayleigh in 1890. Lord Rayleigh. On measurements of the amount of oil necessary in order to check the motions of camphor upon water. *Proc. Roy. Soc.*, 1890, Vol. A., XLVII., p. 364.

Since Hardy's papers, quoted in these pages, their structure and effects have become well known.

(4) Leak, W. N. On the Improvement of Oiling Fluids for Anti-mosquito Work. *Journ. Trop. Med. and Hyg.*, 1921, Vol. XXIV., p. 37.

the surface of water with a trace of oleic acid on a platinum loop. It is important to have an invisible film only and no excess of the acid, since any excess would interfere with the results of the experiments. The excess will be visible as small lens-shaped blobs which should be removed with blotting-paper or lifted out with a clean spoon. After the excess has been removed no trace of film can be seen on the surface, even when it is examined critically, but the surface will differ from that of clean water in the following respects: thymol particles do not move upon it; crude oil and kerosene do not spread on it; and certain kinds of cotton wool (1) are wetted and sink, whereas they float on clean water. That these differences are due to a film on the surface may be shown in a series of experiments:—

(1) Water pipetted or syphoned out from below the surface and placed in a clean dish will allow crude oil to spread.

(2) If the water be poured into a glass-stoppered bottle, vigorously shaken, and then poured out into two clean dishes, the water in the first dish will allow crude oil to spread owing to the destruction of the film. If the second dish be allowed to stand for an hour, the film will have had time to reform and crude oil will not spread.

(3) If a blob of crude oil be placed on the surface it can be made to spread out by removing part of the invisible film. This can be done by scraping the surface of the water with a clean glass microscope slide.

(4) Similarly the invisible film can be removed by adding caustic soda. The sodium oleate formed dissolves in the water and spreading occurs.

(5) In Miss Pockels' trough experiment (2) it can be shown that by increasing the area of the water surface so that the invisible film can no longer cover it completely, the movements of thymol on the water can be started afresh. In this experiment the spread of oil can be used to indicate the limit of extension of the invisible film.

For example, a small part of the surface of water in a photographic developing dish may be separated off by a barrier of clean glass and contaminated with oleic acid. A drop of crude oil will form a blob on this surface, but if the barrier be removed the blob will spread out, although it will not form a complete film since it cannot spread into the area occupied by the invisible film.

(6) The surface of a little water at the bottom of a tall cylindrical jar may be contaminated and a blob of crude oil placed upon it. If water be added gently the invisible film is not destroyed and the blob can be raised to the top of the jar without allowing it to spread. If now the surface of the water be poured off the invisible film will be removed and crude oil will spread on the underlying water.

A native inspector in charge of oiling who had seen these experiments made an interesting observation. He noticed that if the oiler waded through the water the oil did not spread as well as when he sprayed it from the bank. This must be due to contamination by an invisible film from the Tamil labourer, who has a habit of using coco-nut oil extensively on his skin. A similar pollution of the small open pools probably explains why the oil did not spread when it was mopped on to the surface as described in the Introduction.

Thus the effect of an invisible film contaminating the surface of water is seen

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(1) Johnson and Johnson's "No. 1 quality," for instance, but not Baird and Tatlock's "No. 1 quality Raw Cotton untreated with any antiseptic."

(2) Pockels, A. Surface Tension, Nature, 1891, Vol. XLIII., p. 437.

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to be very considerable, but films of substances like crude oil and kerosene do not produce this effect. A thin film of these substances can be detected on the surface of water by the iridescent colours which it produces, and moreover it does not prevent either the movements of thymol or the spread of crude oil. The reason for this difference between visible and invisible films will be discussed in the next section on the nature of the substances which cause oil to spread on water.

### Vb.—NATURE OF THE SUBSTANCES WHICH CAUSE THE SPREAD OF OIL ON WATER.

This part of the investigation was inspired by the work of Irving Langmuir<sup>(1)</sup>, and I have attempted to apply his ideas to the present problem.

He attributes the effect of a "poison" inactivating a catalytic agent to the adsorption of a layer of the "poison" one molecule thick on the surface of the catalyst. The layer is adsorbed by virtue of an active group of atoms in the molecule of the poison which, in addition to the primary valency binding it to its molecule, exerts a secondary valency or attraction outside its molecule. The molecules in the film arrange themselves with the active groups attached to the surface of the catalyst by their secondary valencies, exposing the inactive portion of the molecule on the surface, and thereby stopping the catalytic action.

The effect of an invisible film of oleic acid on water is an exactly similar phenomenon. The  $\text{-COOH}$  group in each molecule of the acid is the active group and becomes fixed<sup>(2)</sup> to the water, exposing the inactive hydrocarbon part of the molecule on the surface. The attraction of the water for active groups in oils is thus eliminated and oils can no longer spread on its surface.

It is a well-known fact that pure saturated hydrocarbons, whether open chain or ring compounds, are inert bodies, with no chemical affinities. They are not soluble in water, nor do they spread on water.<sup>(3)</sup> When placed on water they form blobs which look like the blob formed by an excess of oleic acid. But the water surrounding a blob of pure hydrocarbon shows none of the signs of invisible film described in the last section, since crude oil can spread on it and thymol particles move freely upon it.

Volatile hydrocarbons form films by evaporation and condensation of their vapours on the surface of water, but if evaporation be prevented by covering the dish the films contract into compact blobs.<sup>(4)</sup> These films formed by condensation

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(1) Langmuir, I. The Constitution and Fundamental Properties of Solids and Liquids. Journ. Amer. Chem. Soc., Part I, Solids, 1916, Vol. XXXVIII., pp. 2221-2295; Part II, Liquids, 1917, Vol. XXXIX., pp. 1848-1906.

(2) See *ibid.*, p. 1863. Langmuir regards this fixation of the active group as actual solution, or in other words chemical combination by secondary valence.

(3) Miss Pockels stated in 1894 that "pure petroleum seems to be the only oil that does not spread on a normal surface."

Pockels, A. On the Spreading of Oil upon Water. Nature, 1894, Vol. L., pp. 223-224. Hardy has extended this statement to all pure saturated hydrocarbons.

(4) Hardy, W. B. The Tension of Composite Fluid Surfaces. Proc. Roy. Soc., Vol. A., LXXXVI., 1912, p. 620, and Vol. A., LXXXVIII., 1913, p. 314.

Hardy, W. B. The Spreading of Fluids on Glass. Phil. Mag., Sixth Series, Vol. XXXVIII., 1919, p. 50.

are not one molecule thick like the film of oleic acid, but vary in thickness. The hydrocarbon must be absolutely pure or else it may form a film other than that of condensed vapour. For instance, Merck's pure benzene, guaranteed free from thiophene, behaved in this way, but other samples of benzene formed films which were not affected by covering the dish. Again, most samples of xylene supplied for microscopic work spread on water, but one sample remained as a blob surrounded by an irregular film of condensed vapour, which at once contracted into the blob when the dish was covered.<sup>(1)</sup>

Liquid non-volatile hydrocarbons show no tendency to spread and remain as compact blobs on the surface of the water, provided they are perfectly pure. Most samples of medicinal liquid paraffin behave like this; large quantities of the "Calol" brand of the Standard Oil Company and the "E.H.V." brand of the British Drug Houses have been used without detecting any variation between different samples.<sup>(2)</sup>

Pure solid hydrocarbons melt when placed on water above their melting point and remain as blobs on the surface. Most samples of paraffin wax and naphthalene contain impurities which cause them to spread, but some behave in the above manner.<sup>(3)</sup> The experiment must be done with care, since handling the wax may impart enough impurity to cause it to spread.

The chemically inert hydrocarbons show, therefore, no tendency to spread on water, but it is well known that these substances acquire chemical affinities when other atoms or groups of atoms are introduced into the hydrocarbon molecule. And, according to Langmuir, the following groups impart to the hydrocarbon molecule an affinity for water:  $-\text{OH}$ ,  $-\text{O}-$ ,  $-\text{COO}-$  and  $-\text{NH}_2$ .<sup>(4)</sup>

The introduction of a single  $-\text{OH}$  group into a hydrocarbon with a small molecule is sufficient to make the substance soluble in water as, for instance, when the inert insoluble gas ethane,  $\text{C}_2\text{H}_6$ , is converted into the highly soluble ethyl alcohol  $\text{C}_2\text{H}_5\text{OH}$ . With increase in the size of the hydrocarbon molecule the effect of the introduction of a single  $-\text{OH}$  group becomes progressively less until when octyl alcohol,  $\text{C}_8\text{H}_{17}\text{OH}$ , is reached the substance is only slightly soluble in water. Apparently the  $-\text{OH}$  group cannot drag the larger hydrocarbon radicle into solution and possibly the large hydrocarbon radicles have a greater cohesion between them-

(1) This sample, which was obtained from the British Drug Houses, gave poor results when used in the ordinary technique for mounting specimens of mosquito larvae in Canada balsam. The cloudy appearance of the specimens suggested that dehydration was incomplete, but the absolute alcohol, when tested with anhydrous copper sulphate, proved to be satisfactory; and excellent results were obtained when a little phenol had been added to the xylene. This observation shows that the purer hydrocarbon is not such a good clearing agent as one contaminated with an  $-\text{OH}$  containing substance like phenol, and suggests that the clearing action depends on the mechanism to which the spread of oil is attributed in the immediately following pages. It also explains the reason for saturating xylene with phenol crystals as recommended by G. Mann. *Methods and Theory of Physiological Histology*, Oxford, 1902, page 271. See also W. F. Harvey, *Ind. Journ. Med. Res.*, Vol. X., 1922, p. 373.

(2) Some samples of medicinal liquid paraffin spread, however, and it might be of interest to find out whether there is any difference in medicinal action between these two classes of preparations.

(3) Edser, E. *The Concentration of Minerals by Flotation*, Brit. Assoc., Fourth Report on Colloid Chemistry, 1922, p. 303, "I have found that the medicinal paraffin oil sold as 'Nujol' does not contaminate the surface of water. Melted paraffin wax and melted naphthalene do not contaminate the surface of hot water."

(4) Langmuir. *Journ. Amer. Chem. Soc.*, Vol. XXXIX., p. 1903.

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selves. When octyl alcohol is placed on water it forms an invisible film similar to that described for oleic acid on page 33 in that neither excess of the alcohol nor any oil can spread on the surface.

When the  $-OH$  containing substance is solid it cannot spread on water, but if it is soluble in water its particles show rapid movements on the surface. When crystals of phenol,  $C_6H_5OH$ , are gently placed on the surface of water these movements can be seen, but they are evanescent owing to the rapidity with which the phenol dissolves.<sup>(1)</sup> Thymol,  $C_{10}H_{13}OH$ , is much less soluble than phenol and particles of thymol show these movements most typically. The movements go on until the water is saturated with thymol, and when this point is reached the thymol particles lie inert on the surface and, moreover, oil is unable to spread on the surface.

The movements appear to be due to a mutual attraction between the molecules of water and the active groups in the solid particle. By solution of some of the substance the attraction of the water which is in contact with the substance is satisfied, and the particle is pulled away by the attraction between it and the unsaturated water in the neighbourhood. Saturation of this water occurs and the particle is pulled to a different area of unsaturated water. The particle is drawn thus from point to point over the water until movement ceases, when the whole surface is satisfied.<sup>(2)</sup> With further increase in size of the hydrocarbon radicle the substance is still less soluble and these movements do not occur. For instance, when particles of cholesterol,  $C_{27}H_{45}OH$ , are sprinkled on water they lie inert on the surface. Their presence does not prevent the spread of oil since a drop of crude oil spreads out and drives them to the margin of the dish just as it drives lycopodium spores or talc powder. By heating the water the solubility is increased and slight movements similar to those exhibited by thymol occur. By dissolving the cholesterol in pure paraffin its molecules are rendered mobile and the resultant mixture spreads on water. Also solid cholesterol can be spread mechanically over the surface of the water, either indirectly by shaking in a stoppered bottle, or directly by means of a platinum wire cleansed by heat. The water will then show evidence of an invisible film of cholesterol on its surface in that neither the spread of oil nor the movements of thymol can occur.

So far only  $-OH$  containing substances have been discussed, but the organic acids containing a single  $-COOH$  group supply a similar series of substances, thus:—

With a low molecular weight, for instance, acetic acid, they are soluble in water like the lower alcohols.

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<sup>(1)</sup> Tomlinson described these evanescent movements in 1861.

Tomlinson, C. On the Cohesion Figures of Liquids, *Phil. Mag.*, 1861, Vol. XXII., of Fourth Series, p. 252.

<sup>(2)</sup> This is not the usual explanation of these movements. The substance dissolves and reduces the surface tension of water, and the movements are commonly ascribed to the pull of the higher surface tension of unsaturated water in the neighbourhood.

A similar explanation has already been suggested for the fact that oils are prevented from spreading by an invisible film, which is also usually explained by a reduction of surface tension.

Both the spread of oils and the movement of thymol particles would appear to be due to the same cause, namely, the affinity of water for active groups in the substance; and both can be prevented in the same way, namely, by saturating the water with a substance which contains a single active group.



When liquid and insoluble in water, for instance, oleic acid, they form invisible films like octyl alcohol.

When solid and slightly soluble, for instance, benzoic acid, they show the movements characteristic of thymol.

When solid and insoluble in water, like stearic acid, they lie inert on the surface of water, but can be spread out mechanically by the methods described for cholesterol.

The foregoing facts may be summarised by saying that pure paraffins do not spread, but that substances containing an  $-OH$  or  $-COOH$  group form invisible films when they are liquid and insoluble in water. They are also able to satisfy the attraction of water for active groups and thereby prevent the spread of oils. With pure paraffin as the test material these facts can be used for determining what substances cause spread of oil.

In conducting the test the substance is added to the pure paraffin and a thorough mixture is made by shaking in a drop-bottle. The effect of dropping the mixture on the surface of water is observed and noted, and then the mixture is left standing for a day or two to allow the excess of the substance to separate out. This enables the effect of a saturated solution of the substance in pure paraffin to be studied.

Simple compounds like ethyl alcohol, phenol and acetic acid cause the mixture to spread, but they are so soluble in water that they separate out at once, leaving small globules of paraffin on the surface. These substances are so soluble that the traces of them dissolved out during the process are not sufficient to satisfy the affinity of the water for active groups in oils, and therefore do not prevent the spread of crude oil. Water must be nearly saturated with these substances before it loses its affinity for active groups.

A mixture of 10 per cent. octyl alcohol in pure paraffin spreads, but part of the octyl alcohol leaves the paraffin, and forms an invisible film on the water, which prevents the spread of the octyl alcohol-paraffin mixture. If another drop of the mixture, or one of crude oil, is added, the drop remains as a compact blob until the excess of octyl alcohol has evaporated. A mixture containing 1 per cent. octyl alcohol behaves, however, quite differently. The paraffin is able to retain this amount of alcohol in solution, and the alcohol does not leave the mixture to form an invisible film. The absence of invisible film is proved by the spread of further additions of the alcohol-paraffin mixture. Evaporation of octyl alcohol from the mixture causes the film to alter continually in shape and thickness, but that this is not due to octyl alcohol on the surface of the water can be shown by preventing evaporation by covering the dish with a glass plate, when the film will become stable and of a definite thickness. The difference between the behaviour of the 10 per cent. and the 1 per cent. mixtures shows the disadvantage of adding too much octyl alcohol to the mixture. This will be referred to and other examples will be described in the section on the effect of excess of active material in oils.

Substances like stearic acid, benzoic acid and salicylic acid are not sufficiently soluble in pure paraffin for a concentrated solution to spread, but an intimate mixture of any of these substances with pure paraffin spreads on water. Salicylic acid differs markedly from stearic acid in being unable to prevent the spread of oils by satisfying the affinity of water for active groups. A mixture of stearic acid and paraffin spreads and the film breaks up once, owing to the stearic acid leaving the

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mixture to form an invisible film on the water, which prevents the spread of additional drops of the mixture or of any oil. A mixture of salicylic acid and paraffin spreads and breaks up in a similar manner owing to the acid dissolving out into the water, but each successive drop of the mixture spreads and the water still allows oils to spread. It is easy to prevent the spread of oils by adding stearic acid to the water, but impossible to do this with salicylic acid. Even when the water is saturated with salicylic acid it will allow crude oil to spread across it in a slow continuous fashion, and substances like salicylic acid containing two active groups may partly explain the difference between an invisible film formed by a substance with a single active group and the film which allows other oils to spread upon it.

Another form of film which does not prevent the spread of successive additions of oil is one which shows iridescent colours. These films are formed typically by complicated mixtures like crude oil or kerosene, but they can be imitated by making solutions in pure paraffin of mixtures containing several active substances. Vegetable oils probably spread by virtue of the free fatty acid they contain. Many samples do not appear to spread because excess of the acid forms an invisible film on the water which prevents the spread of any oil. Other samples, however, spread and show iridescent colours; and when this happens there is no limitation to the spread of oil. Because the invisible film consists of a single layer of molecules orientated regularly to the surface it is uniform in thickness, and shows no interference colours. The iridescent colours of the mixed film suggest that it is irregular in thickness<sup>(1)</sup>, and its molecules are arranged irregularly on its surface. The difference in behaviour of these two films is due to the irregularities on the surface of the mixed film, forming points of attraction by means of which the next layer of oil can spread.

This statement can be confirmed by an experiment based on the period necessary for an invisible film to form. The period is very short when the water contains electrolytes, but, as Hardy has shown, it is much longer when distilled water is used.<sup>(2)</sup> If oleic acid is dropped on distilled water it is possible to detect an evanescent display of colours during the brief period before the excess collects together into a blob in equilibrium with its invisible film. It is also possible to make crude oil spread upon the oleic acid if it is dropped at once on, or near, to the developing blob. If any appreciable time is allowed to elapse, the crude oil will not spread, even if it is dropped on the excess of oleic acid; and, if the crude oil is dropped at a distance from the blob of oleic acid it will not spread, because of the rapidity with which the invisible film forms. This experiment shows that a "mixed" film effect can be obtained even when a pure substance is used.

The different types of spread may be grouped as follows:—

(1) An invisible film. This is produced by a pure substance containing one active group in the molecule. It is one molecule thick. The active group is

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(1) Rayleigh, Lord. Studies of Iridescent Colour and the Structure producing it, Proc. Roy. Soc., 1923, Vol. A., CII., pp. 668-674.

Mallock, A. Note on Iridescent Colours of Birds and Insects, Proc. Roy. Soc., 1911, Vol. A., LXXXV., pp. 598-605.

(2) Hardy, W. B. The Influence of Chemical Constitution upon Interfacial Tension, Proc. Roy. Soc., 1913, Vol. A., LXXXVIII., p. 315.

Also Proc. Roy. Soc., 1911, Vol. B., LXXXIV., p. 220, and Vol. LXXXVI., p. 608.

adsorbed to the surface of water and exposes a uniform layer of inactive groups, which prevents the spread of excess of the substance and the spread of any other oil.

(2) A film due to condensation of the vapour of a volatile hydrocarbon. This may be more than one molecule thick. It collects into a blob like that of pure paraffin if evaporation is prevented. It does not stop other oils spreading on the water.

(3) A film containing substances like salicylic acid. This does not prevent the spread of oils. Salicylic acid contains two active groups and the action may be due to one group being adsorbed to the water, and the other exposed to attract a further layer of oil.

(4) A mixed film. This kind of film shows interference colours; and excess of the oil, or of another oil with active groups, will spread slowly across it, but pure paraffin will not spread.

(5) A thick mixed film. This occurs when excess of an oil is added to a film which shows colours. The colours disappear and a uniform film of sensible thickness is formed which may be difficult to see. But it differs from an invisible film in that even pure paraffin will spread by diffusing through the surplus oil on the surface.

So far only the  $-OH$  and  $-COOH$  groups have been mentioned, and now the effect of other active groups must be discussed.

The  $-SH$  group will probably have the same effect as its analogue, the  $-OH$  group.<sup>(1)</sup> It is known that sulphur compounds are present in crude oil, and the behaviour of thiophene free benzene, described on page 35, tends to confirm this view.

The effect of the  $-NH_2$  group has been tested by using freshly distilled pure aniline. That this substance is active is proved, since it both causes spread when added to pure paraffin, and prevents spread when used for saturating water.

The evidence about the  $-CHO$  group is conflicting. Formaldehyde and paraformaldehyde do not cause pure paraffin to spread, nor does the concentrated solution (thirty volumes) of formaldehyde prevent the spread of oils. A sample of benzaldehyde caused pure paraffin to spread, but it is possible that this was due to traces of benzoic acid formed by oxidation.

There is also some doubt whether substances containing the  $-OH$  and  $-COOH$  groups remain active when the hydrogen in the active group is replaced by a metal or an organic radicle, although Langmuir includes  $-O-$  and  $-COO-$  in his list of active groups. Metallic salts, like potassium acetate and sodium benzoate, do not cause paraffin to spread, but this may be due to the difficulty of getting them to dissolve in the paraffin. An intimate mixture of sodium oleate and paraffin spreads, but again this may be due to traces of free oleic acid. A mixture of oleic acid in pure paraffin was diluted down until it contained only 0.009 per cent. oleic acid, and it still spread. After this mixture was treated with metallic sodium the supernatant fluid was inactive. On shaking, however, it became active again.

(1) Hardy, W. B. A Theory of Lubrication, Brit. Assoc., Fourth Report on Colloid Chemistry, 1922, p. 198.

"The group SH acts much as OH, thiophenol  $C_6H_5SH$  and benzyl-hydrosulphide  $C_6H_5-CH_2SH$  resembling phenol and benzyl-alcohol respectively."

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Similar experiments were done on kerosene and crude oil. When sodium is added to these substances bubbles of gas are given off, owing to the sodium replacing hydrogen from  $-OH$  containing substances in the oil, and from the traces of water which are invariably present. The sodium becomes coated with the resultant substances and the spreading power of the oil is reduced. The following table shows the results obtained :—

	Amount of oil necessary to add to pure paraffin in order to cause the mixture to spread.	
	Kerosene.	Crude oil.
Before treatment with sodium	25 per cent. of the mixture.	0·5 per cent. of the mixture.
After treatment... ..	34 " " "	2·5 " " "
After thoroughly shaking up the mixture ... ..	28 " " "	0·1 " " "

The experiment shows that the oils lose activity by treatment with sodium and regain activity when the sodium compounds are mixed in again by shaking. This suggests that the metallic compounds derived from the  $-OH$  containing substances are active, but less soluble in the oil than  $-OH$  compounds. A crucial test of the activity of the  $-COO-$  group as distinct from the  $-COOH$  group would be to determine whether a perfectly neutral vegetable oil spreads on water. If such a substance could be prepared it might be as inert as pure paraffin until contaminated by a trace of free fatty acid.

#### Vc. THE EFFECT OF EXCESS OF ACTIVE MATERIALS IN THE OIL.

The nature of the substances which cause oil to spread has been discussed, and now the effect of adding too much of these materials to the oil will be described.

So much active material can be added to oils that the spread of the resulting mixture is interfered with. One example of this is seen in the behaviour of mixtures of octyl alcohol and paraffin described on page 37. A saturated solution of cholesterol in pure paraffin affords another example. When this is placed on water it spreads at first, but the excess of cholesterol soon leaves the paraffin, and covers the water with an invisible film that prevents the spread of oils. Even when the solution is diluted with pure paraffin until it is only 20 per cent. of a saturated solution it contains too much cholesterol. When it is diluted to 5 per cent. of a saturated solution an entirely different result is obtained. The paraffin is able to retain this amount of cholesterol and successive drops of the mixture spread satisfactorily.

If a little oleic acid is added to a sample of vegetable oil, which spreads well on water, its spreading powers are destroyed, and the excess of oleic acid can be demonstrated as an invisible film on the surface of the water. The same things occurs when oleic acid is added to crude oil, but much less oleic acid is sufficient because it is less soluble in mineral than in vegetable oils.<sup>(1)</sup>

(1) I have not recorded the amounts used in these experiments, but 5 per cent. would be enough for a vegetable oil and 1 per cent. for a mineral oil.

Instead of adding the active material to the bulk of oil the experiment can be done by adding it to a film of oil already formed on the surface of the water. In such a film there is only a small amount of oil, and a minute quantity of active material forms an excess. When oleic acid is added, with a platinum loop to a film of crude oil, the excess spreads on the water as an invisible film, and the crude oil collects together in blobs.

These examples illustrate the disadvantage of adding an excess of active material to the oil. The amount of active material present in the oil must not exceed the amount which the oil can retain in spite of the attraction of water. If this amount is exceeded the excess leaves the oil for the water. Excess breaks up the film, for the active groups satisfy the affinity of the water for active groups in the oil, and this completely prevents the film from spreading. When holes develop in a film, or when a film fails to cover a surface completely, the fact that the exposed areas consist of this inactivated water can always be demonstrated.<sup>(1)</sup>

Observations made by H. C. Fisher <sup>(2)</sup> on the effect of adding the Panama Canal larvacide to crude oil are of interest in this connection. The larvacide, which consists of a crude carbolic acid resin soap, was added to facilitate spraying the oil through nozzles, and the author writes as follows :—

“ It was noticed that, although the mixture flowed with ease through the pumps, when applied to the water the oil failed to spread immediately into a film, but remained floating on the surface in the form of globules, requiring the application of a large amount to thoroughly cover the water with oil. Of course the larvacide present with the oil diffused and did execution on the larvae, and at a later period of some hours, or even days, a tendency to film was noted.”

The explanation of this observation is that the excess of phenol left the mixture, saturated the upper layers of the water, and thereby prevented the mixture from spreading. Later the phenol diffused through the mass of water and the mixture commenced to spread.

From these considerations the value of adding any given material to an oil may be tested. When the resulting mixture is shaken up with water, the water should not prevent the spread of oils. This test has been carried out with a mixture of a sample of Panama Larvacide in crude oil, with the result that the explanation given in the previous paragraph was confirmed. Crude oil and kerosene do not inactivate water. Even the dark, tarry solution of extractives <sup>(3)</sup> which separates out at the bottom of drums of crude oil does not prevent the spread of oil. In crude oil and kerosene, therefore, there is no excess of active material.

If it is proposed to increase the spreading power of an oil by adding active material, this test should be applied to the resulting mixture. Methods proposed for measuring the effect on the spreading power of adding suitable amounts of active material will now be discussed.

<sup>(1)</sup> Pockels, A. On the Spreading of Oil upon Water, *Nature*, Vol. L., pp. 223-224.

<sup>(2)</sup> Report of the Health Department of the Panama Canal for the year 1921.

<sup>(3)</sup> See page 20.

#### Vd. METHODS PROPOSED FOR MEASURING THE SPREADING POWER OF OILS.

Two methods for estimating the spreading power of an oil have been investigated.

The first depends on the amount of oil which must be added to pure paraffin in order to cause the mixture to start spreading. It is an attempt to estimate the amount of active material in the oil, since the amount of oil required will be less proportionately to the amount of active material it contains. This method may be called the "paraffin mixture test."

In the second method water is completely inactivated by saturating it with an active material, and the test consists in finding how much this solution has to be diluted before it allows the oil to begin spreading. The idea underlying the test is that an active oil will spread on a stronger solution than a less active one. Since the material chosen for inactivating the water is thymol this method may be called the "thymol water test."

In both tests the temperature is a factor. The effect of a higher temperature is to increase the activity of spread, but the uniformity of the temperature conditions under which these experiments were done has been described in the Introduction. The effect of a possible variation of 5° C. in temperature is much less than the error of the experimental methods used.

The end-point or limit at which an oil is said to start spreading is the same in both tests. If an oil remains as a blob 1 mm. or more thick for an interval of three minutes it is regarded as not spreading. If the blob shows an average thickness of less than 1 mm. it is said to have begun spreading. This end-point is quite sharp, and, moreover, a blob which is thinner than 1 mm. continues to spread, while those that are thicker remain as blobs.

In practice 0.5 c.c. of the oil is used, and the resulting blob is measured with callipers. If the diameter is anything more than 2.5 cm. the oil has commenced spreading.

This end-point is not the result of the shape of the surfaces of the blob and surrounding water, because the same end-point is reached when the amount of oil is varied. An oil which just reaches the end-point on the test will still form a film 1 mm. thick when 5 c.c. of the oil is used, although the blob will be much flatter and 8 cm. in diameter. This point was proved by doing a series of estimations, using 1 c.c. and taking 3.5 cm. as the limit. The results were the same as those obtained by using 0.5 c.c.

The tests will now be described, and the results of their application will then be discussed.

#### Ve. THE PARAFFIN MIXTURE TEST.

The paraffin used may be either of the brands mentioned on page 35. The results of the test shown in the table on page 50 were obtained from two parallel series of estimations; in one "Calol" was used, and in the other the "E.H.V." brand, and the results were the same in each series. Moreover, each sample of these preparations was tested against a standard oil before it was used, and no difference was found between the results obtained by using different samples of these brands.

The substance to be tested should be soluble in the paraffin, and therefore the test is more applicable to mineral oils which mix in all proportions, than to vegetable oils which form only emulsions in pure paraffin.

The object of the test is to add paraffin to the oil until the mixture only just spreads, and to record the percentage of oil in the mixture when this occurs. Separate tests can be made with mixtures containing 90 per cent., 80 per cent., 70 per cent. and so on of the oil, but such a process takes a long time and wastes a lot of paraffin, therefore a method of continuous dilution of the mixture has been used. A small fraction of the mixture is tested after each addition of paraffin, and the residue is further diluted until the end-point is reached.

A mixture of known percentage is prepared and its volume is kept constant throughout the test. From this a measured fraction is removed, and the volume of the remainder is made up by adding paraffin. The part removed is used for testing the spreading power of the mixture, and the rest forms a lower dilution of known strength to be tested in the same way. In this way the dilution is continued step by step until the end-point is reached. The strength of the mixture at each stage of the dilution can be calculated readily.

The calculations of the test depend on the following general formula which applies to all mixtures of two constituents of different strengths, provided that there is no chemical action and that there is no change in volume when the two substances are mixed together, viz. :—

$$ax + b(v-x) = kv$$

where  $k$  is the strength and  $v$  the volume of the mixture;  $a$  and  $b$  are the strengths of the constituents and  $x$  and  $v-x$  their respective volumes. (1)

Applying this formula we can find the amount,  $q$ , which must be taken from a solution of percentage  $a$  and be replaced by diluent in order to reduce the solution to percentage  $k$ , since  $b(v-x) = 0$  and  $x = v-q$ , and we find that :—

$$a(v-q) = kv$$

$$q = \frac{a-k}{a} \times v.$$

For example, to reduce an 80 per cent. solution to one of 40 per cent. the amount that must be removed and replaced by paraffin is  $\frac{80-40}{80}$  or  $\frac{1}{2}$  of the volume. And to reduce a 0.9 per cent. solution to 0.8 per cent. is  $\frac{0.9-0.8}{0.9}$  or  $\frac{1}{9}$  of the solution.

The method of carrying out the test can now be described. The mixture is made in a 25 c.c. stoppered measured flask, since 2.5 c.c., the result of removing  $\frac{1}{10}$  of the volume, is sufficient for testing the spreading power of the dilution. It is not necessary to run through the whole gamut of dilutions, since all that is required is a mixture definitely just above the spreading point. In doubtful cases this can

(1) This formula expresses an obvious relationship and is therefore easier to remember than the formulæ devised for special cases of percentage and fractional dilutions, for example :—

Stephens, J. W. W., and Christophers, S. R. *Practical Study of Malaria and Blood Parasites*, 1908, p. 441,

Fletcher, W. *Percentage and Fractional Dilutions*. *Lancet*, 1921, p. 223.

Blacklock, R. *Percentage and Fractional Dilutions*. *Lancet*, 1921, p. 377.

Harvey, F. W. *Note on Dilution of Reagents*. *Ind. Journ. Med. Res.*, Vol. VIII., 1921, p. 131.

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be determined by a rough experiment, but a 50 per cent. solution will suffice for most samples of kerosene and a 5 per cent. for crude oils.

The required amount of oil is measured into the flask with a pipette, the paraffin is added up to the 25 c.c. mark from a burette, and the two oils are mixed together. Two clean petri dishes containing water are then prepared, care being taken not to touch the inside of the dish with the fingers. The sample to be removed is measured out in a graduated 1 c.c. pipette. The pipette should be graduated to deliver 1 c.c. and not merely to measure 1 c.c. The first cubic centimetre is rejected and serves to rinse out the pipette with the dilution that is being tested. The next cubic centimetre is divided into two parts of 0.5 c.c. each, and one of these is put in each of the petri dishes. The dishes are covered with a glass plate to prevent evaporation of the oil, and left for three minutes. After this interval the extent of spread is noted. If the mixture has spread no measurement is necessary, but if it remains as a blob it is measured with a dividing compass in order to determine whether its diameter exceeds 2.5 cm.

If the diameter is greater than 2.5 cm. the next dilution is chosen, and the amount to be removed from the mixture is calculated. The removal of this amount is completed by using the 1 c.c. pipette and the new dilution is made by adding paraffin from the burette up to the 25 c.c. mark. The flask is stoppered, the oils are well mixed, and the new dilution is then ready for testing. With a little practice much time can be saved by choosing the dilutions, so as to come quickly to the point at which the blob only just exceeds 2.5 cm. in diameter. The dilution is then continued by smaller gradations until the end-point is passed and the diameter of the blob no longer exceeds 2.5 cm.

The final stage of the test is to make sure that this lack of spread is due to dilution of active material, and not to the presence of inactivating substances on the water. This is done by adding a drop of crude oil to the water on which the last dilution was tested and ascertaining that crude oil spreads perfectly. In estimating the strength of kerosenes or crude oils the only cleansing required between the stages of the test is a rinsing of the inside of the dishes with a stream of clean water because these substances do not inactivate water (see page 34). The precautions, already mentioned, of starting with chemically clean dishes and of keeping the fingers from the inside of the dishes must of course be observed.

The difference in percentage between the last two dilutions should be as small as possible. In other words, the highest percentage at which the mixture does not spread should be found, so as to ensure that the end-point is the lowest possible. If this is not done the percentage quoted as the end-point of the test may be too high, and more oil would appear to be necessary than is actually the case.

Therefore, when the percentage is over 10, the difference should not be greater than 1, and when the percentage is less than 1 the difference should be less than 0.1. If the difference is greater than this standard a new mixture should be made up at the higher percentage and diluted down to confirm the previous result, or to lower the percentage at the end-point.

The test gives the percentage of the oil which is just sufficient to cause spreading, and the strength of the oil is obviously the inverse of this percentage. For example, if an oil A needs 10 times as much as oil B, it must contain a tenth of the active material per unit value contained by B. Therefore the reciprocals of



the values obtained in the test will give a series of figures which are proportional to the strength of the oils tested. For convenience these are multiplied by 100, so that the figure to represent the strength of an oil is 100 divided by the percentage needed to make the mixture spread. This figure also expresses the number of c.c. of the mixture which 1 c.c. of the oil can cause to spread. The unit value on this scale of strengths is the strength of an oil which is so weak in active material that it only just spreads, since the percentage required is 100 and the strength becomes 1.

The method involves experimental error in making dilutions and in measuring blobs. There is also the inevitable error due to the difference in percentage, however small it be made, between the dilution which spreads and the one that does not spread. The results, therefore, must be considered as rough estimations, but, in practice, surprisingly constant results are obtained with any given oil. And the figures denoting strength are proved to be valid, and roughly quantitative, by the fact that they give correct results when used for preparing mixtures of oil. As evidence of this the preparation of two such mixtures will be described.

Samples of kerosene (Crown Brand), solar oil and crude oil were tested with the following results :—

Material tested.	Result of test.		Control observation.
	Percentage required to start spreading the mixture.	Value taken to denote strength.	Highest percentage which failed to cause mixture to spread.
Kerosene ... ..	15	100/15 or 6·7	14
Solar oil ... ..	2	100/2 " 50	1·5
Crude oil ... ..	0·5	100/0·5 " 200	0·45

When these experimental results are inserted into the formula for mixtures, viz. :  $ax + b(v - x) = kv$  the results prove correct experimentally, thus :—

*Mixture I.*—To prepare a mixture of the kerosene and crude oil which will have the same strength as the solar oil.

If  $a$  and  $b$  are the strengths of the kerosene and crude oil respectively,  $k$  the strength of solar oil and  $v$  unit volume, the following results are obtained :—

$x$  or the volume of kerosene is  $45/58$ , and  $x - v$  or the volume of crude oil is  $13/58$ .

And when a mixture was made of 45 parts kerosene and 13 parts crude oil, the resulting oil was made experimentally to have the same strength as the solar oil.

*Mixture II.*—To find the strength of an equal mixture of kerosene and crude oil.

By making  $x$  and  $v - x$  in the above formula each equal to 0·5, the theoretical value for  $k$ , the strength of the resulting mixture, is 103.

By experiment the result was 110.

The test has been described and the results have been proved to have a rough quantitative value. In the next section the theory underlying the test will be considered and a fallacy in its application pointed out.

## VI. EVALUATION OF THE PARAFFIN TEST.

To discuss the composition of 100 c.c. of a mixture at the end-point of the test let  $A$  be the number of c.c. of the oil and  $X$  be the number of c.c. of the active material. The amount  $A$  is found experimentally in the test and from this  $100 - A$  the amount of pure paraffin present is also known. The amount  $X$  is not known, and the theory of the test assumes that it is the same in each test, since it is the amount which is just sufficient to cause 100 c.c. of the mixture to spread. And  $A$ , the amount of oil which contains  $X$ , is used as a basis for comparing the strength of oils.

This assumption is only justified if  $A - X$ , the inactive part of the oil, consists of the same kind of material as the  $100 - A$  c.c. of diluent. If it consists of material which is caused to spread more readily than the diluent, the value found for the strength will be too high. The effect of this fallacy in the test may be great when  $A$  is large, and  $A - X$  consists of paraffins of a smaller molecule and lower specific gravity than the paraffins used as a diluent.

The effect of the fallacy is seen when kerosenes of low specific gravity are tested. It is not obvious when crude oils of high specific gravity are estimated, because the paraffin base of these oils is similar to the diluent. Nor is it obvious when small amounts of kerosene are used, as in the preparation of the mixtures described in the last section. But the following experiment shows that the experimental strength is greater than the theoretical when larger proportions of kerosene are used.

One part of Tarakan crude oil (strength = 100) was mixed with ninety-nine parts of Shell kerosene (strength = 1.7). The theoretical strength of the resulting oil was 2.7, but the experimental strength was 4.8.

The test, therefore, makes the strength of kerosenes too high, because the standard for measuring the spreading power of the active material is lowered during the test. It shows that there is more active material in crude oil than in kerosene, but it does not prove that crude oil spreads better than kerosene. The smaller amount in kerosene might be as effective in spreading the lighter paraffins.

The test would be of value, however, in comparing the spreading powers of kerosenes, since the standard of paraffin would be approximately the same. And the results of testing the same sample of kerosene at different times, as is done later in this paper, would be still more trustworthy.

## Vg. THE THYMOL WATER TEST.

The test depends on the fact that no oil can spread on water saturated with an active substance. It consists in finding the percentage to which this solution must be diluted before the oil begins to spread upon it. The idea underlying the test is that a strong oil will be able to spread on a stronger solution than a weak oil.

Thymol is chosen for inactivating the water for three reasons. It is not too soluble (*e.g.*, phenol), and not so insoluble as to form an invisible film, which would interfere with the result of dilution in the way described in Experiment 6 on page 33. And thirdly, it is not too volatile, so long as precautions are taken to prevent the solution losing strength by evaporation of thymol.

The saturation of water is a tedious process. A large quantity at a time is

prepared by shaking up thymol and hot water in a carboy. The carboy is left for a day and a sample is pipetted out from the centre and tested to see whether it allows thymol particles to move. If thymol particles can still move the saturation of the water is not complete, and the process must be repeated. The water is heated by passing a little steam and is shaken up and left for another day. On one occasion this process had to be repeated three times before the water was completely inactivated.

The inactive water is syphoned out from the centre of the solution and stored in Winchester quart bottles as stock solution. A large crystal of thymol is placed in each bottle to maintain saturation. Bottles of convenient size are filled just before doing a test by syphoning out the solution from the centre of the stock bottle. The syphoning is done in order to avoid excess of thymol in the final solution, since any excess would dissolve when water is added and so interfere with the results or dilution. Most of the excess floats on the surface but some waterlogged crystals sink to the bottom. It is impossible to filter off this excess owing to the risk of adding an invisible film from the filter paper (see p. 32), but by syphoning out the centre of the solution a saturated thymol water, free from excess and free from invisible film, is obtained in the final bottle.

In order to prevent loss of thymol by evaporation the bottles must not be left open longer than necessary. Control experiments have shown that this is important. Ten samples of saturated thymol water were exposed in watch-glasses to the conditions in the laboratory, and at varying periods a sample was tested by adding thymol particles. The one tested after five minutes was still inactive, after ten minutes thymol particles showed slight movements, and after an hour these movements were quite marked.

The method of performing the test is quite simple. A stoppered 100-c.c. graduated cylinder is taken. The percentage dilution to be tested is made by pipetting into this the required number of c.c. of the saturated solution, and adding water up to the 100 c.c. mark. Thus to make a 60 per cent. solution 60 c.c. of the saturated solution is diluted to make 100 c.c. This is shaken up and is sufficient to fill two petri dishes. In each 0.5 c.c. of the oil is placed, the dish is covered and left for three minutes. The blob is then measured: if it is more than 2.5 cm. in diameter a stronger solution is made, and if it is not greater a weaker solution is prepared. In this way the strength of a solution which allows the oil to begin spreading, and the strength of one that does not, are found. If the difference between these two solutions is greater than 5 per cent. it is reduced by testing intermediate strengths. The difference can be reduced to 2.5 per cent. or even to 1 per cent. with a fair degree of accuracy, but the standard suggested is sufficient for practical purposes. The lower strength, that is the one which just allows the oil to begin spreading, is quoted as the result of the test.

Control experiments were done to prove the necessity for covering the dish. Tarakan crude oil, No. 2 (strength on paraffin test = 100), was found to spread on a 55 per cent. solution, but not on one of 56 per cent. A solution of 60 per cent. or 5 per cent. higher than the spreading point was made, and the following table compares the diameter of a blob of Tarakan oil in an open dish with that of a blob under the conditions of the test.

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Time when the blob was first measured.	Diameter of blob.	
	In open dish.	In closed dish.
When first made ... ..	2.2 cm.	2.2 cm.
After the interval of three minutes ... ..	2.35 cm.	2.25 cm.
After ten minutes ... ..	2.75 cm.	2.25 cm.

After 10 minutes the blob in the open dish had begun spreading and was 0.5 cm. wider than the blob in the covered dish, which had not altered since the 3-minute interval allowed in the tests. At the end of 3 minutes the blob in the open dish had not yet spread, but was 0.1 cm. wider than the covered one. This more rapid spread in open dishes shows its effect in another control experiment, in which the result of testing a series of oils in open dishes is compared with the technique described for the test. The results were uniformly 3 per cent. higher in the open dishes. This difference was so constant that it would almost be justifiable to do the test in open dishes and subtract 3 per cent. from the result. In the paraffin test the dish is covered to prevent evaporation of oil from the blob, but in this test there is an additional reason for covering the dish, namely, to prevent loss of strength of the thymol solution.

### Vh. EVALUATION OF THE THYMOL WATER TEST.

This test differs fundamentally from the paraffin mixture test in two ways.

(1) It is not an attempt to estimate the active material in the oil. It treats the oil as a whole and alters the attractive forces in the water outside the oil.

It therefore gives a better idea than the paraffin mixture test of the relation, existing inside the oil, between the active material and the nature of the inactive material on which it acts. The paraffin test shows that there is more active material in crude oil than in kerosene; but it does not prove which oil has the better spreading power, since it does not show whether the effect on the lighter paraffins of the small amount of active material in kerosene is more or less than the effect of the larger amount in crude oil acting on heavier paraffins. The thymol test answers the question definitely, for the crude oils spread on much stronger solutions of thymol than the kerosenes.

(2) The standard used in comparing the strength of oils on the paraffin test is a weak oil, so weak that it only just spreads; but the standard in this test is a hypothetical oil so strong that it spreads on 100 per cent. saturated thymol solution. Therefore a given increase in strength of the oil does not produce the same difference in result at different parts of the scale in this test. The difference in strength between the stronger oils shows smaller gradations than those shown at the lower end of the scale. For this reason the results given by the test cannot be used for preparing quantitative mixtures, even to the extent to which this is possible with the results of the paraffin test.

Although the values given by the two tests are not strictly comparable, both tests place a series of oils in the same order as regarding strength of spreading power. This can be seen from the table in the next section which shows the result of applying each test to a series of different oils.

Vj. APPLICATION OF THE TESTS TO STANDARD COMMERCIAL OILS.

Samples of oils from known localities and of known commercial standards were kindly given for this work by Mr. J. van Rijn van Alkemade, chemist to the Asiatic Petroleum Company at Singapore. The data which he has supplied with regard to the place of origin and physical properties of these materials are summarised below:—

Name of sample showing place of origin.	Specific gravity at 15° C.	Flash point (closed cup).	Viscosity Engler at 30° C.
Balik Papan liquid fuel, No. 1 ...	0·9100	73° C.	1·7 degrees
" 2 ...	0·9100	74° C.	17 "
Tarakan liquid fuel, " 1 ...	0·9303	72° C.	2·6 "
" 2 ...	0·9304	71° C.	2·6 "
Miri liquid fuel, " 1 ...	0·9426	84° C.	2·2 "
" 2 ...	0·9426	85° C.	2·2 "
Ed. extract from Borneo kerosene ...	0·895	—	—
Shell kerosene ... ..	0·810	—	—

*Balik Papan and Miri Liquid Fuels* are the residues of crude oils from which the benzine and kerosene fractions have been removed by distillation. As far as is known *Balik Papan Liquid Fuel* contains 50 per cent. aromatic hydrocarbons and *Miri Liquid Fuel* consists of naphthenic hydrocarbons.

*Tarakan Liquid Fuel* is a crude oil taken direct from the wells, without being subjected to any distillation process, and contains a small quantity of kerosene fractions. As far as is known 65 per cent. of this oil consists of aromatic hydrocarbons.

*The Shell Kerosene* consists mainly of paraffin (aliphatic) hydrocarbons.

*The Ed. Extract from Borneo Kerosene* is not a market product, but consists of aromatic hydrocarbons removed from crude kerosene by the Edeleanu process. The process depends on the fact that the aromatic compounds are more soluble in liquid sulphur dioxide than aliphatic compounds. The aromatic hydrocarbons are removed in order to improve the burning properties of the kerosene, and mixtures of the Ed. Extract and the Shell Kerosene would represent the grades of kerosene on the market. The sample was a brown-coloured fluid resembling a very impure kerosene.

*Petroleum Jelly*.—"It is a soft oily paraffin wax of an amorphous nature. The melting point of this sample is 41·5° C. according to method of Ubbelohde." This sample was a deep yellow colour.

Both tests were applied to this series of materials and the results are shown in the following table. Columns 2, 5 and 6 show the experimental observations on which the results are based. Columns 3 and 4 place the results of the tests side by side, and thus corresponding values on the two tests can be compared.

Substance tested.  (1)	Result of tests.			Control observations.	
	Paraffin mixture test.		Thymol water test.	Paraffin mixture test.	Thymol water test.
	Lowest percentage required to cause spread. (2)	Value taken to denote strength. (3)	Strongest solution on which oil spreads. (4)	Highest percentage which did not cause spread. (5)	Weakest solution on which oil did not spread. (6)
Tarakan, No. 1 ...	0.9	111	58 per cent.	0.8	59 per cent.
"    2 ...	1.0	100	55 "	0.95	56 "
Miri    "    1 ...	1.6	62	34 "	1.5	35 "
"    2 ...	1.7	59	31 "	1.6	32 "
Petroleum jelly ...	1.8	56	—	1.7	—
Balik Papan, No. 1 ...	2.0	50	25 "	1.9	25 "
"    2 ...	2.1	48	22 "	1.9	23 "
Kerosene Ed. extract	10	10	14 "	9.0	15 "
Shell kerosene ...	60	1.7	2.5 "	58.0	3 "

#### Vk. THE EFFECT OF OXIDATION ON THE SPREADING POWER OF OILS.

When the strengths of different samples of crude oil are estimated a considerable variation in spreading power is found, and the same applies to samples of solar oil and kerosene. But often the same sample of oil is found to have gained in strength after it has been kept for some time in a bottle.

This increase in strength was detected when the paraffin mixture test was being worked out. In the early stages of the test a sample of each type of oil—crude oil, solar oil and kerosene—was chosen as a specimen with which to compare unknown samples of oils. These chosen samples were diluted with pure paraffin until the mixture no longer spread, but formed blobs. Then a series of known dilutions was prepared, and the diameter of 0.5 c.c. of the mixture corresponding to each percentage of oil was measured and recorded. This series of dilutions was set aside with a view to using it as a standard for comparing the percentages of the unknown oil needed to produce a similar series. Ultimately one of these dilutions, namely the percentage necessary to cause the blob to exceed 2.5 cm. in diameter, was chosen to be the end-point of the test, since it is the percentage sufficient to cause the mixture to begin spreading.

The standard dilutions were prepared separately in phials. The phials were well corked, but were less than half full, and were left standing on the bench for future use. It was impossible, however, to use them as standards because when re-examined their spreading power had increased, and dilutions which did not spread when they were prepared had acquired the property of spreading.

The percentage dilutions of kerosene which were prepared on June 19th, 1922, were 50, 40, 35, 30, 25, 20, 15, 10 and 5, of which only the 50 per cent. mixture spread.

On the 21st, 23rd and 27th the series was re-tested, and still only the 50 per cent. spread, but the extent of the spread was greater than on the 19th.

On the 30th it was found that the 40 per cent. and 35 per cent. had developed spreading properties, and the lower dilutions were inert.

On July 4th the 30 per cent. commenced spreading; and on July 31st the 25 per cent., 20 per cent., 15 per cent. and 10 per cent. had all become active, only the 5 per cent. remaining inert. This increase of activity was accompanied by a progressive darkening in colour of the dilutions.

The dilutions of solar oil prepared on June 22nd, 1922, were 5 per cent., 4 per cent., 3 per cent., 2 per cent. and 1 per cent., and of these only the 5 per cent. dilution spread.

On the 24th only the 5 per cent. was active, but on the 27th the 4 per cent. commenced spreading, and on the 30th its spread was more extensive.

On July 4th no further change had taken place, but by the 31st even the 1 per cent. showed an active spread and there were no lower dilutions for comparison.

Similar results were obtained with the crude oil dilutions. The strengths prepared on June 19th were 1 per cent., 0·8 per cent., 0·4 per cent., 0·2 per cent. and 0·1 per cent. Of these only the 1 per cent. was active.

By the 23rd the 0·8 per cent. had developed activity, but beyond an increase in extent of spread of the 0·8 per cent., no further change had occurred up to July 4th. This is probably due to the gradations between the dilutions of this series being greater than those of either of the other two series.

By July 31st, however, both the 0·4 per cent. and the 0·2 per cent. had become active, and only the 0·1 per cent. remained inert.

The definite increase in strength can be accounted for in one of three ways, namely:—

- (a) Increase in concentration of active material caused by loss of inactive vapour.
- (b) Contamination with active substances.
- (c) Absorption of oxygen from the air.

The fact that these dilutions were kept in well-corked phials would seem to exclude (a) evaporation, and (b) contamination, and the following experiment was done to exclude the presence of air, in order to test whether the increase in strength was due to (c) oxidation.

A sample of kerosene which spread at 60 per cent. and one of crude oil which spread at 1 per cent. were taken. A phial was filled to the top with each sample, and an attempt was made to cork the phials without including any air. Another phial was only half filled with each sample and tightly corked. Evaporation and contamination were thus reduced to a minimum in these specimens, but the half-filled phials allowed free contact of the oil with air, and in the full phials the amount of air was as small as possible.

After some months the specimens were opened and tested. The attempt at corking the kerosene so as to exclude air was not quite successful, and the "air-free" sample had gained in activity.

It spread at 35 per cent. instead of 60 per cent., but the "oxidised" sample spread at 15 per cent., and had become distinctly yellow in colour. The crude oil excluded from air had retained its original strength and still spread at 1 per cent., but the "oxidised" sample spread at 0·475 per cent.

Another interesting way of showing that kerosene and crude oil are able to absorb oxygen is by the use of a solution of indigo. It is well known that this acts

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as a catalyst when it is in contact with an oxidisable material like sugar, and conveys oxygen from the air to the sugar. If air is excluded the indigo gives up its oxygen, and becomes colourless.

The same thing occurs when indigo solution is placed with kerosene or crude oil out of contact with air. Phials containing indigo and oil, corked so as to practically exclude air, became a much lighter blue. Control phials containing indigo solution alone and indigo with pure paraffin did not become lighter in colour.

The method of excluding air by trying to cork a phial absolutely full of liquid is, however, a crude one, and if this and the preceding experiment had been done by isolating the materials from air in a tube over mercury, much more definite results would probably have been obtained.

By 1924, when the samples were received from the Asiatic Petroleum Company, the danger of the oils altering in strength was known and precautions were taken to prevent this happening when the tins were opened. Bottles were filled to the top with the samples, and the corks were tied in and covered with wax. These bottles were stored and formed the material for the experiments on the strengths of the different oils described in the last section. The residues left in the tins were found to have increased in spreading power when examined later.

There can be little doubt that the increase of strength under discussion is due to oxidation of the oil. The experiments still to be described in this section will confirm this view, although an example due to contamination with active materials, and an example of increase in spread due to loss of inactive vapour, will also be given. As a demonstration of the effect due to this oxidation the following experiment is described:—

One set of the store bottles, which had been kept for a month, was opened and re-tested. The strengths had not changed and the results were practically the same as those shown in the table on p. 50. These are shown in the control column in the following tables.

Oil was then poured out until the bottles were only one-third full. The bottles were re-corked and left for eight days. On examination the oils had gained in spreading power to the extent shown in the following tables.

Oil.	Paraffin mixture test.		Gain in strength.
	Control. <sup>(1)</sup>	Oxidised samples.	
	Strength after keeping for a month in full bottles.	Strength after keeping for 8 days in bottles one-third full.	
Tarakan, No. 1 ...	111	125	14
" 2 ...	100	118	18
Miri, " 1 ...	62	67	5
" 2 ...	59	62	3
Balik Papan, " 1 ...	50	53	3
" 2 ...	48	50	2
Kerosene Ed. ext. ...	10	10	0
Shell kerosene ...	17	18	0.1

(<sup>1</sup>) The figures in this column are the same as the results of the examination a month previously. (See table on p. 50.)



Oil.	Thymol water test.		Change in result.
	Control (1).	Oxidised samples.	
	Result after keeping for a month in full bottles.	Result after keeping for 8 days in bottles one-third full.	
Tarakan, No. 1 ... ..	58 per cent.	63 per cent.	5 per cent.
" 2 ... ..	55 "	56 "	1 "
Miri, No. 1 ... ..	35 " *	36 "	1 "
" 2 ... ..	32 " *	33 "	1 "
Balik Papan, No. 1 ... ..	25 "	26 "	1 "
" 2 ... ..	22 "	24 "	1 "
Kerosene Ed. ext. ... ..	14 "	15 "	1 "
Shell kerosene ... ..	25 "	3 "	0.5 "

(1) The figures in the control column are the same as the results of the examination a month previously (see the table on p. 50) with the exception that the figures for Miri oils (\*) show an increase of 1.

*Deduction.*—Both tests show that the oils increased in spreading power when they were exposed to the action of air for eight days. The increase did not occur in the absence of air.

This increase in spreading power due to oxidation does not occur in pure paraffin. The following materials were left freely exposed to air in a thin layer at the bottom of a petri dish:—

- Pure liquid paraffin.
- Pure liquid paraffin and water.
- Pure liquid paraffin and paraffin wax.
- Pure liquid paraffin, paraffin wax and water.

The petri dishes were covered to prevent contamination by dust and left for a year. After that period no material capable of causing pure paraffin to spread could be detected. On the other hand, when a paraffin wax, pure enough to form a blob when melted on hot water, was kept melted in a test-tube at about 100° C. on a water-bath for any length of time, it invariably developed spreading properties. This was not ascribed to oxidation, but to contamination. The absorption of active material must be very rapid at 100° C. when it is remembered that mere contact with the fingers at ordinary temperatures may impart enough active material to pure paraffin to cause it to spread (see p. 35). Pure liquid paraffin behaves in a similar way and one sample kept on the water-bath for forty-eight hours became so active that it formed a complete film. But a control sample, which was corked directly the temperature had risen to 100° C., remained inactive, and it is for this reason that the activation of pure paraffins is not ascribed to oxidation.

Most samples of tars, whether natural or derived from coal, cause pure paraffin to spread. The recommendation of Sollman that asphalt should be added to oils to increase their spreading powers has been referred to on p. 31. But some samples of tar do not cause paraffin to spread, although they form a deep brown solution. When this solution was kept exposed to air in a corked bottle for a week it was sometimes found to have developed spreading powers. One particular sample of tar was especially active in this respect. It was given by Dr. Quaife, of the Kuala

Lumpur Rubber Company, and all that could be traced of its origin was that its name was Bitumen Compound, and that it was specially prepared for insulating high tension currents. A freshly prepared solution of this tar in paraffin did not spread, but when the solution was kept till the following day it acquired the power of doing so. It was often shown that the solution prepared the previous day spread, but a solution freshly prepared, however strong, formed a blob. That the spread obtained on keeping the solution was not the effect of having a stronger solution was proved by removing the excess on the first day, and observing that the same change occurred in the clear supernatant solution. The change did not take place in the absence of air. The tar did not have sufficient active material to cause the paraffin to spread, but was able to absorb oxygen from the air, and so form sufficient active material to cause spread.

These experiments do not prove that the tar was acting as a catalyst conveying oxygen from the air to the paraffin and so converting it into spread-producing material, since it might itself have become sufficiently active by oxidation. But they are enough to form the basis of a theory that the increase in spreading power by oxidation of kerosenes and crude oils is due to the presence of materials, like the tar, causing oxidation of paraffins by catalytic action. Some support of this idea is derived from the experiments just described, since the increase in activity is more marked in the dilutions prepared by the addition of fresh paraffin as material for the catalyst to work on, than in the oils to which no paraffin had been added. The hypothesis has been applied in the following study of the constitution of kerosene and its vapours. The experiments tend to confirm the presence of catalytic oxidising agents.

Provided that there is no alteration of strength due to chemical change, evaporation of kerosene will have certain definite effects on the strength of the residue, viz. :—

- (a) If the vapour is stronger than the remaining kerosene the strength of the residue will be decreased by evaporation.
- (b) If the vapour is the same strength there will be no alteration of strength.
- (c) If the vapour is entirely inactive, the strength will decrease in a certain definite ratio. The strength multiplied by the volume of the residue will be a constant figure.

The converse of these is also true; for instance, if the strength diminishes after evaporation the vapour is the most active part of the oil. The following three experiments on the constitution of kerosene and its vapours are based on these obvious axioms.

The first experiment was to test the effect of evaporation when there was full opportunity for oxidation to take place. The kerosene was evaporated in free contact with air, by pouring it from a graduated cylinder into an open dish and back again until its volume was reduced to a definite percentage of the original volume. One sample was reduced to 90 per cent., another to 70 per cent., and a third to 50 per cent. The strength of the original sample was 6·7 and the following table shows the increase of strength that took place. The last column shows the increase of strength that would have resulted if the vapour had been entirely inert, that is, the maximum increase of strength which can be attributed to evaporation alone.

Percentage of kerosene left.	Strength by paraffin mixture test.	Theoretical strength if vapour lost was inert.
100	6.7	6.7
90	8.0	7.4
70	13.3	9.6
50	33.3	13.4

The strength is seen to be even greater than can be accounted for by evaporation of inert vapour. The difference is due mainly to oxidation during evaporation, although, in this experiment, absorption of active material from the air cannot be definitely excluded. This difference is seen to increase more rapidly as evaporation proceeds.

The difference would have been still greater if the vapour had been active instead of inert. The second experiment was devised to determine whether the vapour was active, and whether oxidation was sufficient to counterbalance the loss of active vapour.

Oxidation was prevented by evaporating the kerosene in the absence of air. This was done by placing the oil in a graduated cylinder and bubbling carbon dioxide through it. The carbon dioxide had been freed from oxygen by passing it through an acid solution of potassium permanganate and an alkaline solution of pyrogallic acid, and then dried by passing it through calcium chloride.

The kerosene used had a strength of 5, and 50 c.c. of this sample was placed in the cylinder. Part of the vapour was condensed in a graduated centrifuge tube immersed in ice and salt. After 0.5 c.c. had been condensed the experiment was stopped and the residue of kerosene was found to be 48.5 c.c. Thus the residue formed 97 per cent. of the original volume, 2 per cent. escaped as vapour and 1 per cent. was condensed.

The condensed vapour was more active than the original sample and had a strength of 6.7. The residue had lost in strength and gave a value of 4.8. The vapour that escaped must have been stronger as well as more volatile than the vapour that was condensed. By calculation its strength was 13.2.

The carbon dioxide in the cylinder was replaced by air and the cylinder was corked and left standing for a week. The residue had then a strength of 6.7 and had therefore more than regained its loss in strength.

The results may be summarised in general terms as follows: The first vapour given off by kerosene was strong in spreading material and caused the residue to become weaker. When the residue was kept corked, but exposed to air it regained its strength.

These changes in strength closely resemble the changes in the effect of kerosene vapour on larvae (see pp. 24-26).

The fact that the changes in spreading power and in toxicity were so similar was at once noticed, and the third experiment was devised in order to ascertain in greater detail the changes that occur in kerosene during evaporation.

The same technique was used as in the previous experiment, except that no attempt was made to condense the vapour. Precautions were taken to preserve the whole of the vapour in the original sample of kerosene. The cork was removed and

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quickly replaced by a plug of cotton wool, through which the amounts required were removed with a pipette.

A sample of 50 c.c. was placed in the graduated cylinder. The carbon dioxide was passed through slowly and was stopped at intervals in order to remove 1 c.c. for testing the strength of the residue. The amount removed was allowed for and the percentage of kerosene remaining at each stage of the experiment was calculated.

The strength of the original sample was 4. The evaporation was continued in stages until the residue consisted of 50 per cent. kerosene, and the following table shows the changes in strength that occurred. The last column shows the strength of the vapour given off at each stage. This is calculated by applying the formula given on p. 43.

Residue.		Vapour.	
Amount of kerosene left.	Strength of residue.	Amount of kerosene evaporated.	Calculated strength of vapour.
100 per cent.	4	—	—
99 "	3	1 per cent.	103
92.7 "	2.8	6.3 "	5.9
85.8 "	2.9	6.9 "	1.6
74.4 "	3	11.4 "	2.2
49.6 "	4.5	24.8 "	0

The first 1 per cent. lost as vapour was extremely active and caused a marked loss in strength in the residue. The next loss of 6.3 per cent. was less active and only slightly reduced the strength of the residue. In the two following stages the vapour was only slightly active and the residue increased slowly in strength. In the final loss of 24.8 per cent. the strength of the residue increased rapidly and the vapour was inert.

Calculation from the figures in the table shows that the amount of active material in the residual half is slightly greater than the amount in the vapour, the ratio being as 223 is to 177.

The volatile active material is concentrated in the most volatile 25 per cent. of the oil, the next 25 per cent. is inert, and the least volatile 50 per cent. also contains active material. The previous experiment has shown that loss of the volatile active material is regained when the kerosene is kept corked in contact with air. These facts support the hypothesis set forward on p. 54: (a) the active substances in the non-volatile half behave as catalysts; (b) in the presence of air they oxidise the simpler, and therefore more volatile, hydrocarbons, thereby increasing the spreading power of the oil.

These experiments explain an observation made by E. B. Johnson (<sup>1</sup>). He finds that oil-soaked sawdust causes a better film when it is left for twenty-four hours before using it, and also that certain waste from garages did not spread well until they were soaked into sawdust. He suggests that the latter may be due to

(<sup>1</sup>) Johnson, E. B. Oiling and Larvacides. U.S. Public Health Bulletin, 1921, No. 115, pp. 100-102.

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the filtering out of carbon particles by the sawdust; but oxidation must be very rapid when the oil is exposed in a thin layer on the wood particles, and probably causes the improvement in spreading power which he observed.

Another effect of oxidation was seen in attempting to make an oil which would spread to a certain extent and remain as a film of a definite thickness. Such an oil can be prepared, but when the film is exposed to air it gradually expands and on the following day completely covers the dish. If the film is kept in an atmosphere of carbon dioxide this slow spreading does not occur, the film remains stationary for an indefinite time and ultimately shows the appearance of wet oil which was described on p. 18.

But the main interest of the experiments is that the changes observed in the spreading power of oils are the same as the changes in toxicity recorded in Chapter IV. In the next chapter the relation between spreading power and toxicity will be discussed.

## CHAPTER VI.

THE RELATION BETWEEN THE SPREADING  
POWER OF OILS AND THEIR TOXICITY.

THE effect of oxidation upon the spreading power of kerosene, described in the last chapter, throws light upon the experiments on the toxicity of kerosene and petrol vapours described in Chapter IV. When the latter were done, in 1920, the loss in toxicity of the vapour when the oil was exposed to air was due obviously to the most volatile fractions being the most toxic, but the regain of toxicity when the kerosene was corked and stored was not understood.

The spreading power of the non-volatile residue has now been traced to  $-OH$  containing materials which cause an increase of similar substances in the vapour when the kerosene is exposed to the action of air in a corked bottle. The increase in toxicity that occurred (1) when kerosene was kept under the same conditions is so similar that the substances which cause spread would appear to be the same as those which are toxic to larvae. This hypothesis led to a further study of the effect of vapours upon larvae. The experiments were done in the same way as those described in Chapter IV, with three improvements:—

(a) A smaller petri dish was used and placed directly on the sheet of glass, the loss of vapour and entry of air being prevented by a ring of vaseline. The substance to be tested was introduced in a small metal phial cap, and when two vapours were being tested the substances were placed in separate caps.

(b) The larva was not exposed in a watch-glass of water, since this allows it to escape from the action of the vapour unless it stays at the surface all the time. It was exposed on a slide in a film of water, and imprisoned by one of the aluminium rings supplied for mounting thick specimens without pressure.

(c) The reaction was tested regularly at half-minute intervals, so that only the time at which the last reaction occurred need be quoted. It may be taken for granted that at the next half-minute all reaction to stimulus had disappeared.

By this technique slightly more uniform results were obtained, which must be attributed mainly to (b).

In the first series of experiments, Merck's pure benzene was used to represent the hydrocarbon part of kerosene. The time it needed to cause loss of reaction was compared with that needed by two alcohols, ethyl and octyl, and two known anaes-

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(1) See Experiments II and III, p. 24.

thetics, ether and chloroform. The time taken by a combination of each of these toxic vapours with pure benzene was then found, and the results are shown below :—

Effect of each vapour separately.		
Substance.	Minutes taken to cause loss of activity.	Average (minutes).
Merck's pure benzene ...	8, 8·5, 8·5, 10·5	8·9
Ethyl alcohol ... ..	30·5, 31, 32, 33	31·6
Octyl alcohol ... ..	28·5, 31, 33·5, 33·5	31·6
Ether ... ..	3·5, 4, 4·5, 4·5	4·1
Chloroform ... ..	2·5, 3, 3, 3·5	3·0

Effect of each vapour combined with Merck's pure benzene.		
Substance.	Minutes taken to cause loss of activity.	Average (minutes).
Ethyl alcohol ... ..	6·5, 6·5, 7, 8	7·0
Octyl alcohol ... ..	6·5, 7, 8·5, 10·5	8·1
Ether ... ..	6, 6·5, 6·5, 7	6·5
Chloroform ... ..	4, 4, 4·5, 4·5	4·25

The volatile anaesthetics have a rapid effect while the alcohols cause loss of reaction slowly. The pure benzene stops reaction fairly quickly and this can be due only to a condensation of the vapour into a film of Type 2 (see p. 39) on the lining of the breathing tubes. It has no active group to which any anaesthetic action can be ascribed, and the reason for attributing an affinity of the breathing tubes for pure hydrocarbons has been shown on p. 10.

The presence of this film has, however, a very different effect on the action of the alcohols and the volatile anaesthetics. It delays the action of ether and chloroform, but greatly accelerates the action of the alcohols, for example, ether needed 6·5 instead of 4·1 minutes, but octyl alcohol only needed 8·1 minutes instead of 31·6. The film tends therefore to protect the breathing tube from the effects of ether and chloroform, but it acts as a vehicle for the relatively inert alcohols and increases their toxicity. The hydrocarbon film apparently absorbs the alcohols and enables them to penetrate the lining of the breathing tube more rapidly.

The results of the experiments on vapours described in Chapter IV can now be discussed.

Sen's (1) suggestion that kerosene vapour is an anaesthetic is true. The recovery of larvae from the effects of pure benzene vapour is a recovery from asphyxiation, but kerosene vapour is not a pure hydrocarbon and has been shown in Chapter V to contain oxidised substances which cause the oil to spread. The last experiment has shown that a volatile hydrocarbon increases the toxicity of alcohols which are oxidised substances capable of causing oil to spread and having a definite anaesthetic action. This is the explanation of the toxicity of kerosene

(1) Quoted on p. 28.

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vapour and it has been shown in Chapter IV that larvae are able to recover from its effects when placed in contact with air. The larvae that were submerged in water died just as an unconscious man might die who falls with his head in a tub of water when under the influence of alcohol.

The observation of Moore and Graham <sup>(1)</sup> that different samples of kerosene vary in toxicity is confirmed and extended since the vapour of the same sample has been seen to vary from time to time. Macfie <sup>(2)</sup> also states that the action of the vapour is less constant and slower than that of a film; and the evaporation and oxidation which have been described in Chapter IV probably account for this variation in the effect of the vapour.

Freeborn and Atsatt <sup>(3)</sup> lay stress on the toxicity of the volatile fractions of kerosene, and Moore and Graham find that when used as a contact poison the non-volatile fractions killed a greater percentage of aphids than the volatile. Our experiments threw some light on this controversy. The extreme toxicity of the volatile fractions has been demonstrated and the less volatile fractions are less toxic, but the non-volatile residue has been shown to contain a greater proportion of the active (toxic) material than the volatile fractions. Crude oil has been shown to contain a greater proportion of these substances than kerosene, and therefore kerosene need not be added to it in order to increase its toxicity to anopheline larvae since contact with crude oil will kill as certainly, but not so quickly, as contact with kerosene.

In another series of experiments petrol was used as the hydrocarbon basis instead of benzene, and the experiments were repeated with each of the toxic substances.

The petrol used gave off a very toxic vapour when the bottle was first opened, and caused loss of reaction in an average of 16.6 minutes (16, 16, 16.5 and 18 minutes). When a large bulk of this sample was exposed in an open dish for half an hour it gave off a weaker but fairly constant vapour. Four larvae tested at different times throughout the experiments lost reactivity in 30.5, 30.5, 31.5 and 33 minutes, the average being 31.4 minutes.

In the following table the time taken by each substance to cause loss of reaction is quoted from the last experiment and compared with the time which each took when combined with petrol vapour.

Substance.	Effect when combined with petrol vapour.		Effect alone.
	Minutes taken to cause loss of activity.	Average minutes.	Time taken (minutes).
Ethyl alcohol ...	15, 17.5, 17.5, 19	17.25	31.6
Octyl " ...	8.5, 10.5, 11.5, 15.5	11.5	31.6
Ether ...	4.5, 5, 7, 7.5	6.0	4.1
Chloroform ...	2.5, 3, 4, 6	3.9	3.0

(1) Moore, W. M., and Graham, S. A. A Study of the Toxicity of Kerosene. Journ. Econ. Entom., 1918, Vol. XI, pp. 70-75.

(2) Macfie, J. W. S. The Limitations of Kerosene as a Larvicide. Bulletin of Entom. Research, 1916-17. Vol. VII, pp. 227-295.

(3) Quoted on p. 9.



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The results are similar to those obtained with pure benzene, but not quite so uniform. The same increase in toxicity is shown by the alcohols when combined with petrol vapour, and the same decrease in the volatile anaesthetics. Petrol vapour behaves like benzene, therefore, and carries in the — OH containing substances, rendering them more toxic, and we know that such substances are formed in kerosene during exposure to air, and also that they increase the spreading power of oils.

The relationship between toxic substances and the substances which cause spread can now be stated.

A pure volatile hydrocarbon causes loss of reaction by condensation of a film of vapour on the lining membrane of the breathing tube. This effect cannot be regarded as an anaesthesia, although the larva may recover when placed in air owing to evaporation of the film.

The film delays the action of true anaesthetics, like ether and chloroform, but increases the effect of weak anaesthetics, like the alcohols, by aiding their passage through the lining of the breathing tube. This explains the toxicity of the most volatile fractions of kerosene, since they have been shown to contain oxidised substances which cause spread.

It has also been shown that these oxidised substances are re-formed in the presence of air; and this is the cause of kerosene regaining toxicity when kept in contact with air.

Therefore, although a pure hydrocarbon can kill larvae by blocking the breathing tubes, the toxic substances are of the same nature as those that cause spread of oil.

## GENERAL CONCLUSIONS.

(1) Differential wetting has been shown to be an important factor in the life-history of larvae, and in their susceptibility to the effects of oil.

(2) Anopheline larvae place themselves at right angles to a wet object because their tails are wet and their spiracles are dry. The larvae lie parallel to a dry object because their dry spiracles are "attracted."

Oil on the surface of water acts like a dry object and "attracts" the spiracles. When contact takes place the oil is able to wet the spiracles and therefore enters the breathing tubes. Contact with oil is sufficient to kill larvae, and the appearances shown by oil in the tubes have been described.

(3) A 0.1 per cent. solution of soap is able to wet the spiracles so that the larvae can no longer rest at the surface and ultimately drown.

(4) Weak emulsions of oils do not enter the breathing tubes and kill larvae unless there is a film of oil on the surface. Wet oil does not "attract" the spiracles of larvae.

(5) The spread of oil and the movement of thymol particles depend on the affinity of water for active groups in the substance. Both may be prevented by satisfying this affinity by saturating the water with a material containing an active group.

(6) Pure hydrocarbons do not spread on water. Oils that spread contain substances which have active groups, and these substances have an affinity for the oil on one hand and for water by means of their active groups on the other.

If the oil contains too much active material the excess leaves the oil, satisfies the affinity of the water for active groups, and thereby prevents the oil from spreading.

Crude oil has been shown to be a satisfactory mixture because it does not contain an excess. A method of testing whether any given mixture contains too much active material has been described.

(7) Methods for comparing the spreading powers of oils have been suggested. One of these depends on the amount of oil which must be added to pure paraffin in order to make the mixture spread. The other is based on the extent to which inactivated water must be diluted with fresh water before the oil can spread.

(8) The substances which cause spreading are increased by oxidation.

(9) Pure hydrocarbons are able to wet the spiracles. They can thereby enter the breathing tubes to kill larvae.

Volatile hydrocarbons condense as a film on the lining of the breathing tubes and produce a pseudo-anaesthetic effect.

This film increases the absorption of alcohols and thereby enhances their toxic action, which is a true anaesthesia.

(10) The toxicity of kerosene is increased by oxidation in the same way as the spreading power. The toxicity and the spreading power are therefore probably due to the same substances.