SENSITIVITY TO CO₂ IN *DROSOPHILA*— A REVIEW

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I. FOREWORD

THE inherited CO₂ sensitivity in *Drosophila* was discovered in 1938 in an ebony stock of *D. melanogaster* (Ph. L'Héritier and G. Teissier, 1938 and 1939). Normal so-called resistant flies can be kept for hours in an atmosphere of pure CO₂ without permanent injury. When returned to air they quickly recover from the narcosis and return to completely normal behaviour. With flies from the so-called sensitive stock quite different phenomena are observed. CO₂ is very dangerous for them and an exposure of a few seconds to this gas can poison them irreversibly. As with resistant flies, a sort of awakening follows the narcosis, but the sensitive flies remain injured permanently and display a more or less complete paralysis. If they are able to move their legs or wings, they do it in an unco-ordinated convulsive way which is very characteristic of the intoxication. They may succeed in recovering slowly, chiefly when kept in a moist and warm atmosphere; but most of them die quickly. Except in very rare cases it is easy to distinguish a sensitive from a resistant fly.

Soon after the discovery of the phenomenon, the following facts were established. In crosses the sensitivity behaves as a hereditary unit, but, surprisingly enough, is not transmitted in a Mendelian fashion and exhibits no linkage with any chromosome. It seems to be bound to some discrete cytoplasmic agent which, because of its similarity with a chromosomal gene, was called a "genoid." An outstanding feature of this cytoplasmic inheritance is the fact that it is not purely maternal, for the genoid can be transmitted by the spermatozoon. Even supernumerary spermatozoa can sometimes introduce the sensitivity into the eggs of a resistant female (Ph. L'Héritier and G. Teissier, 1945).

This work was interrupted by the war and the sensitive stock was lost at the time of the German invasion of France in 1940. This would have been, the writer feels, a priceless loss for himself and perhaps for genetics in general. He is therefore very much indebted to Dr H. J. Muller, who carried the stock to America at the beginning of the war and had it preserved at Cold Spring Harbor. This allowed the work to start again in France in 1942 under poor war conditions. It was then found that the genoids, both in the somatic and the germ cells, are greatly affected by the temperature. Raising the temperature during different stages of development leads to permanent or temporary cures of sensitivity and also to the cure of the germ cells. The details of these phenomena are rather intricate and not yet entirely understood (Ph. L'Héritier and A. Sigot, 1946).

In 1946, the use of the techniques of organ transplantation of Ephrussi and Beadle (1936) led to the important discovery that the genoid is contagious and can invade the host and its germ cells from a transplanted organ. Soon afterwards, evidence was brought forward for the presence of the infectious agent in the hmolymp and in the supernatant of ground and centrifuged sensitive individuals. These facts raised the question of the similarity between the genoid and a virus and allowed its study by techniques appropriate to virus research, such as ultrafiltration (Ph. L'Hritier and F. Hugon de Scoeux, 1947).

It thus became a simple matter, by injection into the abdomen, to turn resistant flies into sensitive and, since the genoids subsequently get into the germ cells, any stock of *D. melanogaster* and even of other species can be made sensitive. A new field of investigation was then opened, bearing on the possible relation between the chromosomic genotype and the manifestation as well as the inheritance of sensitivity. It remains still scarcely explored.

The singular importance of the inherited CO₂ sensitivity comes from its being on the border line of genetics and virus studies. It seems that its complete understanding, including the working out of the chemical chain of reactions which links the genoid to its phenotypic effect, and of the conditions of its multiplication inside and perhaps outside the *Drosophila* cells, would throw a valuable light upon the relations between genes and viruses and the activities of both. Such a complete understanding is perhaps not out of reach, since many and diverse lines of investigation may converge upon it ; but it will need the co-operation of insect physiologists, biochemists and geneticists. This makes the writer the more anxious for every valuable suggestion which will help him and his colleagues.

The present paper is intended to give a complete account of the present state of the problem, taking into consideration published, as well as unpublished, experimental evidence. The investigations are still incomplete on many points, but a whole team is now working on the problem.

2. PHYSIOLOGY OF SENSITIVITY

(i) *The conditions for the poisoning effect of CO₂*

The poisoning effect of CO₂ on sensitive flies depends on duration of exposure, temperature and concentration.

Duration of exposure to the gas is not important at usual temperatures. After a very short exposure, the sensitive flies may display only a weak form of intoxication, *i.e.* they are capable of active, though unco-ordinated and convulsive movements and they may even, after some hours, recover. Lengthening the exposure, the other conditions being kept constant, leads at first to less active movements and to a more delayed and less certain recovery; then a state of saturation is reached and nothing is gained by a further increase. The minimum duration to get the full effect of the gas is very short, about 15 seconds, when the CO₂ concentration is high enough. It increases somewhat when the latter is near the threshold value, soon to be described, but under no circumstances does it exceed a few minutes. In all the work on the influence of the concentration, a 5 minutes' exposure was always used as a standard; several hours of exposure do not produce any more important effect, when enough time is allowed for the wakening up from narcosis.

From very recent work, it appears that duration of exposure plays a far more important rôle at low temperatures, *i.e.* in the region of the left branch of fig. 1. This branch might, consequently, have a different meaning from that of the other.

When at a given temperature sensitive flies are exposed to atmospheres containing various concentrations of CO₂, the following facts are observed. Too low a concentration produces neither narcosis nor intoxication; then, increasing the concentration, one comes first to a *narcosis threshold*, which seems about the same with sensitive and resistant flies. At first, both types of flies wake up in the same way, when they are returned to a normal atmosphere. Further on there occurs for the sensitive flies a *toxicity threshold*, above which they are injured by the treatment. As the concentration rises above the threshold, the amount of poisoning obtained increases, at first quickly, then very slowly, towards the limit which is reached with a pure CO₂ treatment.

There is some variation of this threshold value according to the flies used. Apart from an important random individual variation, many factors, such as the age of the flies, their genetic origin, the temperature at which they have been raised and kept, and perhaps many other still unsuspected agents seem to be operative. The data used in the drawing of fig. 1 are more or less average values. They refer to the concentration which, at each temperature, is able to poison about 50 per cent. of flies of the original sensitive stock, a few days old at the time of the test.

The temperature at which the test is made exercises a preponderant influence on the threshold value. As shown by fig. 1, there is a minimum at about $+2^{\circ}$, on either side of which the curve rises steeply in a regular linear way. Above $+23^{\circ}$ the CO_2 does not exercise any poisoning effect on sensitive flies, at the usual pressure of one atmosphere. Dr Kalmus tells me he has some evidence that the curve can be extended beyond this temperature by using higher pressures.

FIG. 1.—Toxic action of CO_2 on sensitive *Drosophila*.

The threshold CO_2 concentration does not depend upon the nature of the gas which is mixed with CO_2 . This may be air, oxygen, nitrogen or hydrogen, without altering in any way the phenomena. The only relevant factor seems to be the partial CO_2 pressure.

(ii) *Adaptation of sensitive flies to lethal concentrations*

Striking adaptations are observed, when at the same temperature sensitive flies are subjected to a lethal CO_2 concentration soon after an exposure to a concentration below the threshold (subliminal concentration). The point needs still more careful investigation, but the present evidence can be summed up as follows.

Any subliminal treatment has the effect of enabling the flies to resist exposure to pure CO_2 for some time. This physiological adaptation lasts only a short time, depending chiefly upon the duration of the subliminal treatment and secondarily upon the

concentration used. When the exposure to the subliminal concentration is increased from half a minute to several hours, the adaptation time starts from a few seconds and rises more and more slowly up to about 15 or 20 minutes. It depends very little, if at all, upon the nature of the atmosphere in which the flies are kept when taken out of the subliminal concentration. For instance, when 5 minutes is the measured duration of adaptation the adapted sensitive flies, at their emergence from the adaptational treatment, can sustain equally well an exposure of 5 minutes to pure CO₂ and a 4 minutes' exposure to air or some other gas, followed by one minute of treatment by pure CO₂.

When adapted flies are submitted to some lethal supraliminal concentration, but not to pure CO₂, the result of the adaptational process is not only to make this concentration innocuous but indeed to render it itself adaptational. In some instances a 5 minutes' adaptational treatment enabled sensitive flies to remain for hours, without showing any poisoning, in a concentration which would have killed them in a few seconds if it had been used first. At the end of the experiment such flies were able to resist for some time exposure to pure CO₂.

Adaptation bears no relation to narcosis, as non-narcotic subliminal concentrations may be adaptational, and adaptation usually lasts longer than the narcosis produced by the adaptational treatment. Besides, flies can be narcotised by many agents other than CO₂, for instance by inert gases such as nitrogen or hydrogen, by poisons such as carbon monoxide or cyanic acid, or by anæsthetics like ether. No such narcosis seems to produce any major effect in the reaction of sensitive flies to a lethal CO₂ concentration. CO₂, therefore, seems to be as specific in its adaptational as in its toxic action.

The biochemical mechanism through which such an adaptation effect can be brought about is of course at present as unknown as the mechanism of the toxic effect itself. One may tentatively assume that some chemical precursor, the presence of which is needed for the toxic process, might combine with CO₂, when at subliminal concentrations, to give an innocuous compound. This reaction might be reversible allowing the precursor to be freed, after a time, for the toxic effect.

(iii) *Other physiological investigations*

A great deal of work has been done to get an insight into the physiological mechanism of the CO₂ intoxication of the sensitive flies. It had to be performed in a somewhat unsystematic way, as not much guidance was found in investigating such a puzzling phenomenon.

First, we tried to find out whether sensitive flies exhibit any other physiological difference when compared to resistant ones. The answer was always negative. A sensitive stock does not appear in any way weak or diseased; its fertility and general viability are

excellent. Treatment by many chemicals such as N_2 , H_2 , CO , HCN , H_2S , $H-CO_2H$, CO_2H-CO_2H , HCl failed to show any major discrepancy. Of course minor discrepancies are to be expected between stocks of different genetic origins, but none appeared to be linked with the CO_2 sensitivity. Similarly, no chemical introduced into sensitive flies, either through injection or through the mouth, has been found to change their behaviour with respect to CO_2 . However, so few chemicals have been tried in this way among the many which might be suspected, *a priori*, to be active, that not much stress must be put on this negative result.

Then it has been shown that the CO_2 sensitivity has nothing to do with stigmata or respiratory movements. These are localised in the abdomen, and indeed sensitive flies, bereft of their abdomens, or even an isolated thorax, behave in precisely the same way as entire flies when treated with CO_2 . Sensitivity is only made apparent in the movements of the legs and wings, so that there seems to be no way of showing it in isolated heads or abdomens.

Impressive, though perhaps not entirely convincing evidence has been collected, showing that the sensitivity is not due to a special property of the blood but rather to a reaction taking place at the cellular level.

(i) Resistant flies were injected with a large amount of hæmolymph drawn from sensitives. Treated by CO_2 , either immediately or a few hours afterwards, they never showed any symptom of even a weak sensitivity. As will be said farther on, such flies do actually become sensitive. But, as this takes place 10 or 15 days after the injection, it is interpreted as the outcome of the invasion of the organism by the genoids carried by the injected hæmolymph. It seems that a far more immediate result would have been expected had the sensitivity been related to some property of the blood.

(ii) Moreover, injection into resistant flies of hæmolymph from CO_2 -poisoned sensitives brings out a similar argument. Such flies are not affected by the injection. They should show some kind of poisoning had the hæmolymph of sensitives acquired some poisoning properties following exposure to CO_2 .

(iii) Finally and conversely the CO_2 behaviour of sensitives is not changed by injecting into them some hæmolymph from resistants. The same negative result is obtained when the fly is "washed" with Ringer. This washing is made in driving a large amount of Ringer into the abdomen and letting it pour out of a hole made in the head. This drastic treatment does not injure the fly much and does not make it any less sensitive.

It seems likely that the toxic effect of CO_2 is localised in the thoracic nervous ganglion. The convulsive movements of the intoxicated flies point of course to a nervous injury, and the thoracic centre must be damaged since poisoned flies do not behave at all like decapitated flies and since the latter clearly manifest sensitivity.

Moreover, some tests were made which indicate that the thoracic ganglion is the only nervous centre to be injured, the cerebral ganglia remaining unhurt. The head of a sensitive fly was introduced into a small hole bored through an extended india rubber membrane. When the membrane had been left to contract, the fly head was perfectly isolated from the rest of the body and could be exposed alone to CO₂. The fact that the head bears no stigmata does not prevent, it seems, the gas reaching the cerebral ganglia by diffusion, since, when the head and the thorax are similarly isolated from the abdomen and exposed to CO₂, the fly shows both narcosis and poisoning in the normal way. But to make the access of the gas inside the head easier, one eye of the fly was removed in some experiments. Neither narcosis nor poisoning was ever observed in these experiments, which involved only a cephalic treatment. When freed the flies moved and fed normally and could be kept any length of time. The evidence seems then strongly in favour of the view that it is the motor nervous system alone, localised in the thorax, which is injured by CO₂.

(iv) *CO₂ sensitivity in larvæ, eggs and pupæ*

Most of the work on the physiology of the CO₂ sensitivity has been done with imagos, on which sensitivity is easiest to demonstrate. But larvæ from a sensitive stock are injured also by an exposure to CO₂ under temperature and pressure conditions which are, approximately at least, the same as when imagos are dealt with. As the movements of the maggots are less elaborate and active than those of the flies, their state of poisoning may not be clearly apparent. When they wake up from the narcosis they may creep about, try to feed and even succeed in undergoing the pupal metamorphosis. But usually they die either as larvæ or as pupæ and do not reach the adult stage. A few of them, however, achieve an entire recovery and become flies. These show the normal sensitive behaviour, the larval treatment having produced no curative effect.

It has not been possible to demonstrate any sensitivity in the eggs or pupæ of sensitive stocks. Like those from resistant stocks, these dormant stages may remain in pure CO₂ for hours without any injury.

3. INHERITANCE OF SENSITIVITY

(i) *Empirical rules*

The original sensitive stock, as well as many others which have been obtained by different methods, breeds nearly true for sensitivity, and usually can be kept in mass cultures through many generations without requiring any selection. Nevertheless, even at the optimal temperature, *i.e.* 20°, a few resistant flies appear in the cultures from time to time. They behave, genetically, as individuals of pure resistant stock. When such a fly happens to be picked up among

the female parents, the ensuing generation is found to be a mixture of sensitives and resistants. By selecting sensitive female parents for one or two generations, the original, approximately true breeding, condition is easily restored.

The cross of two resistant flies, whatever their genetic origins, leads to an entirely resistant progeny. This statement is universal; but it must be noticed that it refers only to permanently resistant individuals: flies made temporarily resistant by a temperature cure may have sensitive offspring.

When a female from a sensitive stock is crossed with a resistant male, the whole progeny is sensitive and behaves genetically like individuals from the pure sensitive stock. Recurrent backcrosses to resistant males may lead, for many generations, only to sensitive offspring. Sometimes, however, some resistants appear here and there, but it is not certain that these "mutations" are actually more frequent in such a sequence of backcrosses than within a pure stock, as stated earlier. It may then be stated, as a fair approximation, that sensitivity is regularly carried along without segregation through a line of females.

The constancy of the transmission of sensitivity by females is probably under control of the genotype. Indeed, sensitive stocks of different genotypes differ in their abilities to breed true for sensitivity. But a given stock also varies from time to time with respect to this ability, probably as a result of the fortuitous selection at some generation of individuals poor in genoids. Thus, one has to be careful in attributing to the genotype a difference which may happen to exist between two stocks at a certain time.

The reverse cross of a resistant female to a male from a sensitive stock leads always to a mixture of sensitive and resistant offspring. Among the sensitives, males are only somatically sensitive and in no case transmit the character, whereas females produce a variable mixture of both types of offspring. Starting from such an initial cross it is possible, though sometimes difficult, to restore a good true breeding stock by selecting sensitive females for a few generations. Sensitivity may be also carried along mixed with resistance through an indefinite number of generations, by an alternate sequence of sensitive males and females, the other partner of the cross being resistant.

(ii) *Factors controlling the transmission of sensitivity by males*

The ratio of sensitives among the offspring of a sensitive male crossed with a resistant female varies from 0 to about 90 per cent., but never reaches 100 per cent. It is controlled by many factors, which are not yet entirely understood and are very intricate.

Apart from the action of the temperature on deposited eggs and on the male before copulation, each individual male exhibits a certain intrinsic potentiality to produce a high or low ratio of

“infected” spermatozoa. Part of this variation is apparently random and irreducible, the rest seems inherited. Now, each parent hands down to each male offspring two things—a haploid nucleus or genome and a certain amount of genoids. The genoids inherited from the male parent do not seem to influence the ability of the sons to carry sensitivity in their germ cells. This statement generalises the fact, already pointed out, that a male never transmits sensitivity when it has inherited it only from its father.

In contrast to the sperm, the amount and distribution of genoids within the egg, prior to fertilisation, are certainly important. They may be dependent on the genetic origin of the mother and on the circumstances of its life. Thus the problem is highly complex ; in this peculiar field of investigations no sharp line can be drawn between environmental and genetic factors since, as will be seen farther on, acquired effects are distinctly inherited.

There is some evidence, better than in the case of the females, that the chromosomal genotype may control the transmission of sensitivity by males. Among the stocks which were made sensitive, some were found in which the males transmit the character to only a very small part of their progeny, and even some in which they do not transmit it at all. Within such stocks the inheritance of sensitivity is purely maternal. This influence of the genes is indirect and delayed. The genotype of the reproducing male itself has been demonstrated to exert some action ; the genotype of its mother is active, but the genotypes of former maternal ancestors must also be taken into consideration. When genoids are introduced into a stock of a certain genotype it looks as if, after a few generations, some kind of equilibrium is reached which determines the average content in genoids of both male and female gametes. Any crossing with another stock upsets this equilibrium. As the transmission of the genoids is only remotely connected with the genes, it is not easy to recognise individual actions of genes during the genetic segregation which follows the cross. Until a gene is found which exerts a major influence on the phenomenon, careful and lengthy experiments must be performed to reveal such minor genic actions. Investigations of that kind are being actively pursued.

Another factor which plays a distinct rôle in the transmission of sensitivity by the males is their age. When a set of sensitive males is kept copulating with a fresh batch of resistant females daily, the proportion of sensitives among the progenies at first decreases rather sharply, then this decrease slows down and, finally, an actual slight increase occurs towards the end of the reproductive period of the males, *i.e.* 15 or 20 days. This is but an average course ; there is a large variation between individual males.

A similar decrease in the proportions of sensitives is observed when two identical sets of males are mated with similar resistant females, the first set being mated as soon as they reach the adult stage, the second after a period of ageing, during which they are kept virgin.

Finally, there is also a decrease in the proportion of sensitives among the eggs laid successively, after one copulation with a sensitive male by resistant females.

The last fact seems to imply either that the infected spermatozoa have a greater death-rate than the others, or that the genoids disappear spontaneously within them. The same process, whatever it may be, must occur in the males and account for part of the variation in the proportion of sensitives among their successive progenies; but the phenomena are made more complex in this case by the fact that spermatogenesis being a more or less continuous process, the age of the successive batches of spermatozoa does not correspond necessarily with the age of the male. The final slight increase of sensitives, referred to above, might be accounted for on this ground. The last spermatozoa used by a male are supplied by the imaginal spermatogenesis which has been shown to take place in *Drosophila* (Philip, 1942), and hence these spermatozoa may not be very old at the time of fertilisation.

Finally, the last factor which has been investigated is the genotype of the resistant females used in the cross. When identical sets of sensitive males are crossed with females of different genotypes, significant though rather slight differences in the proportions of sensitives may be observed among the different progenies. It does not seem worth while to review the several hypotheses which might account for this fact, as they must remain at present so highly speculative.

(iii) *Mechanism of transmission of sensitivity by males*

Sensitivity might be supposed to be transmitted by males, a priori, in two ways—genoids may be included within the spermatozoon, as was assumed above, or they may be present only in the spermatid and may be carried into the egg mechanically at the time of fertilisation. Some evidence to decide which hypothesis is true may be derived from polyandric fertilisation. When a resistant female is crossed simultaneously with two males, one resistant and the other sensitive, a few exceptional sensitives, about 5 per cent., are found among the progeny of the resistant father. The offspring of the other male show a variable but always much higher proportion of sensitives, the same indeed as a control cross involving similar resistant females and sensitive males alone. Polyandric females were demonstrated to give birth, day after day, to a thorough mixture of both kinds of offspring; the two samples of sperm must therefore blend in their seminal receptacles. If the genoids were present in the spermatid and not within the spermatozoa, such a difference in the proportions of sensitives should not be observed between the progenies of the two kinds of males. The facts are consistent, therefore, with the assumption that the spermatozoa themselves include the genoids.

If such is the case, the appearance of some sensitives among the progeny of the resistant males must be accounted for by polyspermic fertilisation which is usual in *Drosophila* (Huettner, 1924). There is no difficulty in assuming that a genoid, introduced into an egg by a

FIG. 2.—Transmission of inherited and acquired sensitivity.

supernumerary spermatozoon, may succeed in invading the organism. Now although most of the eggs must be penetrated by both kinds of spermatozoa, there are very few exceptional sensitive flies. Genoids in supernumerary spermatozoa must, therefore, have only a small chance of establishing themselves in the egg. It may be that they are not brought to the right place inside the egg.

4. TRANSMISSION OF SENSITIVITY BY ORGAN IMPLANTATION AND INJECTION

(1) *Somatic acquisition of sensitivity*

Extensive investigations have failed to show any resistant fly acquiring sensitivity by mere biological contact with sensitive individuals. Resistant larvæ may live in the same culture bottle with sensitive larvæ; they may even feed on crushed sensitive larvæ or flies and still keep their resistant behaviour. Also, sensitivity is never carried from one individual to another by copulation. In contrast, artificial procedures, such as implantation or injection, convert any resistant individual very easily into a sensitive one.

The implantation of an organ from a sensitive larva into an individual from a resistant stock can make it sensitive. All the implantations were made at the last larval instar stage. The fly may then display the sensitive behaviour either as soon as it hatches from the pupa or only when a few days old. The following organs were demonstrated to bring sensitivity to the host: ovary, brain, imaginal discs of eye, legs or wings, portion of the gut. In most of the individuals which became sensitive, the presence of the developed implant could be demonstrated by dissection, while nothing was usually found in the flies which remained resistant. As implants may be overlooked, it seems likely that the correlation is absolute and that the appearance of sensitivity is strictly conditioned by the success of the implantation. There is no difference between the readiness with which the two sexes acquire sensitivity in this way.

Injections are as effective and easier to perform than organ implantations. Hæmolymph as well as the centrifuged supernatant liquid of crushed flies of any stage were shown to be infectious. The injection can be made with the same permanent success into either larvæ or imagoes. It takes a certain time for the symptoms of sensitivity to appear after the injection. This so-called *incubation period* is particularly easy to show, when it is the adult fly which has received the injection. It may last at 20° from 10 days up to about 30 days and is not changed by the successive CO₂ tests, which are needed to ascertain its duration. The latter depends on the total amount of genoids introduced initially into the organism, as shown by injection of several dilutions of the same supernatant. It is also controlled by temperature, and increases when temperature is raised from 20° to 25°. Though this point needs fuller investigation, it looks as if the appearance of sensitivity depends upon the attainment of a certain level in the invasion of the organism by the genoids. The time needed to reach this threshold must be of course the shorter, the higher the initial level of infection and therefore the lower the dilution of the infectious liquid.

The infectiousness of a liquid, whether diluted or not, is apt to disappear in a few hours when it is kept at room temperature. At

higher temperatures this inactivation becomes more rapid and must be nearly instantaneous at 60°. Cooling, on the contrary, helps to preserve the activity. Some infectious liquids were kept two days at -7° without entirely losing their activity. In ordinary laboratory routine, the liquids used for the injections are always kept at about 0° and all the necessary centrifugations are made in a cooled room.

We have tried to ascertain the size of the infective particles, using ultrafiltration on collodion membranes. Filtration through a 180 m μ membrane has been found to preserve some of the activity of an infectious liquid. Activity is however much depressed by the process, making it likely that the 180 m μ membrane is not far from the limit below which no infective particle will be found in the filtrate. This still crude measurement will be completed later on ; it already shows that the size of the genoid in *Drosophila* extracts lies well within the usual range of virus particles.

(ii) *Inheritance of the acquired sensitivity*

The most striking aspect of the acquisition of sensitivity by implantation or injection is the ability of the genoids to penetrate inside the germ cells and be carried by the gametes to the next generation. The operation has indeed the unusual consequence of changing not only the physiology of the individual but also that of its progeny, for a potentially unlimited number of generations. The chief features of this phenomenon are schematically summarised in fig. 2, which shows, for comparison's sake, the rules of the normal hereditary transmission of sensitivity as well.

The two sexes, as will be seen, display very different abilities to become germinally infected, and this contrasts sharply with the equal readiness with which they become somatically sensitive. A male never transmits sensitivity, unless it is the offspring of a female in whose germ cells the genoids were already present at an early stage of oögenesis. This early infection is possible in only two cases : first when the mother is sensitive congenitally, *i.e.* has inherited sensitivity ; secondly, when a resistant ovary is grafted into a sensitive foster-mother. Acquired sensitivity then is not inherited in the males, even when this acquisition reaches back to the time of the fertilisation, *i.e.* when the genoid is brought into the egg by the spermatozoon.

A female, on the contrary, in whatever way she has acquired sensitivity, may always produce at least a few sensitive offspring. This is observed even when the somatic infection takes place at so late a stage as the imaginal one. But there is a large variation of the proportions of sensitives among such progenies and the 100 or near 100 per cent point, which is characteristic of females of pure sensitive stock, is reached only in about the same circumstances as those which allow the males to transmit the sensitivity.

To sum up, there appear to be two situations with respect to transmission by the gametes :—

(i) The ovum is already infected at an early stage of the oögenesis. In this case, if the ovum develops into a female, it will have 100 per cent sensitive progeny or nearly so ; if it develops into a male, it will transmit the sensitivity to some of its offspring.

(ii) The infection takes place too late, *i.e.* either during the individual's life, or too short a time before conception. A male will then produce only resistant offspring and a female a mixture of sensitives and resistants.

The ultimate difference between the two cases might well rest upon the inclusion of some genoids in the primordial germ cells at the time of their formation. This might happen only when the ovum is infected long before fertilisation, *i.e.* in the first case. In the second case, a secondary infection of the gonads would succeed, in the females, in compensating partly for the lack of genoids in these primordial germ cells ; but, in the males, such a secondary infection would not take place for an unknown reason. Anyway, it is worth noticing that when the genoids are artificially introduced into a resistant stock they can utilise only one door to enter the germ cells, *i.e.* the oögonial cells. Afterwards, in the later generations, they are of course present in the spermatogonial cells as well. It remains unknown whether the genoids can penetrate the oögonial cells at a special stage only or at any time during the oögenesis.

All that has been said about the inheritance of acquired sensitivity refers to *Drosophila melanogaster*. Inside this species, no evidence was found of a control by the chromosomic genome of the fundamental facts set forth here. Three other species, *D. simulans*, *D. funebris* and *Calliphora vomitoria*, were tested for their abilities to acquire sensitivity. The last seems to be immune, but both *Drosophila* species acquire it very readily. An outstanding difference, however, is that the character is inherited in *D. simulans*, as it is in *D. melanogaster*, but there is no evidence thus far of its inheritance in *D. funebris*. Thus, if the gametes of this species can carry the genoid at all, it must be either a very rare event or must happen in circumstances still to be determined.

5. TEMPERATURE CURES OF SENSITIVITY

Temperatures above 30° are able to cure the sensitivity either temporarily or permanently, according to the genetic origin of the flies and their developmental stage at the time of treatment. A further effect of high temperature is to prevent the regular hereditary transmission of the sensitivity : it cures the germ cells.

(i) Temporary cures

Any sensitive fly, whatever its genetic origin and however it has acquired sensitivity, may be induced very easily to assume for a

time a resistant behaviour by heating it above 30° during either the pupal or the imaginal stage. When the fly is returned to 20° sensitivity reappears in a few days. The transitional stages between sensitivity and resistance are difficult to study, for the organism goes through them quickly either forwards or backwards. It appears from preliminary work that for "half-cured" flies the temperature-CO₂ concentration curve shifts both upwards and towards the right in relation to the curve characteristic of normal control flies, so that its minimum is at a higher temperature. This displacement goes on until the whole curve vanishes; then the flies appear to be resistant whatever the temperature and the CO₂ concentration.

The duration of heat treatment required to produce a complete temporary cure depends on the temperature and on the developmental stage at which it is applied. At 34°, about the highest temperature which can be used without injuring the flies, one day and a half is enough to cure young imagoes. With lower temperatures this duration increases at first slowly, then very quickly in the neighbourhood of 30°. Old flies are somewhat more difficult to cure than young ones.

When the heating takes place during the pupal period 24 hours are sufficient, even at 30°, provided they are at the end of this stage. But such a short treatment, even at a higher temperature, has no effect when it takes place at the beginning of pupation. This does not prove, however, that the temperature is more effective on the last pupal day. The same process, which brings back sensitivity in the imago when the heating ceases, may indeed operate in the pupa, where it is bound to be overlooked as there is no way of showing sensitivity in a pupa.

A very strange, as yet utterly unaccountable feature of the temperature cures can be observed when sensitive flies are subjected to two heat treatments, the first one during the embryonic or the larval stage, the second during the pupal stage. It is found that the last one, which should have cured the flies very easily, had it occurred alone, is made less effective by the first. This *protection effect* (it is a protection against the cure) can be observed only with flies, the sensitivity of which comes from the mother; with flies having inherited sensitivity from their father alone, the embryonic or larval treatment produces a permanent cure.

Finally, a last fact about the temporary cures was secured by injection experiments. It was found that the centrifuged extract from temporarily cured flies is not infectious. Since the flies can recover sensitivity spontaneously, some genoids must of course be stored somewhere in the organism. One is led to assume that they are either rare or in such a location that the grinding of the flies does not allow them to escape in an active condition in the supernatant.

(ii) *Permanent cures*

When the progeny from the cross of a resistant female by a sensitivity-transmitting male is heat treated, during either the embryonic or the larval stage, it is found to include fewer sensitives than if no heat treatment had been used, or none at all. This non-occurrence of the normally expected sensitive offspring is the result of an actual cure and not of a higher mortality of the sensitives. This cure is permanent : sensitivity has never been observed to recur in any such individuals.

The effectiveness of a heat treatment depends on the temperature and even more on the stage of development. The most susceptible stage is the beginning of embryonic development. Heating the eggs for 12 hours at 32°, as soon as they are laid, suffices to cure most or even all of the individuals. Heating the larvæ is far less effective, and the whole larval period, *i.e.* about 4 days, must be subjected to the treatment to get a complete cure. The susceptibility to the cure disappears entirely with pupal metamorphosis ; after this event, as we saw, only temporary cure can be produced.

FIG. 3.—Responsiveness of sensitive flies of different genetic origins to short heat treatments at different stages of development. Full line : permanent cure. Broken line : temporary cure.

It is very striking to compare the effect of heating the embryonic or larval stages of flies, which have inherited sensitivity from their father alone, with its effect on flies from a sensitive stock. With these there is no permanent cure but only the protection effect, which means rather what could be called an exaggeration of sensitivity. A kind of behaviour intermediate between that of the two kinds of

sensitive flies is shown by the second generation from the cross of a resistant female with a sensitive male, which includes, as we saw, both types—sensitives and resistants. When such progeny is subjected to heat treatments at different stages the embryonic treatment produces merely a protection effect, while larval treatment gives some permanent cures. Temporary cure is obtained of course in the usual circumstances.

The rather intricate features of these somatic temperature cures are summarised in fig. 3, where the ordinates are arbitrary scales of units of what can be called the responsiveness to the heat treatment.

(iii) Gametic cures

Investigations are at present being made on gametic cures and it is hoped that the details will soon be worked out.

From preliminary work it appears that any heat treatment of the male from the laying of the egg up to the imaginal metamorphosis lessens the proportion of sensitives among its progeny. But the ability of mature spermatozoa to carry sensitivity is not changed by the temperature, as is shown by submitting adult males or fertilised females to heat treatments. The period of responsiveness to temperature therefore covers the period from the isolation of the primordial germ cells to the completion of the spermatozoon, but does not extend to the life of the sperm itself.

Heat treated females, similarly, are found to give a smaller proportion of sensitives among their offspring, even when it is the egg-laying fly which is heated. The temperature is thus also active during the whole period of development of the ovum.

When a partial or complete gametic cure happens to coincide with a somatic cure, this may be looked upon as a case of inheritance of an acquired character. It occurs regularly with permanently cured flies which never breed any sensitive offspring. The temporary cure may or may not be inherited, according to the duration of the heat treatment and stage in the life cycle at which it is applied. On the other hand, it is known from transplantation experiments that in females the germ cells can acquire the genoids from the somatic cells and *vice versa*. In my opinion these acquired character inheritances have no deep biological significance, in the sense that they do not involve any direct and constant action of the somatic cells on the germ cells. The inheritance of the acquired cure seems to be rather the result of the identity of responses of both kinds of cell to the same external condition, the temperature. The reciprocal infection of somatic and germ cells does not seem to agree any better with the progressive germinal response to somatic adaptative changes, which is assumed to take place by Lamarckian-minded biologists.

(iv) *Mechanism of the temperature cures*

We cannot yet give a definitive account of the mechanism of temperature action, and to work out a plausible hypothesis is only worth while to the extent it may prove to be an incentive to new experiments.

First, it must be noticed that similar phenomena are to be found in Sonneborn's observations (1946, 1947) on "killer" varieties of *Paramecium*. When "killer" *Paramecia* are exposed to a temperature of 38.5° they turn into sensitives. With a long enough exposure a pure sensitive progeny can be grown from them; but with short exposures part of the progeny at least reverts to killers. Sonneborn has accounted for these observations in a satisfactory way, on the basis that high temperature destroys at a fairly low rate the cytoplasmic particles ("kappa" particles) which are held responsible for the killer behaviour. Short exposures allow some particles to remain uninjured, but they are too few for the *Paramecium* to be a killer, as this needs a high kappa concentration. Later, the multiplication of kappa outpaces the cellular division rate, hence the reversion to the killer behaviour. The outpacing is greatly favoured by autogamy.

Both permanent and temporary cures have also been observed with some plant virus diseases, consequent on to heat treatments. In some cases heating the infected plants leads only to a masking of the symptoms of the disease, which reappear when the plants have been returned to a normal temperature. In other cases a true permanent cure is obtained. In the writer's knowledge no thorough study of these cases has been made (Johnson, 1922; Tompkins, 1926; Bennett, 1927; Crainger, 1936; Kunkel, 1936; Martin, 1930).

In the writer's opinion there is a close analogy between the hereditary CO_2 sensitivity in *Drosophila* and the killer case in *Paramecium*, and Sonneborn's kappa particle and the CO_2 genoid are likely to be very similar biological objects. Nevertheless, the temperature action is more complex in *Drosophila*, in relation it seems to the metazoan condition. With *Paramecium* the permanent cure is the extreme stage of the temporary cure; but with CO_2 sensitivity the two cures are never the result of the same treatments. One must then try to account separately for the two cases, although of course they may be related in some obscure unknown way.

Since the temporary cure always takes place in the same circumstances, whatever the genetic origin of the fly, it must be independent of the amount of genoids present in the egg. Now this initial amount is known to control the infection of the germ cells, but may not have any influence on the ultimate somatic concentration and location of the genoids inside the late pupa or adult fly. If such is the case it is not surprising that a heat treatment, which is applied to these late stages, brings out a uniform response. It was formerly assumed

(L'Héritier and Sigot, 1946) that the temperature destroys some intermediate substance in the chain of reactions, which is supposed to link the genoids with their physiological effect. The heated fly was therefore bound to appear resistant, until the substance has returned to its normal concentration. However, it was recently found that the centrifuged supernatant of temporarily cured flies is no longer infectious. Heating must then damage the genoids themselves and not some alleged product of their activity. Some parts of the organism must of course remain infected to allow the reversion to sensitivity, so that the resistance of the genoids to temperature probably varies with their location.

Coming now to the permanent cure, it seems significant that to produce this effect a heat treatment must be applied during the growth of the organism and to individuals whose initial condition of infection is low. This suggests a correlation with cellular multiplication which is found also in the gametic cures, since ripe gametes are not influenced by heat which is active only during gametogenesis. It was believed at first (L'Héritier and Sigot, *loc.*) that the temperature acted in inducing a discrepancy between the rates of multiplication of the genoids and of the cells. At high temperatures the cells would outpace the genoids and thus be freed of them. For this process to succeed in freeing the whole organism, the genoids should not be too numerous at the beginning of development, which means that they should have been carried only by the spermatozoon. It is not surprising that gametic cures are not similarly restricted, since gametogenesis involves so many cell divisions, which is bound to make the outpacing more effective than during somatic development. This hypothesis is very similar to Sonneborn's so-called concentration hypothesis, confirmed by Preer (1946), who found that "killer" *Paramecia* lose the killer character when they are cultivated in a rich and frequently renewed medium. Such conditions allow a rapid sequence of divisions and the kappa particles are not able to keep pace.

To account for the permanent cures of CO₂ sensitivity, this hypothesis is not however entirely satisfactory, since it does not make allowance for the protection effect. Although it may be understood why heating the embryonic and larval stages fails to free the whole organism from genoids, when they are very numerous in the egg, it should at least decrease their amount, and it looks like a paradox that this should make it more difficult to produce a temporary cure. Moreover, no direct evidence of such a decrease was found in injection experiments. When the infective capacity of the extract of larvæ from a pure sensitive stock, which have been heated at 30° through their embryonic and larval life, was compared with a control extract, no distinct decrease of infectiveness was found.

In view of this new evidence, the assumption of a discrepancy between the rates of multiplication of the cells and the genoids, brought

about by the raising of the temperature, cannot be retained, at least in its former crude form. In the writer's opinion some mechanism of this kind probably accounts for the gametic cures, but the somatic permanent cure of those individuals which have received sensitivity from their father alone must pertain to some more complicated mechanism. When the genoids have been brought by the spermatozoon they may be located in some special part of the organism, at least during the embryonic and larval stages, instead of being diffused uniformly, as they probably are in individuals from a pure sensitive stock. This special location might make them prone to be destroyed by heat, as they always are in the pupa and the adult fly, whatever the genetic origin of the individual. Further speculation must be postponed until these assumptions have been tested.

6. GENERAL DISCUSSION

Though much remains to be done to clear up the problem of CO₂ sensitivity, it does not seem likely that any new evidence will alter fundamentally its general outlook and will strip the biological object, which has been called a genoid, of its ambiguous character. The puzzling question to be discussed is indeed whether the CO₂ sensitivity is a virus disease or is a case of cytoplasmic inheritance.

Three features of the CO₂ sensitivity do not appear to fit its assimilation to a virus disease. First, the sensitive flies are not diseased in any of the ordinary meanings of the word. Their viability, longevity and fertility are entirely normal and, though it has been much looked for, no way has ever been found to detect the sensitivity without exposing the fly to CO₂. Moreover, unlike most of the virus diseases sensitivity is not contagious in the natural circumstances. To introduce the genoid from the outside into an organism, artificial surgical means must be used. Finally, the regular inclusion of the pathological agent in the gametes is a very unusual feature for a virus disease.

It must be conceded, however, that none of these three differences will resist any sharp scrutiny when they are taken individually. Several cases are known of plants harbouring a virus without showing any symptom of disease. The presence of the virus in these so-called virus carriers can be detected by introducing it into another organism, which may be performed by needle inoculation, grafting or by an insect vector (Johnson, 1925 ; Smith, 1929 and 1930 ; Salmon and Ware, 1928 ; Thrupp, 1927). The classical case seems to be that of the potato variety King Edward ; it has been shown that every plant of this variety harbours a concealed virus that causes a typical disease to develop in any other potato plant which a King Edward shoot happens to be grafted on (Salaman and Le Pelley, 1930). Moreover, the insects or ticks which play so great a rôle in the spreading of some plant and animal virus diseases, harbour the agent without apparently

being diseased in any way. In some cases at least it has been demonstrated that the virus multiplies inside its animal vector (see *System of Bacteriology*, vol. vii, Medical Research Council, London, p. 444). Some viruses, therefore, are able to invade the cells of an organism without altering its general physiology and morphology ; of course any exceptional and highly specific effect will have every chance of escaping detection.

Coming now to discuss the non-contagious character of the sensitivity, it must be noticed that though some virus diseases are highly contagious many of them depend for their spreading upon an artificial introduction into each of their successive hosts, which in some cases may be effected only by a specific insect. It is commonly assumed that the plant viruses are unable to penetrate the external surfaces of the leaves and stems, unless there happens to be some wound, which may be only the breaking of a trichome. Once inside the plant they spread by diffusion from cell to cell. The genoid is able, similarly, to cross the walls of the cells, since starting from an implanted organ it can invade the whole organism and is found ultimately even inside the gametes. Its inability to cross the hard external chitinal body wall is not surprising, and that it also fails to cross the digestive tract wall may only mean that it is destroyed by the digestive juices. There does not seem, consequently, to be any fundamental difference between the genoid and a pathological virus on this ground.

Concerning the third point, it must be noticed that though most of the virus diseases are never inherited, several cases have been reported where a plant virus is transmitted by seed and even by pollen. The case of the mosaic of bean has been studied rather extensively. Both ovules and pollen harbour the virus and give rise to infected seedlings, in an inconstant and irregular way (Nelson, 1932 ; Reddick, 1931 ; Harrison, 1935). The same general situation holds for the insect vectors. As a rule, the progeny of an infectious individual does not carry the virus, but there are notable exceptions. The wood tick, *Dermatocentor venustus*, which is responsible for the inoculation of Rocky Mountain spotted fever, regularly transmits the virus to its offspring. The presence of the agent has been demonstrated in the eggs, which are heavily infected (see *System of Bacteriology*, l.c.).

There is therefore no final ground on which to reject the assumption that the CO₂ sensitivity might be after all merely the physiological symptom of a very strange virus disease. It might be thought even that sensitive *Drosophila* is the insect vector of some disease, the chief host of which remains to be discovered. On the other hand, there is good ground for looking at the case as one of cytoplasmic inheritance. The character is inherited according to precise and elaborate rules, which allow the outcome of every cross to be predicted with nearly the same accuracy as when Mendelian characters are dealt with. The genoid is carried along by gametes of both sexes in a similar way

to albino plastids in the cases of cytoplasmically inherited variegations in plants and, from this point of view, may be looked at as a cytoplasmic hereditary unit, together with plastids and Sonneborn's kappa particles. Such objects, however, are not supposed usually to be able to cross the cell walls and plastids apparently do not. But the writer has indirectly learned that Sonneborn recently succeeded in showing that *Paramecia* could acquire kappa particles by contagion, without the intimate cytoplasmic contact which takes place during conjugation. Now, the strict dependance of kappa particles upon the genes and the constancy with which many hereditary characters are controlled by such particles in *Paramecium* preclude, apparently, their assimilation to viruses. Should this discovery be confirmed, the ability to penetrate the organism from the outside could not be used as a criterion to separate cytoplasmic units and viruses.

The similarity between the genoid and the autonomous cytoplasmic factors of *Paramecium* seems to end when the relation to the genome is taken into consideration. These cytoplasmic factors have been found in the so-called B group of varieties of *Paramecium aurelia*, where they control several hereditary differences. Each of these is autonomous, in the sense that no gene is able to initiate its formation, but it can multiply only in the presence of a specific dominant gene and is quickly lost when, following a sexual process, the genotype becomes homozygous for the recessive allele. In the A group of varieties of the same species, however, the hereditary characters are controlled directly by the genes, and though there is some sort of temporary cytoplasmic inheritance the cytoplasmic constituents are not autonomous and their formation can be initiated by the genes (Kimball, 1947). In *Paramecium*, therefore, there is a close connection between the hereditary cytoplasmic factors and the genes, whereas no major influence of the genes on the CO₂ sensitivity has ever been demonstrated. The fact that sensitivity can be inoculated into several species of *Drosophila* and, in some of them at least, is afterwards regularly inherited, shows even that the genoid can accommodate itself to very different genomes. The writer, however, is not inclined to hold this difference as fundamental. Some minor influences of the genes have actually been found and the discovery of a gene, which would prevent entirely the multiplication of the genoid, is not a priori an unlikely event. Without altering much the general outlook of the problem, this discovery would bridge much of the difference from the *Paramecium* case.

Too little is known about the kind of cytoplasmic inheritance which is inferred from the non-infrequent dissimilarity between reciprocal hybrids in plants, for any parallelism to be drawn with the CO₂ sensitivity. Therefore, to sum up the discussion, the writer expresses his feeling that no advance will come from classifying the CO₂ genoid in any definite category, since its classification is doomed to remain so much a question of definition and personal feelings. It seems more opportune to rely on future work to disclose in the

same organism or in others more or less similar facts, which will make it less speculative than it is at present to trace the general relations between the biological objects, which are called genes, plasmagenes and viruses.

7. SUMMARY

Sensitive *Drosophilas* display a very peculiar physiological behaviour, when they are exposed to CO₂. A contact of a few seconds with this gas can poison them irreversibly, whereas ordinary resistant *Drosophilas* can remain a long time in pure CO₂ without any permanent injury. The thoracic nervous ganglion is very likely the seat of the intoxication.

Sensitivity is transmitted in crosses as a hereditary unit, but shows no linkage with any chromosome and appears to be bound to some discrete cytoplasmic agent, which has been called a genoid. A female of a pure sensitive stock, crossed with any male, produces only sensitive offspring. A sequence of recurrent backcrosses to resistant males leads similarly to sensitive offspring, with only a few accidental resistants. The cross of a male from a pure sensitive stock to a resistant female produces a mixture of sensitives and resistants. Among the former, the males are only sensitive somatically and never breed any sensitive offspring, whereas the females are able to transmit sensitivity to part of their progeny.

Sensitivity is not contagious by mere natural contact, but can be acquired easily by the implantation of an organ of a sensitive individual or the injection of hmolymp or extract of sensitives. Acquired sensitivity is always inherited in females, but a male never transmits this character unless it arises from an ovum which has been invaded by some genoids at an early stage of ogenesis.

Keeping sensitive flies at temperatures above 30 leads to cures of the sensitivity. These cures may be either permanent or temporary, according to the genetic origin of the flies used and the developmental stage at which they are heated. Moreover, heating can interfere with the normal hereditary transmission of sensitivity.

The question whether CO₂ sensitivity must be looked upon as a virus disease or as a case of cytoplasmic inheritance is discussed.

REFERENCES

- BENNETT, C. W. 1927.
Mich. State Coll. Agric. Exp. Sta., Bot. Sect. Tech. Bull. No. 80.
- CRAINGER, J. 1936.
Nature 137, 31.
- EPHRUSSI, B., AND BEADLE, G. W. 1936.
Am. Nat. 70, 218-225.
- HARRISON, A. L. 1935.
N.Y. State Agr. Exp. Sta. Tech. Bull. No. 236.

- HUETTNER, A. F. 1924.
J. Morph. 249-265.
- JOHNSON, J. 1922.
Phytopath. 12, 438-440.
- JOHNSON, J. 1925.
Wisc. Agric. Exp. Sta. Res. Bull. No. 63.
- KIMBALL, R. F. 1947.
Genetics 32, 486-499.
- KUNKEL, L. O. 1936.
Phytopath. 26, 809.
- L'HÉRITIER, PH., AND TEISSIER, G. 1938.
C.R. Ac. Sc. 205, 1099.
- L'HÉRITIER, PH., AND TEISSIER, G. 1939.
C.R. Ac. Sc. 206, 1193 and 1683.
- L'HÉRITIER, PH., AND TEISSIER, G. 1945.
Pub. Lab. E.N.S., Paris, Biologie Fasc. I.
- L'HÉRITIER, PH., AND SIGOT, A. 1946.
Bul. Biol. Fr. et Bel. 80, 171-227.
- L'HÉRITIER, PH., AND HUGON DE SCOEU, F. 1947.
Bul. Biol. Fr. et Bel. 81, 70-91.
- MARTIN, J. P. 1930.
Hawai Planters Rec. 34, 375.
- NELSON, R. 1932.
Mich. State Coll. Agric. Exp. Sta. Tech. Bull. No. 118.
- PHILIP, U. 1942.
Nature 149, 527-528.
- PREER, J. R. 1946.
P.N.A.S. 32, 247-253.
- REDDICK, D. 1931.
Deux. Cong. Intern. Pathol. comp. 363.
- SALAMAN, R. N., AND LE PELLEY, R. H. 1930.
P.R.S., B. 106, 140.
- SALMON, E. S., AND WARE, W. M. 1928.
Ann. Appl. Biol. 15, No. 3.
- SMITH, K. H. 1929.
Ann. Appl. Biol. 16, No. 1.
- SONNEBORN, T. M. 1946.
Cold Spring Harbor Symp. Quant. Biol. 11, 236-255.
- SONNEBORN, T. M. 1947.
Adv. in Genet. 1, 263-358.
- TOMPKINS, C. M. 1926.
Phytopath. 16, 581-609.
- THRUPP, T. C. 1927.
Ann. Appl. Biol. 14, 175.