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BIOLOGICAL EFFECTS OF APHOLATE ON INSECTS

BY

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The fight against harmful insects, and particularly against those which are carriers of pathogenic germs, has, during these last ten years, attracted the attention of a very large group of research workers. To insecticides as a means of destruction, have been added new types of fighting, such as the use of X rays for the diffusion in nature of sterilized males which, mating with the females, should have considerably lowered the frequency of the population. But this method has proved to be effective only in a few cases, for many biological reasons.

With the discovery of alchilant agents such as aphoxide, aphomide, apholate etc., we have been given new hopes for the attack against carrier insects, but as far as we can see today, their application is still a long way off; the advantage of the alchilant agents in producing sterility, despite their brief stability, is annulled by many factors such as quantity, competition of other males, the possibility of double fecundation and the immigration of new populations (Dame Woodard, Ford, Waidhass, Lewolden, Chapman, Wilden). The effects of alchilant agents on various insects at different stages of life, have also been widely studied in the laboratory. The sterilizing effects has always been found in different quantities and in different conditions. Atrophy of the gonads particulary in the females has been found, and dominant lethal mutations (Rai, Lindquist, Parkiston, Johson, Murray, Bichley). The behaviour of alchilants has also been investigated, but as yet, only in some micro-organisms or in cells cultivated in vivo. Brookes and Lawley have established that mustard gas reacts preferably with

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guanina of DNA in *E. coli* strain B/r. So it seems certain that the loss of mustard gas on the part of the DNA is due to an enzyme and not to a spontaneous hydrolysis of the DNA alchilate. Crathorn and Roberts, studying the action of mustard gas in HeLa cells, have found a depression in the degree of synthesis of DNA which was the prelude to a premitotic block which follows it. We too have begun some research on the biological effects of the alchilant agents, not only for sake of study in itself, but also for a comparison if possible, with our studies of radiogenetics. The species which we have used is the *A. mac. atroparvus*, a species which is easy to rear in the laboratory, and which has been colonized for a long time.

As treatment substance we used apholate with abbreviation Olin W 2774 WSDA Entin 26316 whose grade of lethality for insects was fixed in the concentration of 100 pp.m.

Two sets of research were carried out: in the first we used a dose 5 times stronger than that of lethality, that is, 500 pp.m., but for brief and precise treatment periods. The constant efficacity of the alchilant agent in our opinion allows a better analysis of biological effects. The times used are 12, 8 and 6 hours respectively, selected according to previous tests. Above 12 hours, the shock of the alchilant at the concentration used, is almost lethal. Then, taking into account that all the spermatogenic phenomena take place in the fourth larval stage, it was also interesting to see, from the chromosome viewpoint, the effect of the substance on the meiotic processes which, in anopheles, are easily analysed. Still leaving the concentration of the substance unchanged for the moment, the treatment times were adapted to different ages of development during the fourth larval stage, larvae modified after 24, 48, 72, and 96 hours being treated separately. On the contrary, in the second case the larvae, immediately after the change to the fourth stage, were left for the whole larval period up to the pupa stage, in three different concentrations of apholate, i.e., 100, 50 and 25 pp.m.

The larvae treated for 12 hours with doses of 500 pp.m. revealed the toxic effect of the apholate particularly at the emerging stage, since very few succeed in reaching the adult stage, and those which reach it are in a weak condition so that they do not feed so well as the normal ones. But inside this group, those which experienced the greatest effects are the larvae treated 48 and 72 hours after the change, few of which survived the treatment, giving a very small number of very weak adults. The larvae treated 24 and 96 hours after the moulting show a higher rate of survival. It is probable that the young larvae have a high capacity for recovery, while in those

of 96 hours after the change this power of recovery may be due to the phenomenon of the moulting into pupa form which shows signs of starting at this stage. The toxic action is revealed not only by the death-rate of the larvae, but also by a noticeable increase in the period of development, which may be prolonged up to three or four days. Concerning the tests with doses at a concentration of 100, 50 and 25 pp.m. but with continuous treatment immediately after the moulting to the fourth instar up to the pupa, the highest death-rate is always concerned with the moment of emergence of adults, while the highest rate of survival is met with at the pupa stage. The larval death-rate does not proceed regularly, it is higher 200 hours after treatment, with a second peak at 300 hours. It is opportune to consider whether there is also a difference between the rate of males and females. Considering the fact that the male sex is generally weaker we had expected, at the end of the experiment, to get sex ratio favourable to the female. But the data show clearly that the males survive more frequently than the females. With all probability, this fact is not accounted for by the female's higher sensibility to the toxic substance, but is rather due to the fact that the males, having a more rapid development, remain in contact with the toxic substance for a shorter period than is thought sufficient to justify their higher rate of survival. No adults were obtained by the treatment with 100 pp.m. The males, which survived the two lower doses, were mated with normal females. With the dose of 50 pp.m., a hatching of 1% was obtained, while with the dosage of 25 pp.m. the hatching rose to 8,91%. The unhatched eggs were also tested if they were embryonated. If we take it for granted that the embryonated eggs were fertilized, we must conclude that apholate induces such deep chromosomic aberrations that ovular development is prevented. During the treatment tests, some specimens were withdrawn to observe what happened to the chromosomes during spermatogenesis. The chromosomic aberrations on spermatogenesis are of a different nature. One of the most frequent concerns the effect of glutinousness: the chromosomes generally lose their individuality, sometimes to the point of joining to form a compact mass. Sometimes the action of the apholate is manifested by the breaking up of the chromosomes, and then we see, especially in anaphase, a number of fragments which, in all probability, will be lost. Often we can observe chromosomic bridges, which are due to a localized terminal stickiness of two homologous chromosomes which, at the moment of anaphase, are not able to divide regularly, causing anormal breaks in the chromosomes. Just as apholate delays the development cycle, so it also delays the meiotic processes which

appear to be even more irregular. Whereas, in the larvae at the fourth stage, all the meiotic processes are almost exhausted and there is a great abundance of spermatidi, in the specimens which have been treated and which are at the same stage of development, can be observed chromosomic aberrations and a delay, in the meiotic processes. We tried to correlate the rate of chromosomic aberrations with the intensity of the dose. We have calculated the percentage as between 40 and 50, but this problem must be examined with techniques giving more accurate data.

Owing to the considerable slowing-up of the vital processes occurring during the course of the experiment, we also wished to see how the alkaline phosphates would behave in the treated specimens compared with normal specimens. The determination of alkaline phosphatasi was performed by using two methods: the quantitative and electrophoretic. In the former method the quantitative analysis of the alkaline phosphatasi is expressed in units of enzymatic activity produced in 40 in 50 specimens, while the difference of enzymatic units can be regarded as the expression of metabolic alteration in the larvae. Only two concentrations were used for the treatment of the larvae, i.e., 100 pp.m. Every 24 hours the alkaline phosphatasi were examined on a sample of 50 specimens.

On examination of the data obtained, it can be seen that the concentration of alkaline phosphatasi is directly correlated to the concentration and to the period of the treatment. In the control, the units of enzymatic activity decrease little until the 72d hour, are stable until the 96th hour, and then decrease very slowly until the 120 th hour. In the larvae with 50 pp.m., the enzymatic activity presents a considerable decline between the 72d hour and the 96th hour, still decreasing slightly until the 144th hour. If we then consider the line of treatment to the solutions at 100 pp.m., we notice two points of falling, after 48 hours and after 120 hours, but what is surprising is a resumption of phosphatasi after the 120 th hour. Perhaps we can explain this as being due to highly resistant specimens which are able to recover after a hard selection, eased perhaps by a decreased toxicity of the solution. With electrophoresis the picture is the same; after 24 hours the proteinic bands begin to weaken until they finally disappear completely. These results seem to indicate an interesting means of research if we consider the work of Raychanduri and Butz and other authors who hawe found a high degree of direct corellation between acid and alkaline phosphatasi and fertility in insects and other vertebrates.

LITERATURE

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BIOLOGICZNY WPŁYW AFOLATU NA OWADY

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Ostatnio coraz większe znaczenie zdobywają substancje chemiczne o właściwościach mutagennych, i to zarówno ze względu na możliwość praktycznego zastosowania ich w walce z przenosicielami chorób, jak również ze względu na badania biochemicznego mechanizmu mutacji.

W naszych doświadczeniach stosowaliśmy afolat na larwy *Anopheles atroparvus* IV stadium, działając na nie w dwojaki sposób — duże dawki w ciągu krótkiego czasu i małe dawki przez długi okres. W obu przypadkach okazy doświadczalne wykazywały zmniejszony wzrost w porównaniu z kontrolą.

Większość larw padła, nie osiągając stadium dojrzałego. Badania mejozy u samców, występującej głównie w IV stadium larwalnym, wykazały istnienie licznych komórek z uszkodzeniami chromosomowymi i mostkami, co stwierdzono na podstawie fragmentów chromosomów. Można było zaobserwować również liczne jądra heteropyknotyczne.

Badaliśmy również z punktu widzenia biochemicznego aktywność fosfatazy zasadowej w homogenatach larw IV stadium, które poddano działaniu 0,00005 i 0,0001 części afolatu. W obu przypadkach można było zaobserwować stały i stopniowy spadek aktywności enzymatycznej w porównaniu z kontrolą.