PROGRESSIVE PARALYSIS OF THE NERVOUS SYSTEM OF HOUSE FLIES BY FORMALDEHYDE AND ANESTHETICS

Wm. A. Hiestand, Purdue University

Casual observations on house flies which had been poisoned with formaldehyde led to a closer investigation of the paralytic effects of this substance and certain anesthetics. Formaldehyde has been widely used as a poison for house flies because of its simple application. Flies apparently do not object to formaldehyde in fairly weak concentrations (1-5 per cent) and can easily be induced to ingest solutions of 5 per cent or more by the addition of sweet substances. Stronger solutions produce "unpleasant" reactions on the part of the flies, but nevertheless the insects often return for more.

Material and Methods. The following experiments and observations were conducted on more than 100 house flies. Formaldehyde was fed mixed with honey and water. No difficulty was experienced in inducing the flies to imbibe. Anesthetics used were ether, chloroform, and "chlorotone" (chlorobutanol). For ether and chloroform effects the flies were placed in small test tubes (12 mm. diameter) plugged with cotton. Cotton plugs were placed in such a way that a space of approximately 20 mm. was allowed to permit movement. Observations were then made by means of a binocular microscope. Anesthetics were applied by placing a drop of the substance on the cotton, which allowed the vapor to enter the chamber. A very small quantity of the anesthetic was found necessary to produce complete anesthesia. When completely quiet the flies were removed to fresh tubes and allowed to recover. Chloretone was used in two ways. It was fed in a 5 per cent solution made with honey and water as previously explained for formaldehyde. It was also administered through the tracheae by allowing the flies to come in contact with the vapor of the substance in a test tube. Crystals of chloretone were placed in the tubes with the flies. Being a volatile substance its effects were noticed immediately.

Effects of Formaldehyde Ingestion by Flies. The effects of formaldehyde solutions of various strength are identical except for the rate of occurrence of events. Strong solutions produce effects more rapidly than weak ones. Likewise the succession of events occurs more rapidly with strong solutions. However, if two separate feedings of a weak solution are given the effect of the second is that of strongly augmenting the effects of the first. It was noticed also that large flies reacted more quickly than small ones when allowed the same time for feeding. This is apparently not correlated with sex since large and small individuals of both sexes were used and apparently neither sex was

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affected more than the other. The events following feeding always occur in the same order as follows:

1. The proboscis reflex occurs first. After ingestion of some of the mixture the proboscis is projected and withdrawn repeatedly during the activity of the fly. The movement consists of a ventro-cephalad projection and a withdrawal. This same reflex has been noted when flies were fed other harmful substances and is probably due to the presence of an abnormal sensation. The movements of the proboscis occur regularly at intervals of about two seconds. It has often been noticed that an exudate was present at the tip of the oral lobes which often was deposited on the side of the confining tube.

2. Following the proboscis reflex occurs a repeated "cleaning" of the first pair of legs. An absence of "head cleaning" was observed. The first pair of legs were rubbed over the proboscis and then rubbed together against their inner surfaces. Since this does not always occur, little significance is attributed to it.

The above reactions (1 and 2) are preliminary stages, not representing any paralytic effects. Following these the effects are stages of successive paralysis.

3. The apex of the abdomen is next to be affected. Inability to support it free from the surface of the glass is apparent. The flies walk about dragging the posterior portion of the abdomen.

4. The third pair of legs shortly become paralyzed. These are also dragged as the fly pulls itself by means of the first and second pair. Often while at rest the fly assumes an attitude not unlike the *Anopheles* mosquito with the hind legs elevated. That the legs were actually paralyzed was demonstrated by pinching them with tweezers. This produces no reaction on the part of the fly, indicating an insensibility in these appendages. If any of the other legs are so treated the fly immediately reacts by trying to free itself.

5. Following the paralysis of the third pair of legs a period of apparently no additional effects results, the length of which depends upon the strength of the formaldehyde solution and the amount ingested. This period may vary from a few seconds to over an hour.

6. At the end of the above period the second pair of legs become affected and shortly are paralyzed also. The fly then drags itself by its prothoracic legs.

7. Very soon following the paralysis of the mesothoracic legs the wings and first pair of legs are affected. The interval occurring between the effect on the second and first pair of legs is very brief. In fact, unless the formaldehyde has been given in quite a dilute solution the interval will not be apparent. At this stage the fly can no longer right itself if placed on its back.

8. The final effect is the loss of the proboscis and antennal movements which continue as long as the fly is alive.

Thus we may assume that formaldehyde paralyzes the nervous system of the house fly in a posterio-anterior direction which as will be mentioned later is antagonistic to the physiological gradient theory.

In reference to the definite interval occurring between the paralysis of the third and second pair of legs one might expect to find some anatomical reason such as a gap between the ganglia supplying their respective legs. However, upon dissecting out the thoracic ganglia of the house fly the writer was unable to note any possible explanation in the arrangement of the ganglia since it was observed that the three pairs of ganglia supplying the legs are coalesced into a single large central unit. Therefore, the explanation for the pause must be physiological rather than anatomical.

I have given some evidence to show that the loss of ability to move the appendages is a paralysis, probably nervous since sensation as well as motion disappears. There has been observed also another source of evidence. When formaldehyde is fed to flies in considerable numbers, and they are allowed to fly about at will many can be seen copulating and remaining attached, a condition which does not normally occur. These same flies show a beginning or later stage of paralysis. It is assumed by the writer that paralysis has occurred in the posterior region of the body involving the last abdominal ganglia at least, thereby resulting in an inability to control the muscles of copulation whereby to release themselves.

Effects of Anesthetics, Chloroform. The sequence of events during chloroform anesthesia was observed to be as follows:

1. The proboscis reflex starts as soon as the fly becomes aware of the vapor.
2. At a later period the legs become affected and are paralyzed almost simultaneously. It would be impossible to say definitely in what order the legs are affected, since any pair may be observed to make the last movement.
3. The wings become affected shortly after the legs.
4. The proboscis reflex and antennal twitching are the last actions to be noticed. These may persist for some time after the legs and wings have become immobile.

Recovery from Chloroform Anesthesia. When the insects are removed from the tubes and allowed to recover the actions are manifested in the following order:

1. The proboscis and antennal movements appear first.
2. After some interval of time the legs begin to twitch and definite movements occur. Apparently no definite order exists in recovery of the legs, or possibly the recovery is so rapid that it becomes impossible to detect any definite order.
3. Wing movements follow leg movements until the fly is able to walk or fly. However, the insects are unsteady on their feet for quite a period of time. The legs appear to have lost tonus since instead of being angular and rigid as they are when normal, they are apparently limp with the extremities often appearing curved. This period of unstability ordinarily lasts 30 minutes or longer.

Ether. Very little difference exists between the reactions to chloroform or ether. Recovery is more rapid from ether and the period of instability shorter. It might be mentioned at this point that in spite of the fact that all the legs recover almost simultaneously it appears that the first and second pair are more active than the third pair.

Anesthesia by Chloretone Vapor. Flies were placed in tubes to which were added crystals of chloretone. As soon as anesthesia was complete the flies were removed and recovery observed. The stages of anesthesia are as follows:

1. The proboscis reflex starts immediately after the chloretone vapor is detected.
2. The flies exhibit erratic movements showing lack of co-ordination. Many spasmodic movements occur.
3. The loss of ability to move the legs and wings occurs practically simultaneously as in the cases of ether and chloroform. The final stage in this phase is represented by a twittering of the tarsi of all the legs.
4. The proboscis reflex continues throughout all of the above stages and is the final movement to disappear.

In recovery from chloretone anesthesia the proboscis reflex again appears first followed by leg movements in a disorganized fashion. The legs show loss of muscle tone for long periods following recovery. In very many cases the flies were found dead the following day, indicating incomplete recovery from chloretone.

Effects of Ingestion of Chloretone. Flies which had been fed chloretone (5 per cent solution with honey and water) showed reactions similar to those obtained with formaldehyde. In most cases feeding was difficult due to the odor of the substance. However, the flies returned to imbibe repeatedly although for short intervals only. The sequence of events is as follows:

1. Erratic movements are apparent indicating an abnormal condition.
2. The proboscis reflex starts and continues throughout the successive stages.
3. The third pair of legs become somewhat sluggish.
4. All of the legs become paralyzed almost simultaneously although loss of movement apparently occurs first in the third pair. This stage occurs only when considerable quantities of chloretone have been ingested. Recovery is complete with chloretone feeding, the flies showing no ill effects after complete recovery.

DISCUSSION

Thus we see that the events do not occur in exactly the same order when formaldehyde is ingested by flies as when anesthetics are administered. In the case of formaldehyde, paralysis occurs definitely in an anterior direction. A pause exists between the effects on the last pair of legs and the second pair. This can not be explained by the morphological arrangement of the thoracic ganglia. It is therefore due to some physiological reaction. The proboscis reflex which occurs first is evidently caused by some sensory disturbance and may not be due to the direct action of formaldehyde on the head ganglia.

Since the proboscis movement, together with the antennal twitchings, is last to disappear we can assume that the region of highest metabolic activity, namely the head, is the last to be affected. This result is in direct opposition to the accepted theory of axial, or better, physiological gradients. Since the antennal and proboscis movements are not the first to disappear we must assume either that the head does not represent the region of highest activity or else that the fly is an exception to the accepted theory of susceptibility of physiological gradients.

With anesthetics somewhat similar results have been noted. Here we do not find the clearly marked progression of paralysis of the legs as is the case with formaldehyde poisoning. Nevertheless, here again the movements of those appendages controlled by the cephalic ganglia are the last to disappear.

In the great majority of cases toxic substances, such as HCN, acids, alkalies, etc., affect first the regions of highest metabolic activity. This has been repeatedly demonstrated with simpler animals. Child[1] states that "susceptibility varies directly with, though not necessarily proportionately to, the general protoplasmic activity or rate of metabolism." Evidently the house fly demonstrates regional differences in susceptibility to various toxic agents. The beautiful example of posterio-anterior paralysis by formaldehyde must evidently be accounted for by other means than simply physiological gradients. Could assimilation of the poisonous substance after ingestion be regional and thus affect certain ganglia before others? It scarcely seems probable when we consider that small amounts of the toxin may produce these effects after periods of an hour or more. Certainly in this length of time the formaldehyde would have quite generally penetrated the body substance as a whole.

In return from anesthesia the flies follow the accepted theory of axial gradients, that is to say, the anterior organs revive first and the progression is caudad. This agrees with Child's statement that "the rate and degree of recovery after temporary exposure to a certain range of concentration or intensity varies in the same way," that is, the order and rate of recovery is proportional to the metabolic rate.

CONCLUSIONS

It has been demonstrated that formaldehyde causes a progressive paralysis of the nervous system of the house fly in an anterior direction starting with the posterior end of the abdomen and ending with the mouth parts and antennae.

Anesthetics produce results comparable to formaldehyde although the phases of paralysis are not as distinct as in the case of formaldehyde poisoning.

In recovery from anesthesia the phases occur in the opposite direction, that is to say, the anterior end recovers first and the progression is in a posterior direction.

[1] Child, C. M. Physiological foundations of behavior. 1924. Holt and Co.