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Research Needs and Priorities: Biochemistry

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Aedes aegypti is one of the most thoroughly investigated insect species in the laboratory, and much is known of its nutrition and metabolism and of its general biochemistry and physiology. It is the application of this biochemical knowledge to field problems that is lacking. Moreover, the expansion of knowledge into special areas concerned ultimately with control and eradication is urgently required. These practical needs and priorities are concerned mainly with three aspects of mosquito research—namely, (*a*) choice and suitability of breeding-site, (*b*) choice of host and (*c*) control by insecticides, chemosterilants and toxins.

RESEARCH NEEDS

Choice and suitability of breeding site

It is becoming possible to determine what substances are oviposition attractants (arrestants *sensu stricto*) and to characterize their mode of action. Methyl *n*-butyrate has recently been found to be an oviposition attractant for *A. aegypti.* Proteinaceous material such as crude egg-white proved to be an arrestant for ovipositing *Culex pipiens fatigans*, while water and ether extracts of distillates from breeding-waters are attractants for this species. A combination of protein and methyl propionate constitutes an oviposition attractant for *C. salinarius.* Such studies would allow the mosquito-breeding hazard of urban waters to be evaluated and might even make possible the development of traps to detain gravid females. Conversely, it might be profitable to search for oviposition repellents, since, for example, myristoylpyrrolidine has been found to repel *C. fatigans* for 10 days when added to pools at the rate of 2 ppm.

Although the production of *A. aegypti* larvae suffers in the total absence of micro-organisms, too much pollution is unsuitable for *A. aegypti*, which is essentially a clean-water breeder. Field studies are needed to determine the outside limits of urban water quality that can support this species, attention hitherto having been paid to the type of container rather than to the water it contains. Since the elements of nutrition for *A. aegypti* larvae have been established, it should be possible to ascertain whether some waters and their microflora are deficient in certain amino-acids or accessory factors. The physiology and biochemistry of osmoregulation in *A. aegypti* has been authoritatively characterized, yet it is not on *A. aegypti* but on other species of *Aedes* and *Anopheles* that the limits of osmotic pressure and total-solid content have been investigated in the field.

The relation of temperature to the development of the various stages of *A. aegypti* has been characterized, and cold-hardiness has been increased by selection. Different strains of *C. fatigans* have been found to differ in the resistance of their eggs to desiccation. Some strains of *A. aegypti*, which happen to be the DDT-resistant ones, prefer dark oviposition sites to well-illuminated ones. Much therefore must be learned about the biophysics of drought-resistance, the physiology of skototaxis and the biochemistry of cold-hardiness, as well as the comparison of populations in various parts of the world for these characteristics.

Choice of host

Although there are marked specific and even racial differences in the choice of mosquito species for vertebrate hosts, the factors in the host emanations which decide host selection still await determination. Experiments on female *A. aegypti* have revealed comparative attractiveness, in the statistical sense, when a with-or-without choice is presented, for a number of amino-acids, and especially lysine and others which absorb CO_2, and for several oestrogens. Some authorities, however, do not consider that this species is capable of olfaction for substances of much lower vapour pressure. Mosquito traps have been made progressively more effective by the successive addition of moisture, CO_2, warmth, lysine and oestriol, yet it is generally said that field attractants for mosquitos are non-existent. Here is an area of research that needs the attention of insect ecologists, physiologists and biochemists.

Attractants for male mosquitos are even more urgently needed. At present, honey is employed to

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actattr males to ingest chemosterilants, and clover honey is better than others. Carbon dioxide results in attractiveness for males as for females. Discovery of a good bait to carry chemosterilants should have first priority. Compounds which promote and prolong feeding have been found in adenosine monophosphate (AMP) and adenosine triphosphate (ATP) for *Culex pipiens pallens*, and gustatory attractants for *A. aegypti* have been found in AMP and ATP. These substances are needed for the membrane-feeding experiments now being undertaken in the USA to discover a systemic repellent.

Immunological studies have done much to clarify the nature of the salivary antigens of female *A. aegypti* and the reaction of the host to them. The isolation of lyophilized salivary secretion should permit biochemical studies to evaluate the contribution of protein and/or polysaccharide to the antigenicity. Much is known about the gonotrophic cycle in *A. aegypti* and the differences in hormonal control between West- and East-African strains and about the diel cycle of biting in this species, but investigations are needed on the numbers of cycles and quantities of eggs that field populations in various parts of the world can achieve. The factors affecting the longevity of adults, e.g. the glycerophosphate dehydrogenase activity, have been studied in houseflies, but the process of aging is in need of biochemical study in mosquitos. The precision with which the vectorial capacities of *A. aegypti* for *Plasmodium gallinaceum* and of *C. fatigans* for *Brugia malayi* have been found to be monofactorial genetically should be matched by biochemical studies to determine the mechanisms controlled by such genes and extended to other disease organisms transmitted by *A. aegypti*.

Control by insecticides, chemosterilants and toxins

Perhaps an effort should be made to find more physiological or natural insecticides than the present synthetic compounds. Simulants of the juvenile hormone, such as farnesol and ziram, kill *C. fatigans* larvae at levels of 1-10 ppm and greatly delay their pupation at 0.1-1 ppm. Tyrosinase inhibitors such as phenylthiourea and 4-chlororesorcinol have much the same effect. Biochemical studies should be extended to isolate and identify the toxins of those species of algae that hinder the development of larvae in certain pools, or of well-known micro-organismal parasites such as *Coelomomyces* and *Thelohania*, as is already being done for the toxin of *Bacillus thuringiensis*.

Biochemical studies that would be of more immediate benefit are those characterizing the reaction of *A. aegypti* to insecticides at present in use or in process of development. The general importance of esterases, capable of hydrolytic detoxication of ester linkages, has already been realized in the housefly, from which many protein fractions with this activity have been separated by starch-gel electrophoresis. Association of the different fractions with individual gene alleles has been commenced in *Drosophila*. Mutant esterases, by genic conversion of the normal into the iso-enzyme (isozyme), have been detected in the aliesterase-phosphatase conversion by an allele on chromosome 5 in diazinon-resistant strains of *Musca* and what is probably an aliesterase-carboxyesterase conversion in malathion-resistant housefly strains. An isozyme of carboxyesterase has been partially characterized in malathion-resistant *Culex tarsalis*. It is possible that the paper-chromatographic studies of the amino-acids and fluorescent materials to differentiate species and strains of mosquitos and, more especially, immunological investigations of antigens that are discovering marked differences between species, can be taken from the empirical to the basic biochemical level and thus greatly elucidate the ability of various mosquito populations to produce enzymes that can withstand chemicals used for their control.

The type of aliesterase that is present in the housefly is also present in *A. aegypti*, although it is absent in *Culex* and *Anopheles*. Thus it is remarkable that malathion-resistant strains of *A. aegypti* have not developed increased phosphatase or carboxyesterase activity but only a decreased absorption. On the other hand, parathion-resistant populations of *Aedes nigromaculis* in California have developed increased detoxication activity, particularly of paraoxon. *Culex tarsalis*, having no housefly-type aliesterase to convert, has proved unable to develop a significant parathion-resistance. Therefore, further study of the esterases of a mosquito such as *A. aegypti* are necessary for scientific understanding, as has already been emphasized by van Asperen.

The various biochemical factors making for physiological vigour and vigour tolerance need sorting out. Why is it that laboratory-reared larval offspring do not show the tolerance levels which their field-collected parents did? This phenomenon appeared in malathion-tolerant populations of *C. fatigans* from Douala, Cameroon, and Freetown, Liberia, and is evident in *A. aegypti*; for example, material at Montego Bay, Jamaica, which showed an LC_{50} of 1.0 ppm malathion for field-collected larvae, showed an LC_{50} of only 0.45 ppm for the F_1 laboratory-reared larvae. It is still unknown why it is that selection with the malathion or other organophosphorus (OP) compounds leads to much stronger cross-resistance to DDT, in mosquitos as in houseflies. The upper limits of the capability of *A. aegypti* larvae for OP resistance should be probed by long-continued selection and by making composite strains; it is probable that this resistance will be attained in the absence of specific genes for detoxifying enzymes. The possibility of the development of resistance to the pyrethroid dimethrin should also be investigated, and it should involve increased esterase activity as well as vigour-tolerance factors.

It is now evident that mosquitos, unlike houseflies, can detoxify dieldrin, since normal strains of *A. aegypti* and of *C. fatigans* gradually produce metabolites from small doses of dieldrin. The mode of action of dieldrin may involve its property of capturing electrons, as has been indicated for DDT, and the mechanism of dieldrin-resistance may be connected with it. Certainly, more must be learned of the biophysics and biochemistry of insect ganglia in connexion with resistance to dieldrin and to gamma-HCH. In view of the cheapness and effectiveness of lindane formulations, HCH-resistance in *A. aegypti* is well worth study, since it probably involves factors additional to dieldrin-resistance.

The DDT-dehydrochlorinase enzyme of *A. aegypti* has yet to be associated genetically with the DDT-resistance gene on chromosome 2. Although the same gene appears to be involved for all strains hitherto studied, many populations of this world-wide species should be tested for the chromosomal location of the main DDT-resistant gene or even for its presence at all, since it appears to be absent in Upper Volta material. The fact that deutero-DDT is not as insecticidal to field DDT-resistant populations as to laboratory DDT-resistant strains indicates vigour-tolerance factors that demand investigation, for these may be similar to those that make DDT-resistant field populations no longer so phenomenally susceptible to the remarkable OP larvicide Abate.

Biochemical and histological studies are needed to characterize the after-effects of insecticide treatments at the completely sublethal level, already investigated in houseflies, on ovulation and egg-hatch of *A. aegypti*. The association of an increased DNA content with the development of resistance to the chemosterilant apholate by *A. aegypti* and the known detoxication of metapa by the housefly emphasize the importance of biochemical studies in the new genetical methods of control.