THE EFFECTS OF OXYGEN POISONING ON THE POST-EMBRYONIC DEVELOPMENT AND BEHAVIOR OF A CHALCID WASP[1]

MARY HELEN M. GOLDSMITH[2] AND HOWARD A. SCHNEIDERMAN

Department of Zoology, Cornell University, Ithaca, N. Y.

Aerobic organisms survive only within a narrow range of oxygen tensions. In very low oxygen, respiration becomes impossible; in excessively high oxygen tensions, oxygen poisoning occurs. This report describes the effect of high pressures of oxygen on the development and behavior of the chalcid wasp, *Mormoniella vitripennis*. Few higher organisms are as suitable subjects for studies of oxygen poisoning as insects. The design of their respiratory and circulatory systems is such that insects are less prone to carbon dioxide accumulation and aeroembolism ("bends") than vertebrates. The small size of *Mormoniella* also tends to minimize such complications. Further, *Mormoniella* is a particularly attractive experimental animal because the histological details of its post-embryonic development are well know (Tiegs, 1922).

In preliminary notes (Goldsmith and Schneiderman, 1956, 1958) we reported that the sensitivity of *Mormoniella* to oxygen poisoning changed in a systematic way during post-embryonic life. The present paper describes these changes in detail and identifies the organ systems which are the main targets of oxygen poisoning at successive stages in the life history.

Materials and Methods

1. *Experimental animals*

Mormoniella vitripennis Walker is parasitic on pupae of muscoid flies. Tiegs (1922) has described the developmental anatomy of this wasp; Whiting (1955), and Schneiderman and Horwitz (1958) have recently reviewed its life-history. The adult female pierces the puparium and lays her eggs on the developing fly pupa. A few days later the eggs hatch and the larvae begin feeding on the host. The cells of a larva grow in size throughout the several instars, but according to Tiegs, there appears to be no larval cell proliferation. At 25° C. a larva feeds for about four days and molts several times but is unable to defecate. Finally, it ceases feeding and enters a resting stage during which the larval tissues begin to break down; simultaneously the imaginal discs proliferate. After only a few cell divisions the thin partition between the midgut and the invaginated rectum breaks down, defecation occurs, and the greyish larva becomes white. During the 24 hours immediately before and after defecation, the so-called prepupal period, the

[1] This investigation was supported by a research grant (H-1887) from the National Heart Institute of the Public Health Service.

[2] Present address: Biological Laboratories, Harvard University, Cambridge 38, Mass.

larval cells break down and the cell divisions necessary for the formation of the adult integument, appendages, nervous system, and certain muscles occur. Except for the thoracic and abdominal muscles, whose myoblasts continue dividing for about 15 hours after pupation, adult development is a period of refinement, molding, and differentiation of tissues and organs into the final imaginal form.

A time-table, descriptive of the development from egg to adult, is given in Figure 1. Following Snodgrass (1935) and Hinton (1946, 1948, 1958), an instar is considered ended when the epidermis retracts from the cuticle. In *Mormoniella,* hours or days may intervene between the time of detachment and the actual ecdysis, which unveils the new instar. Thus the pupal stage actually begins just prior to defecation when the cuticle of the last instar larva separates from the epidermis (evidenced externally by wrinkles). The pupa itself is not

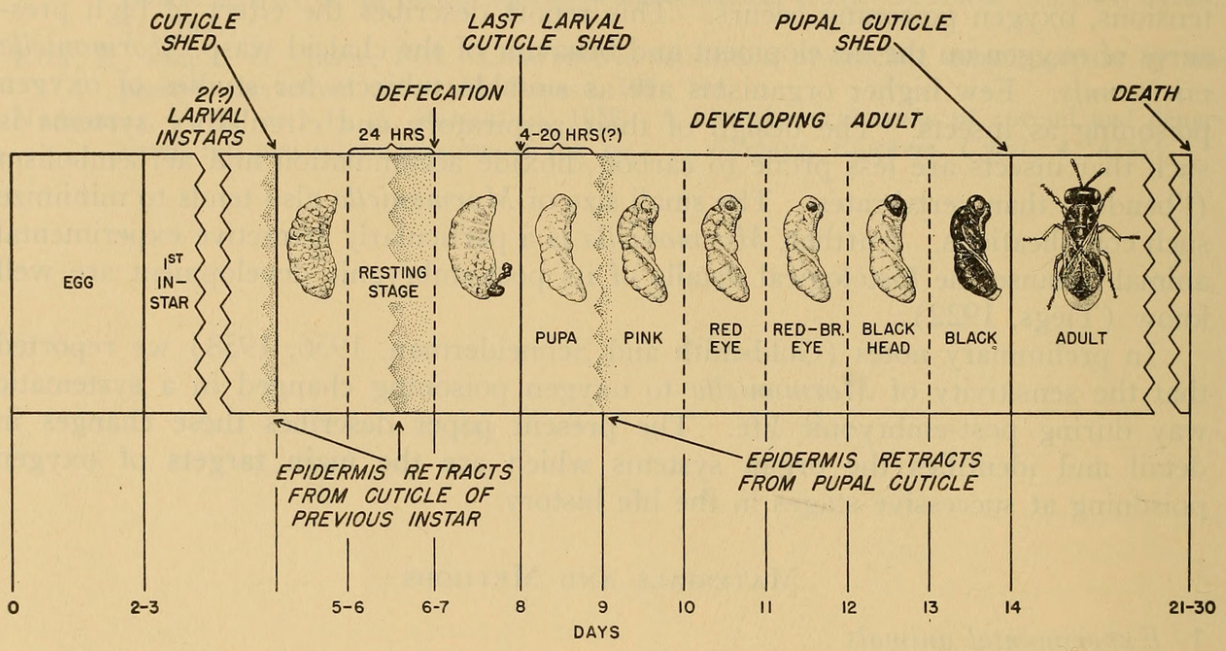

FIGURE 1. Time-table for the development of *Mormoniella.* The approximate times at which the cuticle detaches from the epidermis (signaling the end of an instar) are indicated by stippling.

revealed until the cuticle of the last larval instar is shed, an event occurring 24 to 48 hours later. The so-called prepupa is simply the pupa enclosed within the last larval cuticle. Likewise, the pupal stage ends with the detachment and retraction of the pupal cuticle, although the adult does not emerge for 4 or 5 days. During this period the wasp, which is properly called a developing adult, is enclosed in two cuticles—an inner adult cuticle and an outer detached pupal cuticle, which is shed at eclosion. The exact time at which epidermis retracts from the pupal cuticle, marking the end of the pupal period, has not been definitely determined for *Mormoniella.* Our observations agree with those of Tiegs in suggesting that this occurs within 24 hours after shedding the final larval cuticle.

Under certain conditions, just prior to entering the resting stage the larva ceases development and enters diapause (Schneiderman, 1957; Schneiderman and Horwitz, 1958). At room temperature diapause persists for a year or more

until the animal dies. If the diapausing larva is chilled at 5° C. for 3 months or longer, diapause ends and development resumes after return to 25° or 30° C. Chilled diapausing larvae may be stored in the cold for a year or more and provide a convenient source of experimental animals.

Mormoniella were reared at 30° C. on puparia of the fly *Sarcophaga bullata*. Since the wasp's development is accompanied by changes in epidermal pigmentation which are readily seen through its transparent pupal cuticle (Fig. 1), it was easy to select animals at specific stages of development and to observe the progress of development after experimental treatment. To assure that all insects used in a single experiment were of the same age and were developing at the same rate, only developing adults that reached a specified developmental stage within six hours of each other were used in experiments. Unfortunately larval development, unlike adult development, is not marked by gross external changes. Diapause, however, always occurs at the end of larval life; consequently, all diapausing larvae are at precisely the same stage of development. In the experiments to be described, larvae which had entered diapause were collected and stored at 5° C. for 4 to 8 months before use. These larvae initiated pupal development within 24 hours after return to 30° C. For the present investigation, animals at five stages of development were used: (1) chilled diapausing larvae (equilibrated at 30° C. for one hour); (2) early prepupae (chilled diapausing larvae placed at 30° C. for 24 hours prior to the experiment and thus just beginning pupal development); (3) "pink stage" developing adults (24 hours after ecdysis from the final larval cuticle); (4) "black stage" developing adults (12 to 24 hours prior to adult emergence); and (5) adults (males and females). In each experiment groups of about ten animals were placed in one-dram shell vials loosely plugged with cotton. Several vials were compressed in each pressure chamber. After exposure and decompression the vials were removed from the chambers and placed at 30° C., where the progress of development was observed periodically until the adults emerged or the insects died.

In every experiment, 25 to 50 insects were kept in air at atmospheric pressure during the experimental period. These air controls indicated the normal rate of development and percentage of adult emergence for a given population. The adult emergence of these control insects varied between 75 and 100 per cent. Therefore, following an experimental treatment only consistent reductions of 50 per cent or more in adult emergence were considered different from the control values. To permit a comparison of the effects of different experimental conditions, a number of groups of insects at the same stage of development were selected from the same population, and each group exposed simultaneously in separate chambers to specific experimental conditions. The data from each group were compared with those of other groups in the same experiment. Each experiment was repeated one to several times. The data presented in this paper are representative of that amassed from almost 10,000 animals studied in a total of about 350 separate compressions (Goldsmith, 1955).

2. *Compression and decompression*

Commercial cylinders of compressed oxygen, nitrogen, and helium (Airco or Matheson) were used. All gases contained less than 0.5 per cent impurities

(Schneiderman *et al.,* 1953). The vials of wasps were sealed in transparent polymethylmethacrylate (Lucite) chambers fitted with brass end-plates and needle valves (Schneiderman and Feder, 1954) and compressed with a specific gas. In all cases the gases were superimposed on the atmosphere of air initially in the chamber. Throughout this paper all pressures are given as gauge pressure. The final pressure in each chamber was checked on the same gauge, both after compression and before decompression, and the insects from any chamber showing greater than ± 5 per cent variation in pressure during the experimental period were discarded. The sensitivity of the gauges at the pressures employed was about ± 3 per cent.

Compression was accomplished in one minute, whereas decompression was performed stepwise over a period of five minutes. The pressure was reduced to half in the first minute of decompression and then held constant during the second minute. In the third minute, the pressure was gradually reduced to one fourth and then again held constant during the fourth minute. During the final minute, the pressure was gradually reduced to atmospheric.

The chambers were kept at a specific temperature in a thermoregulated water bath. Most experiments were conducted at 30 ± 1° C. and positive pressures (gauge) of 5 atmospheres (atms.) oxygen. Under these conditions, adult wasps survived for 1 or 2 hours. Hence, the time required for compression and decompression occupied only a small fraction of the total exposure time.

Results

1. *The effect of pressure on Mormoniella*

In each experiment, wasps at various stages of development were exposed to positive pressures of nitrogen or helium to determine if elevated pressures of metabolically inert gases had any toxic effects. As Table I shows, prolonged exposure for 16 hours to 5 atms. of helium had, at most, a slight effect on survival and development of any stage of the life cycle. Since 5 atms. of helium did not impair the development of *Mormoniella,* we may conclude that this pressure *per se* does not adversely affect *Mormoniella.* By comparison with either helium or nitrogen [3] the toxicity of 5 atms. oxygen is striking. For example, in experiment No. 104, 78 per cent of the control chilled diapausing larvae ultimately emerged as adults. After 16 hours of exposure to 5 atms. helium, a somewhat smaller percentage emerged (70 per cent), but after the same exposure to oxygen only 13 per cent emerged.

2. *The effects of compression and decompression on Mormoniella*

As Paul Bert (1878) first pointed out, the adverse effects of rapid decompression on vertebrates result in large measure from nitrogen being released from the tissues too fast to be dissolved. Since *Mormoniella* is small in size and possesses a tracheal system, rapid decompression might affect this insect differently from a vertebrate. To test this, a total of 1,440 chilled diapausing larvae, early prepupae,

[3] The survival and development of insects exposed to 5 atms. nitrogen was somewhat more variable than that of insects subjected to helium, an effect which can be attributed to nitrogen narcosis (Frankel and Schneiderman, 1958).

TABLE I

Effect of exposing Mormoniella at specific stages of development to 5 atms. of He or O_2 at 30° C. for various periods

Expt. No.		Per cent emerging			
		104*	109*	114**	115**
Duration of exposure (hrs.) \ Stage of devel.		Chilled diapausing larvae	Early prepupae	"Pink stage"	"Black stage"
Air (controls at atm. pressure)		78	90	96	100
He	2	88	86	—	—
	4	94	85	—	—
	8	64	94	—	—
	16	70	82	—	100
	24	—	—	56	88
	32	—	—	84	100
	64	60	78	80	92
O_2	1	72	51	—	—
	2	73	55	88	96
	4	70	54	80	94
	8	63	65	40	0
	16	13	20	0	—
	24	—	—	0	—
	64	—	0	—	—

* About 50 individuals used in each exposure.
** About 25 individuals used in each exposure.

"pink" and "black stage" developing adults were compressed with 10 atms. nitrogen and then decompressed. The following procedures were used to study the effects of rapid compression or decompression:

Group I: Controls—maintained at atmospheric pressure.
Group II: Thirty seconds for compression and thirty seconds for decompression.
Group III: Thirty seconds for compression and five minutes for decompression following the regimen outlined in the section on Methods.
Group IV: Five minutes for compression and five minutes for decompression.

In each group, two tanks were compressed and ten minutes elapsed between compression and decompression. Regardless of the regimen, wasps exposed after the start of the prepupal period invariably emerged as normal adults in about the same time as air controls. By contrast, chilled diapausing larvae proved exceedingly sensitive to compression. Though 85 per cent of air controls in this experiment completed adult development, fewer than 40 per cent of the chilled diapausing larvae emerged as adults when subjected to any of the above procedures. Because of the unusual sensitivity of this stage to compression and decompression, only one successful experiment was conducted with some 750 chilled diapausing

TABLE II

Effect of exposing Mormoniella prepupae to O_2 at 30° C. for various periods

Expt. No.	49* (10 atms.)	115** (5 atms.)	116** (5 atms.)
Exposure (hours)	Per cent emerging as adults		
Air (control at atm. pressure)	85	85	82
O_2 $\frac{1}{3}$	90	—	—
$\frac{2}{3}$	45	—	—
1	59	—	—
2	—	50	44
4	35	40	44
8	—	46	40
16	0	4	11
32	—	—	0

* Each figure is based on about 20 individuals.
** Each figure is based on about 25 individuals.

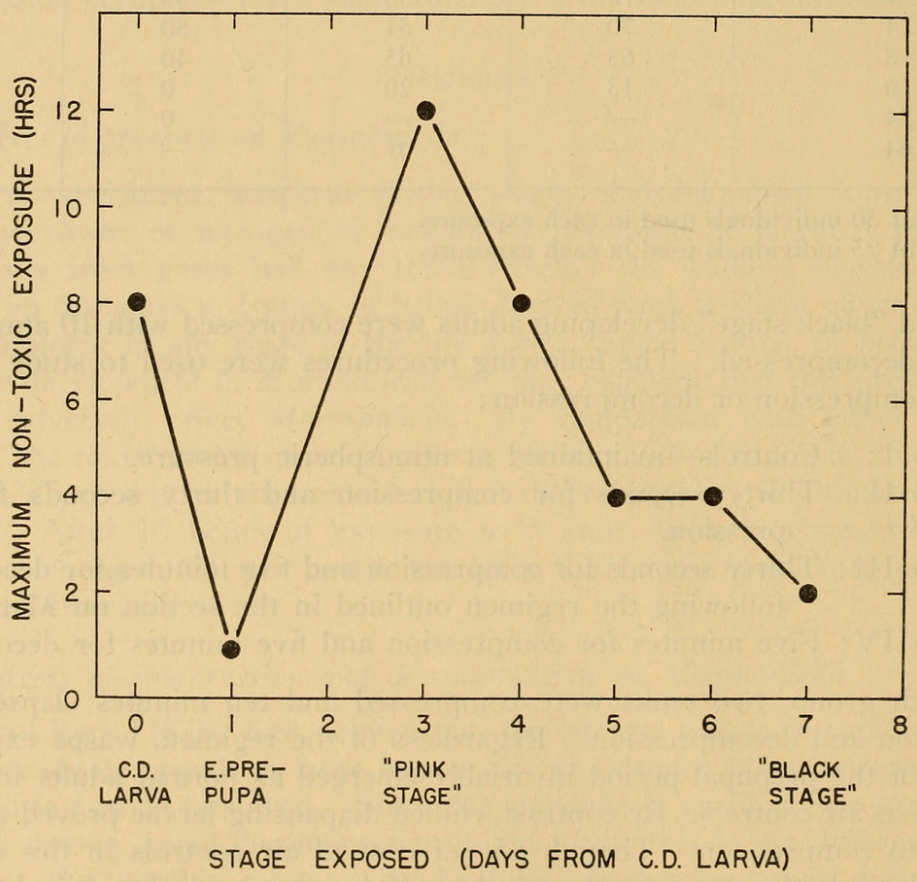

FIGURE 2. The sensitivity of different developmental stages of *Mormoniella* to 5 atms. O_2 at 30° C. The maximum exposure that had no effect on adult emergence is plotted as a function of developmental stage from chilled diapausing (C.D.) larva to "black stage." Stages withstanding only a brief exposure are the most sensitive to oxygen poisoning.

larvae (Exp. 104, Table I). In this experiment, for reasons which are not clear, the controls exposed to 5 atmospheres helium showed no evidence of an adverse effect of pressure or decompression and compression.

3. *Sensitivity of different developmental stages to oxygen poisoning*

We have established that, excepting chilled diapausing larvae, pressure as such, as well as compression and decompression, has only minor effects on the development of *Mormoniella,* and can now consider oxygen poisoning itself. In the first series of experiments we examined the effects of five atmospheres of oxygen on various developmental stages. The toxic effects of oxygen were appraised in terms of: (a) the rate of development of the wasps, (b) the percentage reaching maturity and emerging, and (c) the duration of exposure necessary to halt all development completely. Tables I and II give the effects of exposure to oxygen at several developmental stages on the ultimate emergence of the adults. Figure 3 and Table II record the final stage of development reached by wasps exposed to oxygen at a specific developmental stage.

To measure the inhibition of adult emergence, we determined for each developmental stage the maximal exposure time that had no effect on emergence. It is clear that this insect's sensitivity to oxygen changes markedly during development (Fig. 2). Chilled diapausing larvae and "pink stage" wasps were the least sensitive and tolerated more than 8 hours of oxygen. Early prepupae were many times more sensitive and could barely withstand one hour of oxygen. Thus, during the 24-hour period in which the chilled diapausing larva transformed into an early prepupa, sensitivity to oxygen increased many-fold. Likewise, after the "pink stage," sensitivity progressively increased during adult development, reaching its maximum in the adult wasp which could tolerate only one-sixth the exposure of a "pink stage" wasp.

4. *Oxygen poisoning in chilled diapausing larvae and early prepupae*

The above experiments disclosed that chilled diapausing larvae were far less sensitive to oxygen than early prepupae. To reduce adult emergence of chilled diapausing larvae by 50 per cent, an exposure to oxygen for 8 to 16 hours was required; for early prepupae, however, an exposure of less than one hour at 5 atms. must have been sufficient (Table I). Since at 10 atms. an exposure of 20 minutes had no effect on the subsequent emergence of prepupae (Table II, Experiment 49), it may be inferred that at 5 atmospheres of oxygen an exposure between 20 minutes and one hour would be required to reduce emergence by half.

Curiously, exposing early prepupae to 5 atms. oxygen for 2, 4 and even 8 hours had no greater effect on the subsequent emergence of adults than did one hour of exposure (Table I). Thus, although the early prepupa was the most sensitive developmental stage studied, this sensitivity was manifest in only half the population. The other half showed a sensitivity comparable to chilled larvae. These results were confirmed in numerous experiments with early prepupae (for example, Table II, Exp. 115 and 116). The explanation of this phenomenon is not clear; there is no marked difference in sensitivity of males and females, nor does this result stem from effects of compression or decompression. A likely explanation

is that the resistant members of the population were slower in initiating pupal development and at the time of exposure to oxygen were still in a stage comparable to the insensitive chilled diapausing larva.

Eight hours' exposure to oxygen caused a retardation in the rate of development of both chilled diapausing larvae and early prepupae; both lagged behind the controls by 1 or 2 days. Though adult emergence was reduced, prolonged exposure to oxygen failed to cause an arrest in development comparable to diapause. In fact chilled diapausing larvae and early prepupae defecated, and many continued to

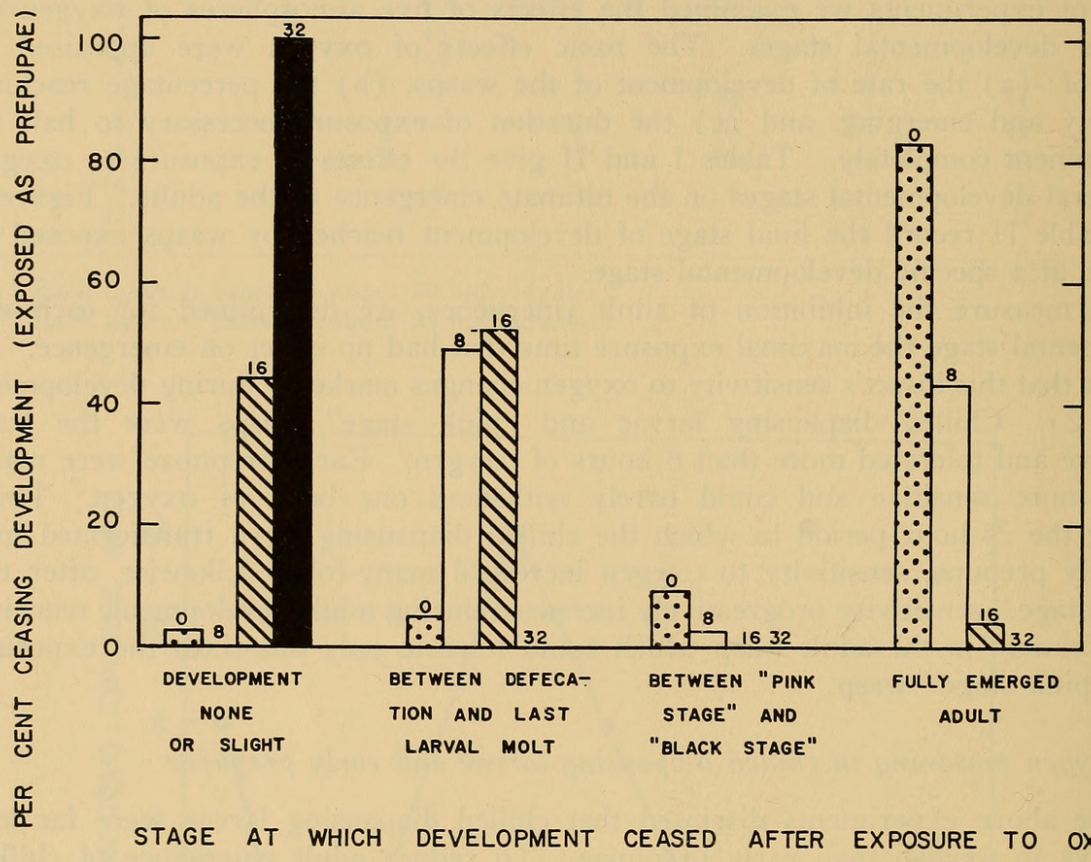

FIGURE 3. The stage of development attained by early prepuae following exposure to 5 atms. oxygen at 30° C. About 50 early prepupae were subjected to each exposure. The duration of exposure is indicated above each bar. Wasps which completed the larval-pupal transformation after exposure to oxygen also successfully completed development and emergence, and almost none of them ceased to develop at stages intermediate between emergence and the larval-pupal transformation. A similar result was also obtained in the experiment with chilled diapausing larvae. For early prepupae, the effect of exposures between 1 and 8 hours duration was not significantly different from an 8-hour exposure.

develop after exposure which markedly reduced adult emergence. As exposure times were increased to 16 hours, fewer animals developed beyond defecation; an exposure somewhere between 16 and 32 hours prevented all further development (Fig. 3). Examination showed that those animals that failed to emerge as adults ceased developing before or soon after defecation. Thus treating larvae and prepupae with oxygen blocked the developmental changes which immediately precede the transformation from larvae to pupae; animals that successfully pupated almost invariably completed adult development and emerged (Fig. 3).

5. *The effects of oxygen on "pink stage" developing adults*

Approximately 80 per cent of the wasps exposed in the "pink stage" to 5 atms. oxygen for 8 to 16 hours appeared to complete adult development (*i.e.*, reached the "black stage") (Table III). At these doses then, increasing the length of exposure to oxygen had no effect on the normal differentiation and pigmentation of the epidermis and appendages. That the longer exposures in this range were toxic, however, was revealed by a decrease in the ability of the adults to complete emergence. Thus after exposures in the "pink stage" for up to approximately 12 hours, most of the resulting "black stage" wasps emerged; however, after 14 to 16 hours only a fraction of these "black stage" wasps escaped completely from their pupal cuticles (Table III). This is in striking contrast to results obtained with

TABLE III

Development of "pink stage" wasps after exposure to 5 atms. of O_2 at 30° C.

Expt. No.	Exposure (hrs.)	Per cent of wasps exposed*			
		Ceasing development at		Completing adult development**	Completing emergence
		"Pink Stage"	Intermediate stages (between "Pink" and "Black Stage")		
300*	8	15	4	81	77
300	10	14	0	86	65
300	12	19	0	81	19
302*	12	Not determined	Not determined	80	60
301*	14	Not determined	Not determined	85	23
302	14	Not determined	Not determined	82	23
300	16	45	38	17	0
301	16	Not determined	Not determined	73	7
302	16	Not determined	Not determined	79	13
114	24	100	0	0	0

* About 30 individuals used in each exposure in experiment 300; about 25 individuals used in each exposure in experiment 301 and 302.

** "Black stage," partially emerged adults, and adults.

chilled diapausing larvae and prepupae where most animals that failed to emerge also failed to complete adult development (Fig. 3). Exposing "pink stage" wasps to oxygen for longer than 16 hours commonly prevented further development, and the animals remained in the "pink stage" until death (Table III).

The results obtained with the "pink stage" wasps suggest that although exposures up to about 16 hours of oxygen fail to inhibit epidermal differentiation during adult development, some internal system necessary for ecdysis is damaged. Since proper functioning of muscles and nerves is necessary for emergence, and since the development of the muscular system can be easily observed, the effects of oxygen on this system were studied. This proved a fortunate choice.

To study the extent of muscle development, wasps that completed adult development were immersed in Zenker's fixing solution. After 24 hours, the animals were dissected and their thoracic muscles examined. In normal "black stage" wasps and in adults, the thoracic muscles are grouped in 5 longitudinal and 5 dorsosternal pairs. These thick white bands almost fill the thorax and are the most prominent tissue in this part of the animal. Oxygen had a striking effect

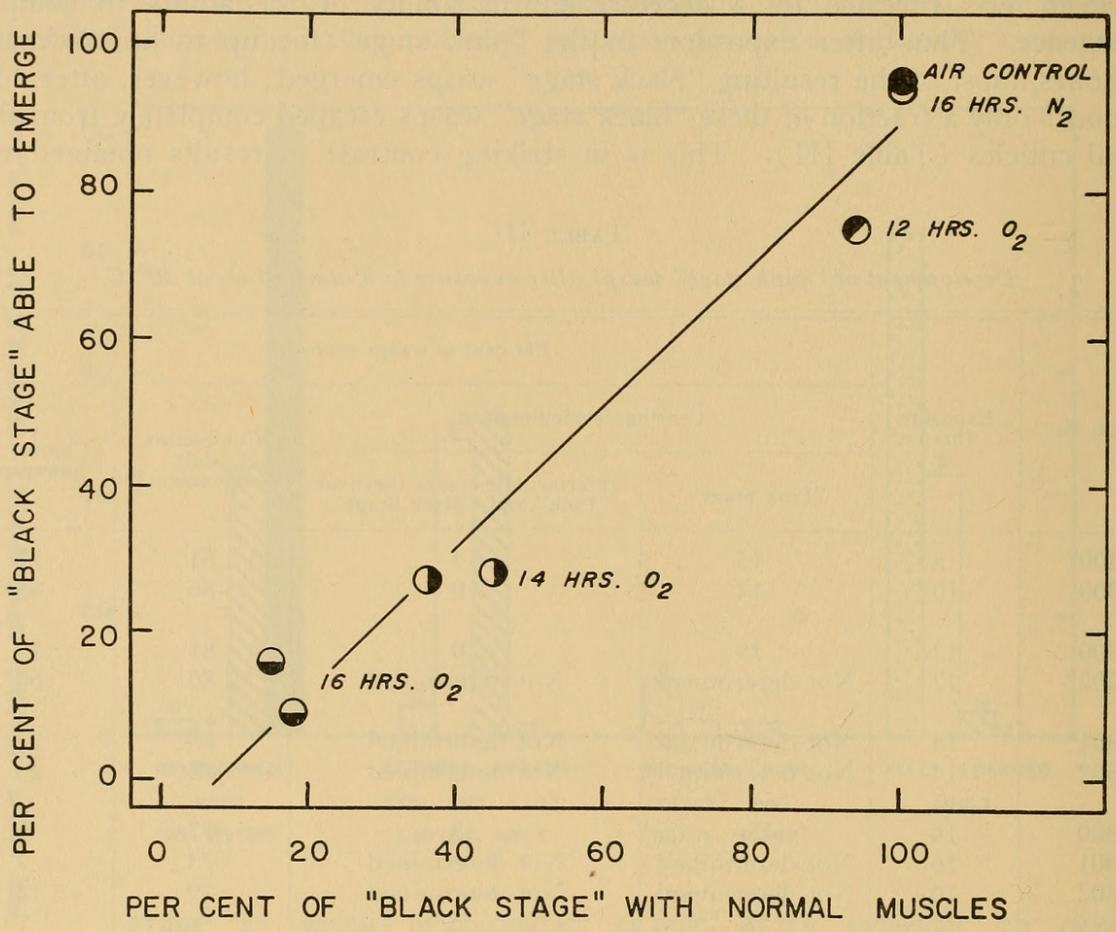

FIGURE 4. The percentage of "black stage" adults which emerge as a function of the percentage which develop normal thoracic muscles. In two experiments (Nos. 301 and 302; see also Table III), "pink stage" wasps of uniform age were exposed to oxygen (5 atms. at 30° C.) for periods that allowed most of them to differentiate to the "black stage." The duration of exposure indicated in the figure varied from a period so brief that almost all adults emerged, to a period so long that no adults emerged. After exposure, the "pink stage" insects were divided into two groups; in one group, the emergence of wasps was observed while in the other group each animal that completed adult development was dissected and its thoracic musculature examined. About 110 insects were dissected.

on the development of these muscles. In "black stage" wasps unable to emerge because of oxygen poisoning in the "pink stage," the thoracic musculature ranged from thin strands of differentiated muscle to a yellowish, jelly-like mass with no distinguishable strands of muscle. The amount of differentiated muscle present was easily recorded as none, small, moderate, or normal.

In the two experiments recorded in Figure 4, it is clear that as oxygen exposure increased from 12 to 16 hours, the decrease in number of adults able to emerge was

paralleled by a decrease in the number of adults that had normal muscles. As previously mentioned, the percentage of animals that completed external adult development after 12, 14, and 16 hours of oxygen was the same (Table III) whereas the percentage of animals that emerged decreased steadily between 12 and 16 hours of oxygen exposure (Fig. 4). After 12 hours of exposure, all of the wasps developed at least a few strands of thoracic muscles. After 16 hours of exposure, however, only 55 per cent of the wasps had any detectable strands (of muscle), and the remainder had only a jelly-like mass in place of the thoracic muscles. Thus, with longer exposures, not only did fewer wasps develop normal thoracic muscles, but the amount of muscle development occurring in animals that failed to emerge also decreased. In our opinion, inability of the oxygen-treated "pink stage" wasps to emerge can be adequately accounted for by the observation that oxygen impaired the development of adult muscles.

6. *The effects of oxygen on "black stage" developing adults*

In most experiments, 2 to 4 hours' exposure to 5 atms. of oxygen at 30° C. caused a detectable decline in the emergence of insects exposed during the "black stage." Several lines of evidence indicate that these wasps were not dead but paralyzed: (a) 24 to 48 hours after exposure of less than 8 hours duration, at the time when they normally emerged as adults, many of the animals moved their appendages weakly; (b) after exposures longer than 8 hours, no movement was observed, but the molting fluid was partially resorbed; (c) animals exposed as long as 8 hours retained a healthy, shiny, black appearance for at least five days, whereas animals killed by ether or alcohol fumes soon became dry, shriveled and dull in appearance. To show that the immobile wasps were still alive, the respiratory rate of several groups of animals was measured before, immediately after, and at intervals of 24 hours following exposure to five atms. of oxygen at 30° C. for various periods. All groups were the same age when oxygen uptake was measured. Since there was no way of knowing how many of the animals in each group were dead or dying as a result of treatment with oxygen, the data summarized in Figure 5 refer to the average oxygen uptake of a group and may not accurately mirror the respiratory behavior of any individual.

The oxygen uptake of the controls was recorded until the adults began to emerge. As seen in Figure 5, the oxygen consumption of the air controls rose sharply on the day of emergence. In the group exposed to oxygen for 4 hours, respiration dropped slightly immediately after treatment, but by the next day had risen to the level of the control. Although adults began to emerge during the next 24-hour period, fewer than 10 per cent completed emergence. Thus, although respiration returned to normal levels, the paralytic symptoms of oxygen poisoning remained. Immediately after 5 hours of exposure, oxygen consumption was only slightly less than after 4 hours of exposure. The initial effects of 8 hours of exposure were more dramatic, and respiration almost ceased. However, 24 hours after exposure to oxygen for 5 or 8 hours, respiration had noticeably increased. This increase in oxygen uptake continued until the second day after exposure. In the case of 5 hours of exposure to oxygen, respiration returned to the pre-treatment level, and yet none of the insects ever emerged. After the second day, respiration gradually declined, probably signifying that the insects

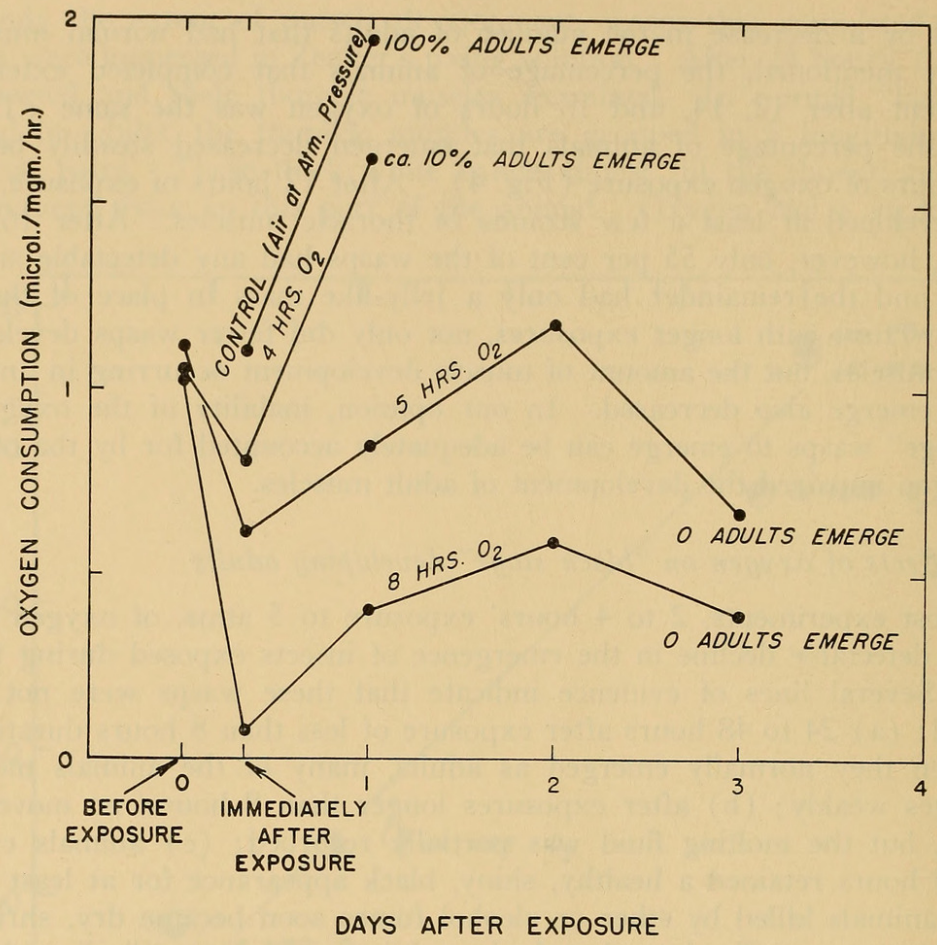

FIGURE 5. The effect of 5 atms. O_2 at 30° C. on the respiration of "black stage" wasps. Groups of 50 wasps were enclosed in perforated gelatin capsules and placed in 20-ml. Warburg vessels, the center wells of which contained 2% KOH. Oxygen uptake was measured immediately before and after exposure of wasps to 0, 4, 5, and 8 hours of O_2. Following this the respiration was measured daily until adults began emerging or until the gradual decline in respiration indicated that poisoned animals were dying.

were dying. Similar results were obtained in each of four separate experiments involving a total of 1100 "black stage" animals. That animals subjected to a short treatment regained a respiration comparable to controls but still failed to emerge, suggests that the system poisoned by threshold oxygen exposure accounts for only a small fraction of the total respiration of the insect.

7. Oxygen poisoning of adults

About 600 adult wasps of known ages were compressed with oxygen to various pressures at 30° C. Males and females alike exhibited successive stages of oxygen poisoning similar to those described by Williams and Beecher (1944) for *Drosophila:*

1. motor activity increased and then gradually declined;
2. walking ceased but a standing position was maintained;
3. the ability to right and to stand disappeared;
4. all spontaneous movements ceased;
5. reflex excitability disappeared.

Since it was difficult to determine the difference between "slight" movement and "no" movement, the time required for loss of the righting reflex was chosen as a convenient measure of oxygen poisoning. In a group of ten adults, the last insect to lose its righting reflex usually did so no more than 5 minutes after the first animal had lost its reflex. Hence it made no appreciable difference in the results whether we measured the exposure time which caused loss of righting reflex in 50 per cent or 100 per cent of the animals.

Figure 6 plots the time for loss of righting reflex as a function of oxygen pressure. In 10 atms. oxygen, adult *Mormoniella* lost their righting reflex after about one-half hour. Poisoning was 8 times faster at 10 atms. than at 3.3 atms. Even at 1.6 atms., the lowest oxygen pressure used in any experiment, poisoning occurred. The relation between pressure and duration of exposure for loss of

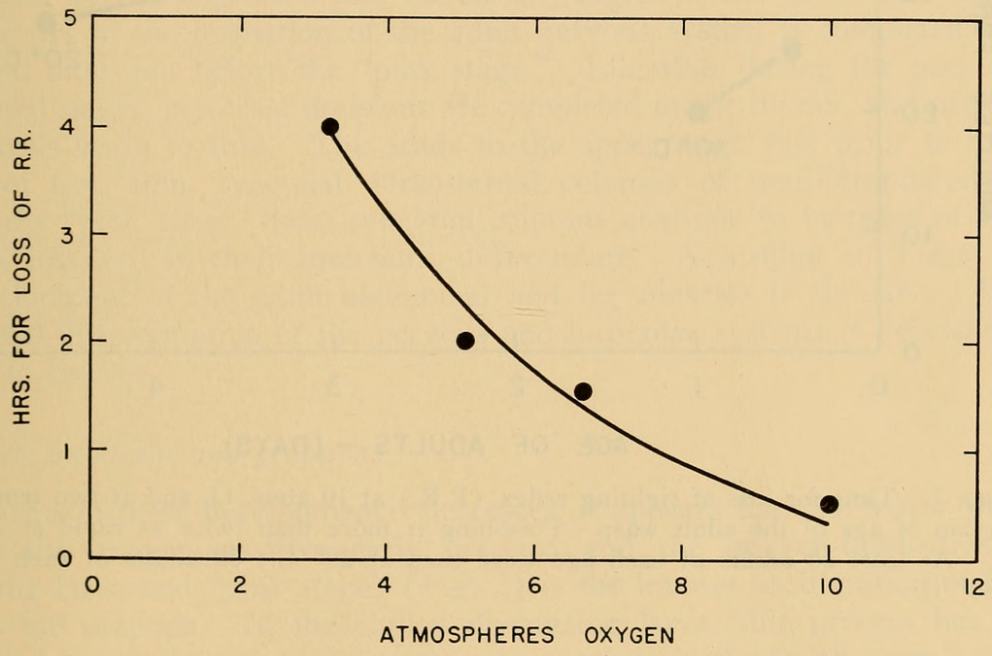

FIGURE 6. Time for loss of righting reflex (R.R.) in 100 per cent of 1- to 2-day-old adult wasps as a function of oxygen tension at 30° C. Twenty adults were exposed at each oxygen tension.

righting reflex appears to be logarithmic, similar to findings of Williams and Beecher (1944) on *Drosophila.*

Both temperature and age affected the sensitivity of adults to oxygen (Fig. 7). Thus the time required for poisoning decreased at higher temperatures; at 10 atms. and 20° C. adults lost their righting reflex in about 60 minutes, whereas at 10 atms. and 30° C. they lost their righting reflex in 20 to 30 minutes. Furthermore, old animals were more sensitive than young ones. Thus 0- to 1-day-old wasps lost their righting reflex in approximately 60 minutes, 1- to 2-day-old wasps in about 40 minutes, and 4- to 5-day-old wasps in about 30 minutes (*cf.* Williams and Beecher, 1944).

Adults removed from oxygen promptly after the loss of righting reflexes generally recovered completely within 24 hours. But animals kept in oxygen for

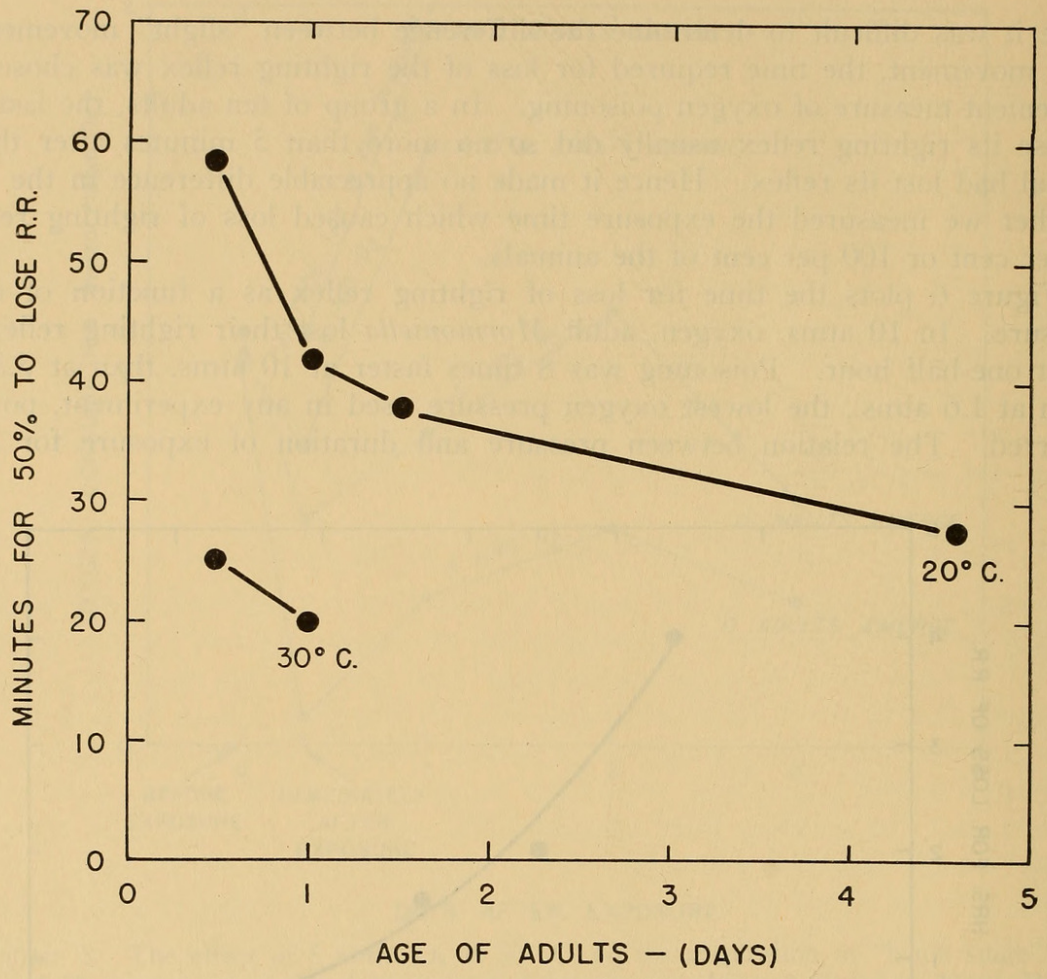

FIGURE 7. Time for loss of righting reflex (R.R.) at 10 atms. O_2 and at two temperatures as a function of age of the adult wasp. Poisoning is more than twice as rapid at 30° C. as at 20° C. At least 50 adults of each age were used at 20° C.; 30 adults of each age were used at 30° C.

as little as 10 minutes after the righting reflex was lost usually recovered only partially and failed to regain normal coordination and motor ability.

DISCUSSION

1. *The sites of oxygen poisoning in Mormoniella*

A. *Developmental processes involved*

The present experiments reveal that oxygen can have a variety of toxic effects on *Mormoniella:* it can prevent pupation, it can prevent imaginal muscle development, it can paralyze adults. These different effects on various stages of the life cycle can be understood best by considering the processes occurring at each developmental stage which are likely to be affected by oxygen. We know from ligature experiments on chilled diapausing larvae that at this stage the neurosecretory cells of the insect's brain have not secreted brain hormone and initiated the events leading to pupal development (Malamud, 1958). Thus, for the first few hours after their return to room temperatures, chilled larvae are engaged in

minimal developmental activity, a quiescence which sharply contrasts with the larval-pupal transformation occurring 24 hours later. During the prepupal period the cells of the integumentary imaginal discs divide and spread rapidly, and the discs of the legs, wings and antennae grow out of their sacs. This activity is followed by defecation, whereupon the appendages evert, and a distinct head, thorax and abdomen become visible. Shortly thereafter the last larval cuticle is shed and the antennae, legs and mouth parts promptly attain their full length and adult aspect. During adult development itself the epidermis produces a new cuticle, with ridges, grooves and bristles, which gradually becomes pigmented.

It is noteworthy that as far as external features are concerned, the most marked transformation occurs in the prepupa, not in the developing adult. Furthermore, the prepupal and very early pupal stages are the periods of greatest development of the muscular and nervous systems and of the proliferation of the imaginal discs. Thus, most of the migration and fusion of ganglia in the nerve cord and brain which occurs in the formation of the adult nervous system is completed after the last larval molt and before the "pink stage." Likewise, during the prepupal and early pupal stages, myoblast divisions are completed in the thorax, and presumptive muscle cells begin to fuse. This leads to the appearance, just prior to the "pink stage," of five, thin, syncytial dorsosternal columns of undifferentiated muscle. During the "pink stage" these syncytial columns continue to increase in size, and the cells involved in their insertions differentiate. According to Tiegs (1922), the development of the adult abdominal and leg muscles is similar. Obviously, the normal differentiation of the nervous and muscular systems is necessary if the adult is to emerge.

B. *Effects on larvae and prepupae*

In terms of these developmental processes, it appears that the major difference between the oxygen-sensitive early prepupa and the oxygen-insensitive chilled diapausing larva and "pink stage" (Fig. 2) is the intense proliferation of imaginal discs in the prepupa. In the chilled diapausing larva, this process has not yet begun, and imaginal cells are comparatively inactive; in the "pink stage," proliferation has already been accomplished. Animals with actively dividing cells appear more sensitive to oxygen poisoning than animals in which the division of the imaginal cells has not begun or has just been completed.

C. *Effects on "pink stage" wasps*

The major morphogenetic processes during adult development that determine the ability of the adult to emerge are the differentiation of the muscular and nervous systems. Preventing the differentiation of either muscle or nerve or damaging these systems after differentiation would prevent emergence. The present experiments reveal that oxygen poisoning in the "pink stage" interrupts muscle differentiation and so prevents adult emergence.

The failure of muscle development in "pink stage" wasps exposed to oxygen could be the result of direct damage to the developing muscles. However, it is worth recalling that several investigators have reported that adult muscle development in Lepidoptera is prevented by removal of the pupal nervous system, al-

though development into an otherwise normal adult proceeds (Nüesch, 1952; Williams and Schneiderman, 1952). These observations confirm the original finding of Kopeć (1923) that deganglionation of thoraces of *Lymantria dispar* prepupae prevented the development of thoracic muscles. Kopeć also found that adult development of the integument, as well as the external adult appearance of wings and legs, was not affected by deganglionation. From these results, one might anticipate that if oxygen poisoning severely damaged the nervous system of the "pink stage" *Mormoniella,* muscle differentiation would cease. Whether the failure of muscle differentiation after oxygen poisoning is caused by a direct action on the muscle itself or by an indirect inhibition that disrupts the trophic role of the nervous system remains to be decided.

D. *Paralytic effects on "black stage" developing adults and adults*

During adult development the neuromuscular system appears to become more sensitive to oxygen poisoning than in the young "pink stage." Thus, when development was allowed to proceed beyond the "pink stage" before oxygen was applied, oxygen sensitivity progressively increased until the "black stage," where even brief exposures prevented emergence. It is of special interest that although these "black stage" wasps were unable to emerge, their respiration recovered and was comparable to the controls. The animals were apparently paralyzed and unable to escape from their pupal cuticles. The observation that this paralysis had no effect on the insect's respiration suggests that either (a) the system first affected by oxygen is responsible for only a small fraction of the total respiration of the animal, or, alternatively, that (b) the respiratory activity of the affected system was not severely damaged. (For interpretation of similar effects in vertebrates see Stadie and Haugaard, 1946.) After exposures to oxygen for 8 hours, the respiration of "black stage" wasps immediately declined, never to return to normal. This permanent decrease in oxygen uptake, evoked by prolonged exposure to oxygen, suggests that the systems of cellular respiration suffered irreparable damage. The inhibition by oxygen poisoning of the respiration of developing insects has also been reported by Clark and his colleagues (Clark and Papa, 1958; and Clark, Harwitz and Rubin, 1958). These workers examined the effects of one minute of exposure to two atmospheres of oxygen on the respiration of the braconid wasp *Habrobracon.* Not unexpectedly, this brief oxygen exposure had no effect on "pigmented *Habrobracon* pupae," which correspond to the "black stage" *Mormoniella.* However, in stages whose subsequent development was adversely affected by these brief oxygen exposures, respiration was immediately inhibited. Clark and his colleagues do not report a recovery of respiration such as we found.

The adult *Mormoniella* was paralyzed by oxygen in much the same way as the "black stage" wasp. The experiments revealed that increasing exposure to oxygen caused an increasing loss of motor activity and coordination. Here again, either the nervous or muscular systems might be the site of oxygen damage.

It seems more likely to us that oxygen acts on the nervous system rather than directly on the muscle. Such a view is consistent with the fact that in oxygen poisoning of vertebrates, when pulmonary damage is avoided, the nervous system is the system first affected and most sensitive to oxygen. Further support for this

opinion stems from the findings, already discussed, that oxygen poisoning of "pink stage" wasps has the same effect on muscle development as deganglionation.

2. Differences in the sensitivity of various organ systems during development

From the foregoing analysis it seems likely that the differences in sensitivity of chilled diapausing larvae, early prepupae, and "pink stage" developing adults (Fig. 2) reflect an increased sensitivity of dividing cells to oxygen. It also seems probable that the gradual increase in sensitivity to oxygen which begins during adult development and continues uninterrupted during adult life reflects an increasing sensitivity of the nervous system to oxygen poisoning. It is interesting to speculate that impulse conduction and transmission are the functions disrupted by threshold oxygen poisoning of "black stage" and adult wasps, while the trophic influence of nerve on muscle is the function impaired in early stages of adult development.

It is noteworthy that exposure to oxygen of "pink stage" wasps may inhibit muscle development, and yet have no effect on the further differentiation of the integument and appendages. This indicates that in the "pink stage" the integument is not as sensitive to oxygen poisoning as is muscle. However, it is important to emphasize that there are times in development when the epidermal differentiation is sensitive to oxygen poisoning. Thus, when early prepupae were exposed to oxygen, many animals failed to develop beyond defecation. This was also true of the less sensitive, chilled diapausing larvae. The larval cuticle was not shed, and the appendages, head, thorax, and abdomen of the adult insect failed to form. We can scarcely attribute the lack of development at this stage to myoblast or nerve cell damage. Failure to develop in the chilled larva and prepupa apparently reflects a failure to the epidermal imaginal discs to fulfill their role.

Differences in the oxygen sensitivity of the various developmental stages of insects have also been reported by Clark and his co-workers (Clark and Herr, 1954). In *Habrobracon* they report that emergence of older prepupae and white pupae was reduced by exposure to one atmosphere of oxygen for one hour at 30° C. This same exposure had no effect on younger larvae-in-cocoons and little or no effect on developing adults. Recently, Clark and Papa (1958) have made the remarkable discovery that exposure to two atmospheres of oxygen for *five seconds* arrested development of 100 per cent of white pupae of *Habrobracon!* From their results it appears that this stage is exceedingly sensitive to oxygen poisoning. In our experiments, higher pressures and much longer exposures (10 atms. for 40 minutes, Table II) were required to inhibit adult development and emergence of *Mormoniella* exposed as early prepupae. (In our experience, the early prepupa is the stage most sensitive to oxygen poisoning.) Although we have not studied the effects of oxygen on *Mormoniella* immediately after the last larval molt, the stage which corresponds to Clark's white pupae, it seems most likely that *Habrobracon* and *Mormoniella* differ considerably in their absolute sensitivity to oxygen poisoning.

In their studies of oxygen poisoning during adult development, Clark and his co-workers did not report a rise in sensitivity during adult development (as reflected in the failure of adults ultimately to emerge), as was found in the case of *Mormoniella*. This is not unexpected, since the exposures they employed were much lower than the ones used in the present experiments.

3. *A comparison of the sensitivity to oxygen poisoning and x-irradiation*

Gerschman *et al.* (1954) suggest that x-irradiation and oxygen poisoning have a common mode of action, both agents causing the formation within cells of large amounts of peroxide and free oxidizing radicals such as OH and OH_2. They support this view with the finding that exposure to high pressures of oxygen after x-irradiation acts synergistically with x-irradiation in decreasing the survival of mice, and that reagents which protect organisms from radiation damage also protect mice from lethal effects of oxygen. The argument gains further support from the fact that oxygen can mimic many of the effects of x-rays, *e.g.* chromosome breakage (Conger and Fairchild, 1952). During the development of *Mormoniella,* however, the sensitivity to x-irradiation (Kuten, 1955; Schneiderman *et al.,* 1956, 1957) does not parallel the sensitivity to oxygen. Although the effects of oxygen poisoning and x-irradiation on *Mormoniella* are strikingly similar during the late larval and pupal stages, during adult development sensitivity to oxygen gradually increases while sensitivity to x-irradiation steadily decreases. In "pink stage" wasps, 4000 r reduced adult emergence by half; in the "black stage" doses as high as 64,000 r failed to inhibit emergence at all (Schneiderman *et al.,* 1956). Similarly, Clark and Mitchell (1951) showed that resistance of *Habrobracon* to x-irradiation increases during adult development. Apparently during adult development systems involved in emergence of the adult wasp become increasingly resistant to x-irradiation while becoming more sensitive to oxygen poisoning. Thus, although x-irradiation and oxygen poisoning may have a similar mode of action on dividing and undifferentiated cells, oxygen has a specific effect on the adult which is not produced by x-irradiation. If oxygen poisoning, like x-irradiation, leads to the formation of free radicals, it is not clear why oxygen has such a toxic effect on adults, while x-irradiation does not.

The type of analysis employed in the present study permits us to define the systems which are the target of oxygen and to recognize that the sensitivity of a developing system can change during its differentiation. Thus although gaps exist in our understanding of the biochemistry of oxygen toxicity, oxygen offers itself as a useful tool to the experimental morphologist (*cf.* Nelsen, 1950; Malamed, 1958). Moreover, for the student of insect growth, the fact that oxygen poisoning appears to provoke many of the same developmental consequences as deganglionation suggests new approaches to investigations of the trophic function of nerve.

We wish to thank Professor Carroll M. Williams for his most helpful comments on the manuscript, and Dr. Timothy H. Goldsmith for his interest in discussing many aspects of this work and care in reading the manuscript. Dr. Williams kindly provided equipment and space for the experiments on muscle development in "pink stage" wasps.

Summary

1. Experiments were conducted to determine the effects of high pressures of oxygen on the development and behavior of an insect, the chalcid wasp, *Mormoniella vitripennis.*

2. More than 10,000 wasps in five different stages of post embryonic development were compressed with 5 and 10 atmospheres of oxygen, nitrogen, or helium. Rapid compression and decompression were detrimental to chilled diapausing larvae but not to other stages. Although the high pressures of helium or nitrogen

had no, or only slight, adverse effect, high pressures of oxygen were exceedingly toxic at certain stages of development.

3. The chilled diapausing larva was but slightly sensitive; about 12 hours at 5 atmospheres oxygen was required to reduce adult emergence by 50 per cent. Sensitivity reached a maximum during the early prepupal stage, when less than one hour of exposure to 5 atmospheres of oxygen reduced adult emergence by 50 per cent. Sensitivity decreased after pupation, and in the "pink stage," at the outset of adult development, about 14 hours of exposure were necessary to reduce adult emergence by 50 per cent. During the rest of adult development and throughout imaginal life, the sensitivity to oxygen again increased. The organ systems attacked by oxygen during these different stages were examined.

4. Exposing "pink stage" wasps to 5 atmospheres of oxygen for about 16 hours stopped development in the "black stage" just prior to adult emergence. Dissection of these "black stage" wasps revealed that they failed to emerge because oxygen poisoning had prevented their thoracic muscles from differentiating. The possibility that this block to muscle development resulted either from direct injury to the muscle cells or from damage to the trophic function of the nervous system was discussed, and an action on the nervous system was favored.

5. Exposure of "black stage" wasps just prior to eclosion caused an immediate and abrupt decline in respiration. Even when respiration returned to control levels within a day, the wasps remained paralyzed within their pupal cuticles and never emerged.

6. The first visible sign of oxygen poisoning in adults was loss of motor activity and coordination. After brief exposures, wasps often recovered completely, but longer exposures evoked permanent paralysis.

7. It was suggested that in the early stages of adult development from the "pink stage" onward, the trophic influence of nerve on muscle is disrupted by oxygen; and that just prior to emergence, and in the adult, impulse conduction and transmission are prevented by oxygen. The gradual increase in oxygen sensitivity during adult development and imaginal life appears to reflect an increasing sensitivity of the nervous system to oxygen poisoning.

8. In contrast to the oxygen-sensitivity of the nervous system, the epidermis of the developing adult was highly resistant to oxygen poisoning. However, prior to the initiation of adult development, epidermal differentiation was blocked by oxygen poisoning. The extreme sensitivity of the early prepupa compared with chilled diapausing larva or the "pink stage" wasp probably reflects the high sensitivity of dividing epidermal cells.

LITERATURE CITED

Bert, P., 1878. La Pression Barometrique, Recherches de Physiologie Experimentale.

Clark, A. M., and E. B. Herr, Jr., 1954. The sensitivity of developing *Habrobracon* to oxygen. *Biol. Bull.*, **107**: 329–334.

Clark, A. M., and C. J. Mitchell, 1951. Radiosensitivity of haploid and diploid *Habrobracon* during pupal development. *J. Exp. Zool.*, **117**: 489–498.

Clark, A. M., and M. J. Papa, 1958. Some effects of oxygen on the white pupae of *Habrobracon*. *Biol. Bull.*, **114**: 180–187.

Clark, A. M., G. A. Harwitz and M. A. Rubin, 1958. Sensitivity to oxygen during postembryonic development of the wasp *Habrobracon*. *Science*, **127**: 1289–1290.

Conger, A. D., and L. M. Fairchild, 1952. Breakage of chromosomes by oxygen. *Proc. Nat. Acad. Sci.*, **38**: 289–299.

FRANKEL, J., AND H. A. SCHNEIDERMAN, 1958. The effects of nitrogen, helium, argon and sulfur hexafluoride on the development of insects. *J. Cell. Comp. Physiol.*, **52**: 431–451.

GERSCHMAN, R. D., L. GILBERT, S. W. NYE, P. DWYER AND W. O. FENN, 1954. Oxygen poisoning and x-radiation: a mechanism in common. *Science,* **119**: 623–625.

GOLDSMITH, M. H. M. (née M. H. Martin), 1955. Studies on the mechanism of oxygen poisoning in insects and the effect of carbon dioxide on recovery. Thesis, Cornell University.

GOLDSMITH, M. H. M., AND H. A. SCHNEIDERMAN, 1956. Oxygen poisoning in an insect. *Anat. Rec.,* **125**: 560.

GOLDSMITH, M. H. M., AND H. A. SCHNEIDERMAN, 1958. Oxygen poisoning in an insect. *Proc. 10th Intern. Cong. Ent.,* **2**: 337.

HINTON, H. E., 1946. Concealed phases in the metamorphosis of insects. *Nature,* **157**: 552–553.

HINTON, H. E., 1948. On the origin and function of the pupal stage. *Trans. Roy. Ent. Soc. London,* **99**: 395–409.

HINTON, H. E., 1958. Concealed phases in the metamorphosis of insects. *Sci. Progress,* **46**: 260–275.

KOPEĆ, S., 1923. The influence of the nervous system on the development and regeneration of muscles and integument in insects. *J. Exp. Zool.,* **37**: 15–23.

KUTEN, J., 1955. Effect of x-irradiation upon the metamorphosis of the chalcid wasp, *Mormoniella vitripennis.* Unpublished thesis, Cornell University.

MALAMED, S., 1958. Gastrular blockage in frogs' eggs produced by oxygen poisoning. *Biol. Bull.,* **114**: 226–246.

MALAMUD, JEAN G., 1958. An analysis of the mechanism of diapause termination in *Mormoniella vitripennis* (Walker) by ligature and other techniques. Unpublished thesis, Cornell University.

NELSEN, O. E., 1950. Temperature and oxygen pressure inhibition of the early development of *Rana pipiens. Anat. Rec.,* **105**: 599.

NÜESCH, H., 1952. Über die Einfluss der Nerven auf die Muskelentwicklung bie *Telea polyphemus. Rev. Suisse Zool.,* **59**: 294–301.

SCHNEIDERMAN, H. A., 1957. Onset and termination of insect diapause. Physiological Triggers (Ed. T. H. Bullock). American Physiological Society. Pp. 46–59.

SCHNEIDERMAN, H. A., AND N. FEDER, 1954. A respirometer for metabolic studies at high gaseous pressures. *Biol. Bull.,* **106**: 220–237.

SCHNEIDERMAN, H. A., AND J. HORWITZ, 1958. The induction and termination of facultative diapause in the chalcid wasps *Mormoniella vitripennis* (Walker) and *Tritneptis klugii* (Ratzeburg). *J. Exp. Biol.,* **35**: 520–551.

SCHNEIDERMAN, H. A., M. KETCHEL AND C. M. WILLIAMS, 1953. The physiology of insect diapause. VI. Effects of temperature, oxygen tension and metabolic inhibitors on *in vitro* spermatogenesis in the *Cecropia* silkworm. *Biol. Bull.,* **105**: 188–189.

SCHNEIDERMAN, H. A., J. KUTEN AND J. HORWITZ, 1956. Effects of x-irradiation on the post-embryonic development of a chalcid wasp. *Anat. Rec.,* **125**: 625–626.

SCHNEIDERMAN, H. A., J. WEINSTEIN AND J. HORWITZ, 1957. Recovery of diapausing larvae of a chalcid wasp from x-irradiation. *Anat. Rec.,* **128**: 618.

SNODGRASS, R. E., 1935. The Principles of Insect Morphology. Pp. 64–68. McGraw-Hill Book Co., New York.

STADIE, W. C., AND N. HAUGAARD, 1946. Oxygen poisoning. X. The effect of oxygen on the oxygen consumption of the intact mouse. *J. Biol. Chem.,* **164**: 257–263.

TIEGS, O. W., 1922. Researches on the insect metamorphosis. Part I: On the structure and post-embryonic development of a chalcid wasp, *Nasonia. Trans. Roy. Soc. S. Aust.,* **46**: 319–527.

WILLIAMS, C. M., AND H. K. BEECHER, 1944. Sensitivity of *Drosophila* to poisoning by oxygen. *Amer. J. Physiol.,* **140**: 566–573.

WILLIAMS, C. M., AND H. A. SCHNEIDERMAN, 1952. The necessity of motor innervation for the development of insect muscles. *Anat. Rec.,* **113**: 77.

WHITING, P. W., 1955. A parasitic wasp and its host for genetics instruction and for biology courses. *Carolina Tips,* **18**: 13. (Published by Carolina Biological Supply Co., Elon College, N. C.)