Some observations made by Vacha and Harvey[1] indicated a high toxicity of ethylene oxide to plant tissues. More recent work by the author has shown that ethylene oxide has many properties which make it suitable for killing noxious plants. By the use of a rod it can be introduced into the soil beneath the bushes. The "depth charge" can be regulated to certain levels of roots in the soil. The materials injected are not accessible to animals. Ethylene oxide is liquid at ordinary temperatures at pressures between eight and twenty pounds per square inch. This gives pressure sufficient to drive it into the soil directly from the tank. A special measuring device fitted to an injecting rod has been devised, which may be called a "gopher stick." Such a device is of use also in killing gophers by similar toxic agents. The ethylene oxide is volatile enough to allow a quick spread through the soil and a relatively short period of its effect in the soil. It is soluble in water, and dilutions with ice-cold water can be made with little loss when it is desired to use a water dilution or a mixture with other toxic agents. Dilutions can be handled in the usual knapsack sprayer with a "gopher stick" in place of the spray nozzle. Mixtures with chlorates or formaldehyde may be used without chemical reaction destroying the toxicity.

The ethylene oxide penetrates quickly up through the tissues, causing marked discoloration of the leaves within a few days. The effect in sterilizing the tissue through which it passes is being investigated since it may be of use in killing fungi or insects within the wood or bark to prevent their dissemination. The use of such a penetrating sterilizing agent would decrease the labor of removing trees which are infected with such pests.

The use of ethylene oxide alone and in water solution has been shown by the killing of several hundred bushes of barberry,[2] currant, gooseberry, poison ivy, prickly ash, scrub oak, popple, boxelder, etc. The size of the charge or dose must be adjusted to the bush to be eradicated. Determinations have been made on the charge required in different types of soils and with various soil moisture contents. Indications are that at the present price of ethylene oxide the cost of materials is about the same as for eradication by common salt while the labor is considerably reduced. Other oxides of the unsaturated hydrocarbon series are being tried for their toxicity and effectiveness.

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[1] *Plant Physiology*, 2: 187–193, 1927.

[2] The author is indebted to Dr. L. W. Melander and the Office of Barberry Eradication, U. S. Department of Agriculture, for cooperation and assistance in the experiments.

SPECIAL ARTICLES

THE BIOLOGY OF THE PETROLEUM FLY

RECENTLY a well-known systematic zoologist, Dr. W. T. Calman,[1] gently admonished physiologists for their tendency to neglect "the unending diversity of structure and habit among animals" and emphasized the danger of making far-reaching generalizations from experiments carried out upon the still somewhat restricted fauna of the physiological laboratory. In no group in the animal kingdom are such generalizations more unsafe than in the Insecta, for nowhere is physiological diversity more marked. And perhaps no insect is more aberrant physiologically than the petroleum fly.

Considering the fact that the existence of this insect has been known to entomologists for over thirty years, it might be thought that all the details of the life history and physiology would have been described long ago. Owing perhaps to the restricted distribution (it appears to be confined to the oil-fields of S. California) this is far from being the case, and although some of the main facts of its structure and life history have been described by Howard[2] and Crawford[3] there still remain many points of interest to be investigated. The subject is one of interest not only to the entomologist but also to the physical chemist and general physiologist, for, as is well known, paraffin hydrocarbons, owing to their power of rapidly penetrating cell membranes, are highly toxic to living tissues.

The writer had the opportunity while working at the Citrus Experiment Station of the University of California at Riverside in 1928 and 1929 of investigating certain matters concerning the nutrition of this insect and its adaptation to a life in oil. The main results of these investigations are here summarized. Full details of the experiments will be published shortly.[4]

The larvae of *Psilopa petrolii* go through their entire development in shallow pools of waste oil, breath-

[1] W. T. Calman, presidential address, Section D—Zoology, British Association for the Advancement of Science, 1930.

[2] L. O. Howard, *Scientific American*, 80: 75, 1899.

[3] D. L. Crawford, *Pomona College Journal of Entomology*, 4: 687–697, 1912.

[4] *Trans. Ent. Soc. Lond.*, 1930.

ing by means of the posterior spiracles which they can project above the surface of the oil at will, each spiracular process being supported by four fan-like structures which rest upon the surface film and which are closely similar to those found in many aquatic ephydrid larvae. The oil is the residue left when the more volatile elements, such as gasoline and petroleum naphtha, have evaporated off. Tests kindly carried out for me by Dr. R. H. Smith showed that at 310° C. only 10 per cent. of the oil had distilled over.

Howard[2] naturally assumed that the larvae must feed on the remains of insects or other organisms caught in the viscous oil, but some doubt was thrown on this by certain experiments carried out by Esterley and described by Crawford,[3] who reported that young larvae could be reared to maturity in filtered sterilized oil without any extraneous organic matter—the inference being that they could derive their energy from the digestion of hydrocarbons! The variety of digestive powers among the insects is well known, but such an astonishing conclusion as this seemed to demand further investigation.

By keeping the larvae in pure, transparent medicinal paraffin it was soon found that they readily devoured small pieces of animal tissue, and in this medium their exact method of doing so could easily be observed. But experiments were undertaken to test the matter further.

Sixty young larvae were placed singly in tubes each containing a small quantity of the natural oil which had been filtered through fine cloth by means of a suction pump. To thirty of these tubes was added every three days a small piece of a crushed tuber-moth larva, the larvae in the remaining tubes being starved. Of those which received food, 50 per cent. pupated and 33 per cent. emerged as adults, whereas none of the starved larvae lived to maturity. In another experiment in which 180 larvae were reared in filtered oil in petri dishes in lots of ten, the results were:

	Per cent. pupated	Per cent. emerged as adults
Oil alone	6.3	1.8
Oil plus food	53.0	41.4

and similar results were obtained from other experiments. It was found that the figures in the first line were due to the cannibalistic tendencies of these larvae when starved, those that die providing sufficient food material to bring a small percentage to maturity, and since details of Esterley's experiments have never been published and are not available, we must assume that this is the explanation of his results.

We may conclude then that the larvae can not develop without extraneous organic matter, and there is little doubt that under natural conditions small insects trapped in the sticky oil are the chief source of food. That they derive no nutriment at all from the oil is more difficult to prove, but experiments with a variety of oils of different composition gave no suggestion that they are able to do so. It was, however, interesting to find on sectioning that the hind gut contains enormous numbers of bacteria-like bodies. Without fresh material it is unfortunately not possible at present to identify them with certainty, but stained with Murray's toluidine blue—Van Gieson, they have the appearance of a coccobacillus. They are Gram-positive and approximately 1 μ in length, and are often present also in the mid gut within the peritrophic membrane, although always in much smaller numbers. What part, if any, these organisms may play in the processes of digestion it is at present impossible to say.

The Ephydridae is a group the larvae of which are characterized by ability to live in a variety of unusual situations—brine, alkaline waters, urine, etc. This ability appears to be due to the presence of a very impermeable cuticle and a very efficient mechanism of hairs and bristles protecting the spiracular openings. It is very largely by the same means, and *not* by any tolerance of the tissues themselves to hydrocarbons, that the petroleum fly is enabled to exist in its extraordinary environment. Experiments with various lighter and more volatile oils which are able to enter the tracheal tubes as vapor and condense as a film upon the walls, thus overcoming the protective mechanism, show clearly that when once the oil enters the tracheal system the toxic hydrocarbons are absorbed and, having got into contact with the tissues themselves, are as poisonous to the petroleum fly as to any other insect.

The impermeability of the general cuticle of the larva to substances usually regarded as highly penetrating is well shown by its resistance to fixatives; for instance, the larva will remain active in strong alcoholic picro-formol (Bouin's fluid) for twenty minutes. But why is it that the oil does not reach the tissues by absorption through the walls of the alimentary canal, which is filled with oil from end to end? The answer is that the oil never gets into contact with the gut epithelium at all. The fore and hind guts of course are protected by a chitinous layer, and in the mid gut there is the peritrophic membrane. The latter is well developed, as is the case with most cyclorrhaphous larvae, and from dissections it seems that the oil itself does not actually pass through the peritrophic membrane. The characteristic dark color of the oil is seen only within it, the region between the membrane and the digestive epithelium, as well as the latter itself, being clear and unstained. Moreover, the wall of the mesenteron can be dissected away without difficulty, leaving intact the tough peritrophic membrane with its oily contents.

It might be thought that the water supply would be an acute problem for an animal living in such a medium, but this is not so, for distillation tests reveal that there is a considerable amount of water contained in the oil in the form of minute droplets, and, moreover, in many cases the oil pools overlie shallow pools of water, those most frequented by larvae being those in which there is no great depth of oil.

One would have imagined that an animal living in oil would be immune from the attacks of parasites, and it is interesting to find that a protozoan has been able to follow its host into such an environment. Certain of the epithelial cells of the mesenteron are seen in section to be distended with spores of a sporozoan parasite, apparently a microsporidian, the nucleus often being pushed to one side by the mass of spores. These bodies are approximately 4 μ in length and stain very intensely with toluidine blue, hematoxylin and methyl blue-eosin, the multinucleate nature of the spore being most clearly seen with the latter stain. Lack of material exhibiting further stages in the life history has so far prevented closer identification.

It is a remarkable thing that *P. petrolii* should exhibit no structural peculiarities correlated with its unique mode of life. With the possible exception of the unusually great muscular development which enables the larva to swim actively in such a viscous medium there are no *essential* morphological differences between the petroleum fly and a typical aquatic ephydrid. Such adaptations as must have occurred are physiological rather than morphological and are of a puzzling nature. What, for instance, can be the peculiarity about the spiracular structures which prevents the oil from spreading into them? Possibly the peristigmatic glands yield an aqueous secretion in place of the more general waxy or oily substances. Similarly, what substance can be produced by the glandular hairs of the tarsus of the adult fly which enables it to walk on oily surfaces which will entangle other insects almost immediately? Again what can be the change which must have taken place in the composition of the digestive juices to enable them to act upon food saturated with petroleum? These are the problems which concern the biochemist and the physical chemist rather than the entomologist, and appear to the writer to have a very considerable theoretical interest.

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THE MORPHOLOGICAL BASIS FOR CERTAIN TISSUE RESISTANCE

TISSUE resistance against bacterial invasion generally finds an explanation in the production of specific bactericidal and antitoxic substances on the part of the organism or in the increased activity of certain wandering cells of the infected host. The resistance which may develop in the higher animals against certain drugs and chemical poisons can not be explained by any of the above-mentioned mechanisms.

The fact has been known for many years that when either the acetate or the nitrate of uranium is given subcutaneously to dogs they develop an acute experimental nephritis in which the injury to the kidneys is at first, in so far as structural changes are concerned, very largely confined to the epithelium of the proximal convoluted tubules. Suzuki,[1] working in Aschoff's laboratory, was of the opinion that this acute injury was confined to the epithelium in certain segments of these tubules.

In a recent Harvey Lecture[2] and in two investigations[3,4] which formed the basis for the experimental data presented in this lecture, a discussion was undertaken of the toxic effect of uranium on the kidney and the mechanism of repair in the injured kidney, and certain observations were presented relative to the resistance which the kidney, the seat of a chronic uranium injury, developed against subsequent acute injuries from the same nephrotoxic substance.

During the past two years these studies have been continued by using repeated injections of uranium with the object in view of obtaining more evidence regarding the morphological changes developing in the kidneys which give to them both a structural and functional resistance to this poison. These experiments have been conducted by first anesthetizing normal dogs and removing from the left kidney a small wedge-shaped piece of tissue which, in its study, has served as the normal control for the acute and chronic changes developing in the kidneys fol-

[1] T. Suzuki, "Morphologie der Nierensekretion," Jena, 1912.

[2] Wm. deB. MacNider, "Urine Formation during the Acute and Chronic Nephritis Induced by Uranium Nitrate," *The Harvey Lectures*, 1928–1929.

[3] Wm. deB. MacNider, "The Development of the Chronic Nephritis Induced in the Dog by Uranium Nitrate. A Functional and Pathological Study with Observations on the Formation of Urine by the Altered Kidneys."

[4] Wm. deB. MacNider, "The Functional and Pathological Response of the Kidney in Dogs Subjected to a Second Subcutaneous Injection of Uranium Nitrate," *Jour. Exp. Med.*, XLIX, 411, 1929.