CARBON DIOXIDE SENSITIVITY IN DROSOPHILA AFFINIS AND DROSOPHILA ATHABASCA

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TABLE OF CONTENTS

INTRODUCTION ---------- 1

Review of literature ---------- 1

Purpose of the study ---------- 16

MATERIALS AND METHODS ---------- 19

PROCEDURES AND RESULTS ---------- 27

CO_2 testing of laboratory stocks and wild-caught flies ---------- 27

Laboratory stocks ---------- 27

Wild-caught flies ---------- 30

Study of the mode of inheritance of CO_2 sensitivity in *Drosophila athabasca* ---------- 33

Injection experiments with *Drosophila athabasca* ---------- 36

Study of CO_2 sensitivity in *Drosophila affinis* ---- 40

Injection experiments with *Drosophila affinis* ----- 45

CO_2 testing of a stabilized strain of *Drosophila affinis* at different temperatures ---------- 49

Action of a rise in temperature on the virus in *Drosophila affinis* and *Drosophila athabasca* ------- 50

Injections of resistant *Drosophila melanogaster* and *Drosophila pseudoobscura* with extracts of CO_2 sensitive *Drosophila melanogaster* ---------- 56

DISCUSSION ---------- 58

SUMMARY ---------- 63

BIBLIOGRAPHY ---------- 65

INTRODUCTION

Review of literature

As is true of various insects, flies of the genus Drosophila placed in an atmosphere rich in carbon dioxide normally become inactive in a few seconds. These flies can be kept in CO_2 for hours without injury, and, when returned to free air, they quickly recover from the narcosis and completely regain their normal behavior. L'Héritier and Teissier (1937) discovered in a stock of Drosophila melanogaster that happened to be homozygous for the third chromosome recessive gene ebony that certain flies placed even for a short time in an atmosphere rich in CO_2 remained in a more or less paralyzed condition when returned to free air. Treated flies exhibited some uncoordinated movements but were unable to fly or walk and soon died. This class of flies was called "sensitive", while their normal counterparts were referred to as "resistant".

L'Héritier and Teissier (1938) demonstrated that the CO_2 sensitivity trait was inherited independently of the chromosomes, the first such case known in Drosophila. Crosses of sensitive females by resistant males gave progeny all of which were sensitive and which behaved genetically like flies from a pure sensitive stock. The reciprocal cross, resistant females by sensitive males, gave a mixture of sensitive and resistant flies, with the

frequency of sensitives ranging from 10% to 90%. The frequency of the transmission of sensitivity by sensitive males mated to resistant females has been called the "valency of the males", and has a value characteristic of each sensitive stock (Brun and Sigot, 1955). Among the sensitive flies from the latter cross, males were only somatically sensitive and never transmitted the character, whereas females produced a mixture of both types of offspring.

L'Héritier and his co-workers have investigated various aspects of the CO_2 sensitivity phenomenon. In his 1948 review, L'Héritier states that at usual temperatures the duration of exposure of flies to CO_2 does not change the final result of the experiment. Sensitive flies after a very short exposure to CO_2 may exhibit only a very weak form of toxication, i.e. they are active, though their movements are uncoordinated and convulsive, and they may even recover after a few hours. Lengthening the exposure leads at first to less active movements and eventually to certain death. At high CO_2 concentrations the minimum duration to get the full effect of the CO_2 is about fifteen seconds (L'Héritier, 1948). The minimum duration increases as the CO_2 concentration drops but never exceeds a few minutes at CO_2 concentrations that kill sensitive flies.

L'Héritier and Teissier (1937) used five minutes as the exposure time for their work on the relation of tem-

perature and concentration. They demonstrated that at each temperature there is a concentration below which sensitive D. melanogaster behave like normal flies and exhibit no signs of toxication. Above this level the percentage of poisoned flies increases rapidly with the concentration, and when the latter reaches a certain value, no individuals survive. Between 2° and 23°C. the lethal concentration increases linearly with the temperature. Thus, for example, at 5°C. all flies survive a 25% concentration of CO_2, while a 35% concentration is lethal; at 21°C., all flies survive a 90% concentration of CO_2, but a 95% concentration of CO_2 is definitely lethal. Above 23°, it is impossible, even with pure CO_2, to kill sensitive flies. Below 0.5°C. the toxicity of CO_2 decreases rapidly with the temperature and, at -2°C. and below, it is again impossible to kill the sensitive flies with CO_2. Recently however, Professor L'Héritier (personal communication) reports that when the exposure time is prolonged to 15 minutes or more, at low temperatures the flies are killed by a relatively low CO_2 concentration.

The threshold CO_2 concentration seems to be dependent only on the partial CO_2 pressure, for air, oxygen, nitrogen, or hydrogen may be mixed with the CO_2 without altering the phenomenon (L'Héritier, 1948).

Not much is known about the physiological mechanism

involved in the toxication of CO_2 sensitive flies. Some evidence has been accumulated which indicates that sensitivity is not due to some kind of poison present in the hemolymph following exposure to CO_2 but that the toxic effect is more probably localized in the thoracic nervous ganglion (L'Héritier, 1948). Resistant flies injected with hemolymph from sensitive flies (including those already killed by CO_2) did not show any symptoms of sensitivity when tested with CO_2 either immediately or a few hours afterwards. They did, however, eventually become sensitive, but, as will be discussed later, this was not until 10 or 15 days after the injection and is interpreted as being due to the invasion of the organism by infectious particles present in the injected hemolymph.

Conversely, the behavior of sensitive flies was not changed by injecting them with hemolymph from resistant flies. The same negative results were obtained when sensitive flies were flushed with Ringer's solution by forcing a large amount of solution into the abdomen and allowing it to pour out a hole in the head. This drastic treatment did not kill the flies, and moreover, did not make them less sensitive.

Sensitive flies which have had the abdomen removed, or even an isolated thorax, behaved exactly like entire flies when treated with CO_2. However sensitivity is only made apparent in the movements of the legs and wings of CO_2-treated sensitive flies, so there is no way of

demonstrating it in isolated heads or abdomens. The convulsive movements of CO_2-poisoned flies indicate that there is some damage to nerve tissue. Some tests were made which indicate that the thoracic ganglion is the only nerve center injured, the cerebral ganglia remaining undamaged. To demonstrate this, the head of a sensitive fly was introduced into a small hole bored through a stretched india rubber membrane. When the membrane was allowed to contract, the head was isolated from the rest of the body and could be exposed alone to CO_2. Neither narcosis nor poisoning was ever observed in the flies treated in this manner, and, when freed, the flies moved and fed normally. However, when both the head and thorax together were similarly isolated from the abdomen and exposed to CO_2, the fly showed both narcosis and poisoning in the usual manner (L'Héritier, 1948).

Although most of the work on CO_2 sensitivity has been done on the adult stage, in which sensitivity is easiest to demonstrate, larvae from a sensitive stock are also injured by an exposure to CO_2 under approximately the same conditions as cause death of adults (L'Héritier, 1948). Since the movements of the larvae are less active in general than those of adults, the symptoms of CO_2 poisoning of larvae may not be very apparent. Larvae treated with CO_2 awaken from the narcosis, moving about and trying to feed, and perhaps even undergoing pupal metamorphosis. They usually die, either as larvae or as pupae,

but a few of them may completely recover and become adult flies. These show the normal sensitive behavior, the larval treatment having produced no curative effect.

Eggs or pupae of sensitive stocks do not demonstrate any sensitivity whatever; as in resistant stocks sensitive flies at these quiescent stages may remain in pure CO_2 for hours without any injury (L'Héritier, 1948).

As far as is known, CO_2 sensitivity is the only symptom of the presence in the fly of the cytoplasmic self-reproducing agent responsible for the phenomenon. L'Héritier and Teissier called the cytoplasmic agent a "génoïde" (genoid) at a time when the only known way to transmit it was by inheritance. However, from the beginning of their work they suspected that the sensitivity trait could possibly be transmitted from one individual to another by means other than normal inheritance. Sensitivity appeared as though it might be the manifestation of an infectious disease, and the genoid responsible for the condition was compared to a virus capable of being transmitted by the gametes of the two sexes. Nevertheless, resistant flies cannot acquire sensitivity by mere biological contact with sensitive flies (L'Héritier and Hugon de Scoeux, 1947). It was found that resistant larvae may live in the same culture bottle with sensitive larvae and even feed on crushed sensitive larvae or adults and still maintain their resistance to CO_2. In addition, sensitivity was never transmitted to resistant flies by copulation.

However, it was shown by L'Héritier and Hugon de Scoeux (1947) that sensitivity can be transmitted artificially from sensitive flies to resistant flies through organ implantation or the injection of hemolymph or an extract of sensitive flies. For their work on organ implantation these workers employed third (last) instar larvae. The flies that had received the implants then displayed sensitivity symptoms soon after eclosion or at least a few days afterwards. These workers demonstrated that the following organs could induce sensitivity in a resistant fly: ovary, brain, imaginal discs of eye, legs or wings, and a portion of the gut. In most of the individuals which became sensitive, the presence of the developed implanted organ could be demonstrated by dissection of the adult. In resistant individuals, the implant was usually not found, indicating that the appearance of sensitivity depended on the success of the implantation.

As briefly mentioned earlier, injections of sensitive fly hemolymph into resistant flies will bring about the development of sensitivity in these flies. L'Héritier and Hugon de Scoeux (1947), discovered that injections of hemolymph as well as the centrifuged supernatant of crushed sensitive flies were as effective as organ implants in transmitting CO_2 sensitivity. In this study they found that larvae or adults could be injected with equivalent success, and that after an incubation period, sensitivity symptoms appeared in the injected individual. This

incubation period is clearly shown when the adult fly has received the injection. At 20°C. this period may last from 10 days up to about 30 days and is not changed by the successive CO_2 tests required to determine its duration. (L'Héritier, 1948).

L'Héritier and Hugon de Scoeux (L'Héritier, 1948) employing a method of dilutions, worked out a procedure for the biological assay of the amount of virulent material contained in an extract. The method is based on the following observations. Flies injected with a concentrated extract all develop sensitivity. However, when a sufficient dilution is used, a fraction of these injected flies remain resistant and this fraction increases with the dilution. The logarithm of the percentage of resistant flies was found to vary linearly with the inverse of the dilution (Plus, 1950, L'Héritier, 1951).

Provided with a method of assaying the titer (number of infectious units per unit volume) of an infective extract, Plus (1950) studied its relation to the incubation time. She was able to show that the incubation time is linearly related to the logarithm of the titer. When inoculated flies are crushed and the virus extracted within 3 hours after inoculation, their yield is about equal to the amount of introduced virus, but afterward a decrease is observed. With poor inocula the yield decreases to zero. With richer inocula, the yield begins to increase before reaching this limit. This lowering stage

is followed by a period of rapid increase, which some days later slows down and comes to a plateau. Afterward, the yield remains about constant as long as the flies survive (L'Héritier, 1958).

Attempts to obtain a pure and concentrated suspension of the agent responsible for CO_2 sensitivity have, as yet, been unsuccessful. It has thus been impossible to measure the size of the agent directly, although some indirect information has been obtained from X-ray irradiation and ultrafiltration. Inactivation of the agent by X-rays fits a one-hit curve, with a target diameter of 39 mu (L'Héritier and Plus, 1950; L'Héritier, 1958). Passing an active suspension through a collodion membrane with a pore size of 180 mu reduces the activity of the suspension to one-hundredth the initial value (L'Héritier, 1951). Though these results are apparently conflicting, this evidence does show that the size of the genoid in Drosophila extracts lies within the range of virus particles.

The most striking aspect of the acquisition of sensitivity by injection or implantation is the ability of the genoid to invade the germinal line and to be carried by the gametes to the next generation. This penetration of the germinal line by the genoid takes place only in females. Although male and female D. melanogaster of resistant origin are both able to acquire sensitivity by injection or transplantation and, when injected with the

same extract, show only a slight difference in incubation time, their offspring behave quite differently. Females with induced sensitivity always have at least some sensitive offspring. However, in the case of males, acquired sensitivity is never passed on to their offspring (L'Héritier and Hugon de Scoeux, 1947). When the sensitive offspring of a female with acquired sensitivity are used in breeding experiments with resistant males, the females have only sensitive offspring, and at least some of the males may transmit the trait. Pure sensitive strains can be established from the progeny of the females.

A fly which has inherited its sensitivity from its father alone, behaves exactly like flies which have acquired sensitivity through organ implantation or injection (L'Héritier, 1951). If it is a male, all its offspring are resistant, but if it is a female, some of its offspring are sensitive. Brun and Sigot (1955) have proposed the terms "stabilized type" for the flies of pure sensitive stocks, and "nonstabilized type" for inoculated flies or flies that acquire their sensitivity from their fathers only.

As L'Héritier reports in his review of 1958, the CO_2 sensitivity agent fits every characteristic of a virus, based on the general behavior of the CO_2 sensitivity agent (inheritance, transfer through graft and injection), its size, the course of its multiplication, and its ability

to undergo mutation (to be discussed below), and since pathogenicity can hardly be a necessary criterion for this class of biological elements, its inclusion in this category seems unavoidable. Bearing the latter point in mind, L'Héritier and his co-workers feel that the word genoid, with its suggestion of a gene-like element, was an unfortunate choice. Since L'Héritier's review of 1954 and in all subsequent reports, the word genoid has been replaced, and the CO_2 sensitivity agent has been called virus σ.

The following observations on the action of high temperatures on the host-virus relationships have been obtained from L'Héritier's reviews of 1948 and 1958.

Flies inoculated with the CO_2 sensitivity virus and placed at 30°C. immediately afterwards usually do not develop sensitivity (except for the thermal resistant form of the virus discussed below). If these inoculated flies are kept at 20° for a few days prior to the high temperature treatment, the effectiveness of the treatment sharply decreases. No viral growth takes place at 30°, but when the flies are returned to 20°, the infection may begin again in at least some of them.

With nonstabilized flies derived from a cross of a resistant female by a stabilized male, the effects of temperature depend upon the stage of development. Freshly laid eggs placed at 30° develop into flies almost all