THE RELATIONSHIP OF OXYGEN CONSUMPTION TO AGE AND WEIGHT DURING THE POST-EMBRYONIC GROWTH OF *LOCUSTA MIGRATORIA* L.

By KENNETH U. CLARKE
Department of Zoology, University of Nottingham

(*Received* 16 *June* 1956)

INTRODUCTION

The novel features of this work on the respiratory metabolism of the growing locust are (1) that successive measurements are made on individual locusts and (2) there is no period of enforced starvation preceding the measurements on oxygen consumption. The first point enables the changes of absolute values of weight and oxygen consumption with age to be considered as no allowance need be made for population variation, the measurements being linked together by being made on the same individual. For this reason, too, the relationship between weight and oxygen consumption can be considered without first of all having to express oxygen consumption as some function of weight (Weymouth *et. alii* 1944). The second point implies that the normal growth of the animal is uninterrupted by enforced starvation, hence the oxygen consumption of the growing animal rather than that approximating to a basal metabolism is being studied.

The disadvantages of these methods are that only a few individuals from each instar can be studied, hence statistics for population values are founded on small samples; also that the individuals studied may not be 'normal' locusts. Some discussion on this latter point will be given after the results have been presented.

THE MATERIAL AND THE ENVIRONMENTAL CONDITIONS FOR GROWTH

The insects for study are females of *Locusta migratoria migratorioides* (R. & F.). Populations of both sexes are supplied by the Anti-Locust Research Centre, London.

The stock and the experimental animals are kept in an insectarium at a temperature of 28° C. and a relative humidity of 70%. Abundant food is always available to them. The lights are controlled to give alternate periods of 12 hr. light and 12 hr. dark with an intensity of 17 foot-candles on the floor of the cages. The presence of these lights causes the temperature of the room to vary from 30° C. during the day to 28° C. at night. Measurements in the cages show that they closely follow the room in temperature, but the introduction of fresh food each morning causes the R.H. to rise to 80% and provides a region of high humidity (90%) into which the locusts can move.

THE SAMPLING OF THE STOCK POPULATION

The stock population is prepared for sampling by removing from it all locusts older than the penultimate instar required for study. The population is then examined at frequent intervals, and those insects that have moulted to the required instar are removed at each observation. The time of ecdysis must lie between the time the animal is removed and that of the previous observation. This interval is referred to as the initial ecdysial range, and its mid-point taken as the time of the initial ecdysis. The newly moulted locusts are sexed (Dirsh, 1950) and the females used in the experiments.

Each individual female is studied throughout the stadium and for one observation at the beginning of the subsequent instar. The period in which measurements are to be made is restricted to this duration for two reasons: (1) the handling of individuals at frequent intervals over long periods of time produces abnormalities in their growth; (2) the possibility of phase change (Faure, 1932) and its effects on respiratory metabolism (Butler & Innes, 1936) is minimized. The isolated females show no evidence of phase change at the commencement of the subsequent instar. Tests were carried out to see if weight and oxygen consumption are changed when the locusts are reared at different population densities. An analysis of four experiments in which weight and oxygen consumption were measured on individuals taken from different population densities, at intervals throughout the stadium and at the commencement of the next instar, failed to show any significant difference in these qualities. It is therefore concluded that any effect of phase change on the growth of the locusts studied may be ignored. Population densities studied range from 1 to 20 per cage of 21 sq. cm. floor area and 450 c.c. volume.

THE DETERMINATION OF AGE DURING A STADIUM

The observations to be made on each individual are commenced as soon after the locust is removed from the stock as is possible. The age of the animal at this observation is taken as being equal to the difference in hours between the mid-point of the initial ecdysial range and the time the observation is made. Thereafter measurements are made at precisely known intervals of time, the series terminating with the one made just before the final ecdysis. From this set of measurements trend lines may be calculated to express the changes undergone during a stadium. Thus the time data on which these trend lines are based are precise, but the relationship between these data and the times of the initial and final ecdysis will be uncertain and the age of the animal at any one observation will not be accurately known. It will not be wrong by a value greater than half the initial ecdysial range. Similarly, the trend-line data can be related to the final ecdysis, the error being not greater than half the final ecdysial range.

Measurements are made at approximately 12-hourly intervals for the first five instars and at 24-hourly intervals for the adult. In every case the duration of the interval between one observation and the next is exactly known, and no approximation is made in the calculations of the trend lines based on them.

THE TECHNIQUE FOR THE MEASUREMENT OF OXYGEN CONSUMPTION

The oxygen consumption of the locust is measured by the direct method of carbon dioxide absorption with potassium hydroxide using the constant-volume type of respirometer of Warburg. The measurements are made at $28 \pm 0{\cdot}01^\circ$ C. The procedure is based upon the standard technique as described by Dixon (1943) with the following modifications introduced to meet the special needs of the material. Shaking of the manometer during the experiment is omitted, as trial measurements show that this omission does not alter the rate of carbon dioxide absorption by the caustic potash. The volume of the locust is large compared with the amount of air the flask can contain, so that the amount of air available to the animal (V_g of

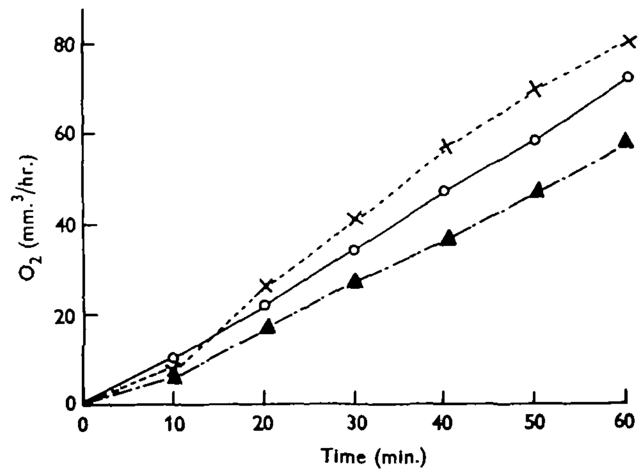

Fig. 1. The oxygen consumption for three individual locusts for a 60 min. period.

Dixon, 1943) must be measured afresh at each experiment. The volume of gas in the flask which is at atmospheric pressure can have its volume and pressure altered by changing the level of the manometer fluid. Since the capillary of the manometer limb is calibrated in the usual way, the amount of change, volume in mm.^{3} and pressure in mm.^{3} of Brodie's solution is known. By the application of Boyle's law the initial volume of air in the flask is calculated from these measurements. I am indebted to Mr A. Aitken for the use of this method. With the value for V_g known, the amount of oxygen used by each locust at each experiment in mm.^{3}/hour at N.T.P. is calculated from the formula given by Dixon (1943), except that his expression for the volume of fluid in the flask is ignored as being irrelevant in this case.

During its life the locust undergoes considerable changes in size which necessitates using flasks of different volume for the different sizes. Based on preliminary measurements flasks are chosen of such a size to ensure that the animal does not use up more than 5 % of the available oxygen during the 90 min. it is in the manometer.

On each occasion the manometer is read at 10 min. intervals for the period of 1 hr. These readings are plotted against time to check that the course of oxygen uptake over the hour is linear. Examples of these readings are given in Fig. 1. These show that constant rates of uptake occur, indicating that the technique used is satisfactory.

RESULTS

The results obtained consist of a series of observations on the changes in weight and oxygen consumption an individual locust undergoes during a stadium, and before and after its subsequent ecdysis. Six individuals have been studied for each of the first five instars and for the growing period of the adult stadium. An example

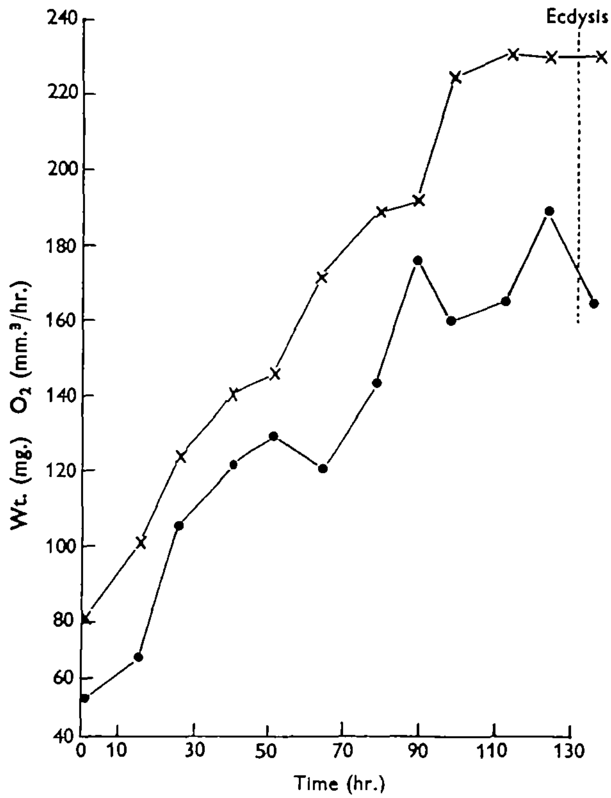

Fig. 2. The changes of weight and oxygen consumption of an individual during the third stadium. Zero time marks the estimated time of the initial ecdysis, and the word 'ecdysis' that of the final ecdysis. × = weight (mg.), ● = oxygen consumption (mm.³/hr.).

is illustrated in Fig. 2 for an individual of the third instar. This graph is quite typical of those obtained for other individuals, showing neither the greatest nor the least amount of oscillation observed.

Further examination of the data is concerned with the changes of weight and oxygen consumption with age, and with the relationship between changes of weight and changes of oxygen consumption. These two aspects are studied at three levels of time: (1) changes occurring in periods of less than 36 hr. duration, (2) changes occurring over the duration of a stadium, (3) changes occurring over the whole of the growing period of the locust's life. Each of these levels requires a different treatment of the results. The short-period changes are concerned with the values observed of weight in milligrams and oxygen consumption in mm.^{3}/hr. and are linked together by reason of their being made on the same individual.

When weight and oxygen consumption are plotted against time over the complete duration of a stadium straight lines can be fitted to the data. These straight lines can be represented by the general formula,

$$y = a + b(x - x'),$$

where y is the value of the quality (weight or oxygen consumption), x is the time, x' is the mid-point of the stadium in time, and a and b are constants which are calculated by the method of least squares for each quality and for each individual. In certain special cases to be discussed later two straight lines are necessary to get a reasonable fit of the trend line to the observed values. In these cases the changes in slope are fairly abrupt and division of data for the calculations is made from an examination of the graphs.

From these trend lines a curve showing the changes of weight and oxygen consumption that occur during the growing period of the locust's life is constructed.

(*a*) *The short-period changes*

In any one individual the pattern of the actual values for weight and oxygen consumption during a stadium shows the irregular manner in which these qualities increase (Fig. 3). A comparison of the pattern of oxygen consumption with that for weight shows that increments or decrements of the former are usually accompanied by similar changes in the latter (Fig. 3), though quite often the reverse is true.

In the presence of abundant food and under optimum environmental conditions the fluctuations of live weight are thought to be consequent upon the normal feeding behaviour of the locust, which consists of periods of eating separated by periods of 'resting'. These irregularities in weight increase are accepted as one of the uncontrollable variables in the growth of the locust and the limits of their magnitude compatible with normal growth must be determined. Certain changes in weight such as decrements due to defaecation or increments due to the taking of a mouthful of food occur so quickly that they might be regarded as instantaneous. For example, the act of defaecation takes less than 5 sec. and can bring about a loss of weight equal to 1·2% that of the animal. The other changes in weight take appreciably longer to occur and a time factor must be given along with the magnitude change. Observations under the conditions in which these locusts are reared show that periods of fasting of 12 hr. and of steady eating for 3 hr. occur sufficiently frequently to be considered as part of the normal behaviour of the animal.

The loss of weight due to a 12 hr. period of fasting is $9 \pm 3{\cdot}3$% of the initial weight of the locust. The percentage loss of weight is comparable for all stages and the figure given is the mean for seventy-two individuals, twelve from each instar. Similarly, the increase of weight due to 3 hr. eating is $15 \pm 6 \cdot 3\%$ of the initial weight of the locust. These values apply to locusts taken from stock which will be in a moderate nutritional state. If the animals have fasted for 12 hr. and are then placed with abundant food an increase of $26 \pm 4 \cdot 2\%$ of the animal's original weight in 60 min. occurs.

These observations suggest that losses of 12·3 % (mean + standard error) in 12 hr. and gains of 21·3 % in 3 hr. may be expected in a normally growing locust. The irregularities of weight increase of the animals studied here do not exceed these limits, and it is held that growth is normal regardless of whether its rate is fast or slow or the amount great or small.

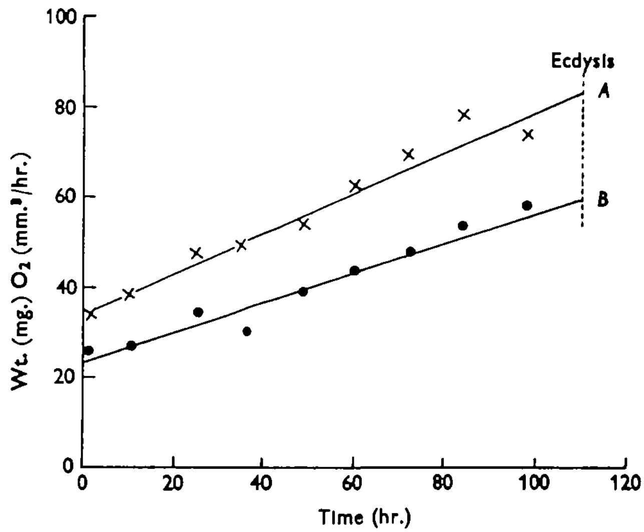

Fig. 3. *A*, the trend for weight and *B*, the trend for oxygen consumption throughout a stadium for an individual of the second instar. Formula for the trend line is $y = a + b(x - x')$. In this individual: for weight, $a = 53 \cdot 8$, $b = 0 \cdot 4554$; for oxygen consumption, $a = 39 \cdot 02$, $b = 0 \cdot 3325$; for both $x' = 47 \cdot 93$. Actual values at each observation, × = weight (mg.), ● = oxygen consumption (mm.^{3}/hr.).

The change in oxygen consumption for a unit change in weight due to fasting or feeding is determined. This change is found to be comparable in all stages and is $-2 \cdot 899 \pm 1 \cdot 01$ mm.^{3}/ hr. for each milligram of weight lost for a 12 hr. period (eighteen individuals measured, six from each of the second, fourth and adult instars). Similarly the change for a milligram of weight increase is $+1 \cdot 319 \pm 0 \cdot 72$ mm.^{3}/hr.

These figures are used to set limits to the range of oxygen consumption that can be regarded as normal for any given weight. Due to the irregularities of weight increase a sample of growing locusts of the same instar and of the same weight will include animals whose feeding histories, immediately prior to the sample being taken, are diverse. For example, the trend line values for weight and oxygen consumption suggest that a locust weighing 100 mg. will have an oxygen consumption of 100 mm.³/hr. But, if the weight is a 'low' due to the animal having fasted for the 12 hr. preceding the measurement, the oxygen consumption will fall below this level. If the weight has fallen from 109 to 100 mg. (approximately 9 %), then the oxygen consumption will also fall from 108 to 82 mm.³/hr., and may fall to 73 mm.³/hr. (mean value of the decrease in oxygen consumption plus standard error). If, on the other hand, the weight is a 'high' due to an animal weighing 85 mg. eating for some hours prior to the observation and thus raising its weight to 100 mg. (approximately 15 %), the oxygen consumption will increase from 85·5 to 105·3 mm.³/hr. and may rise to 115·9 mm.³/hr. The oxygen consumption of seventeen individuals whose weights lie between 96 and 104 mg. was measured

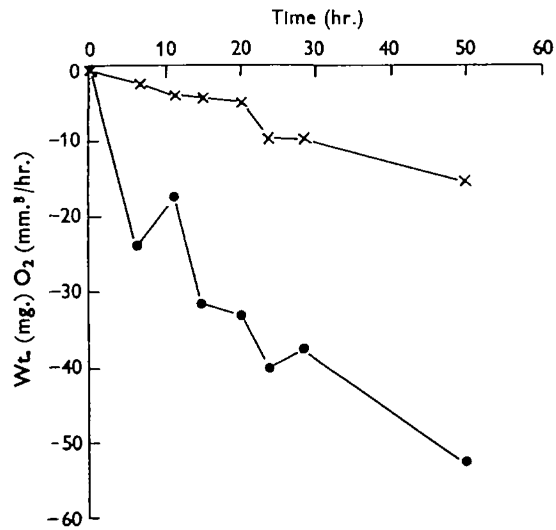

Fig. 4. Loss of weight and decrease in oxygen consumption in a starving locust of the second instar. Measurements made when starvation is commenced are taken as zero and the difference between this and subsequent measurements are plotted above. × = weight (mg.), ● = oxygen consumption (mm. ³/hr.) No allowance is made for the probable time lag between the commencement of starvation and the start of the fall of oxygen consumption.

and was found to range from 71·3 to 122·9 mm.³/hr., which agrees fairly well with the ranges calculated on the above assumptions. The range of oxygen consumption is not further limited by specifying the age of the animal as a sample characteristic since the feeding cycle is independent of age. This situation is also true for the individual which, having the same weight on successive occasions, may show an equally wide range in its oxygen consumption.

The oxygen consumption of the locust does not begin to fall off for at least 1½ hr. after the animal stops feeding, although there is often a decrease of weight due to defaecation during this time. If, however, the loss of weight continues there is a considerable drop in oxygen consumption after 5 hr. (Fig. 4). Similarly, the oxygen consumption does not increase the moment the locust begins to eat. This is most clearly shown in animals that have been fasting (Fig. 5). This shows the changes in oxygen consumption in an individual of the fourth instar following a meal given after 50 hr. starvation. Five other individuals of the fourth instar and six individuals of the second instar show an exactly similar response. From the figure it will be seen that oxygen consumption continues to fall during the 4 hr. period that the locust was feeding, but shows a considerable increase $5\frac{1}{2}$ hr. after the meal has commenced.

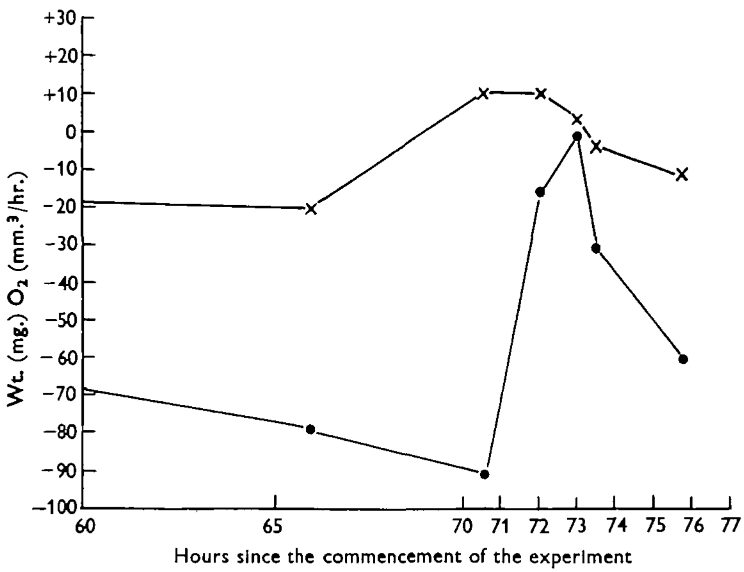

Fig. 5. The effect of a meal on oxygen consumption in a starving locust of the fourth instar. Time is in hours since the animal was isolated from its fellows; weight and oxygen consumption plotted as the differences between the first and subsequent observations. For further explanation see text. × = weight (mg.), ● = oxygen consumption (mm.³/hr.).

(b) Changes occurring during a stadium

The trend of weight is represented by a straight line which expresses it quite well, although this is not the case for data given for other species of insects by previous workers (Yagi, 1926; Calvert, 1929; Teissier, 1931). The differences are due to: (1) a loss of weight due to emptying the gut which occurs at other times during the stadium as well as just before the ecdysis; (2) moulting, which takes place in suitable environmental conditions very soon after the locust has emptied its gut which it can do in 9 hr. (Voskresenskaja, 1936).

In the adult the weight trend is represented by two straight lines, one for the growing period and one for the period of steady weight (Fig. 6). This abrupt change occupying only 2% of the animal's life is masked in population studies (Davenport, 1931) but is clearly shown in all individuals studied.

The trend for oxygen consumption during a stadium is well represented by a single straight line for instars 1–4 and for the adult, but for the fifth instar the matter is more complicated.

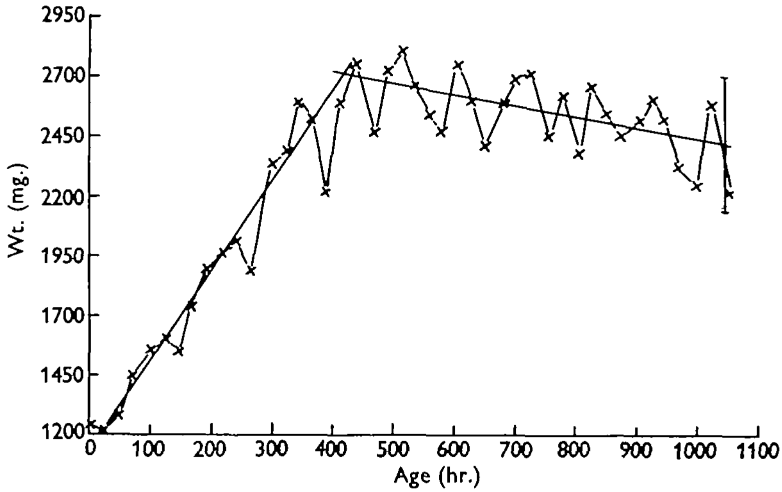

Fig. 6. Changes of weight in an individual during the adult stadium. Trend line values for the formula $y=a+b(x-x')$, for the growing period $a=1938$, $b=3\cdot955$, $x'=208\cdot5$.

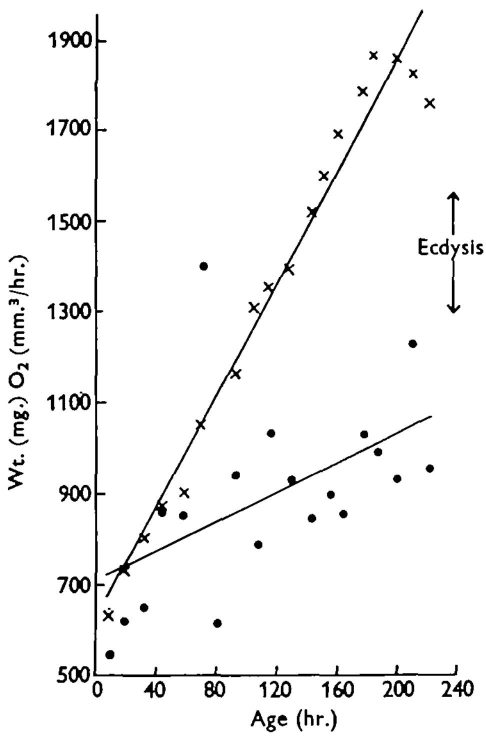

Fig. 7

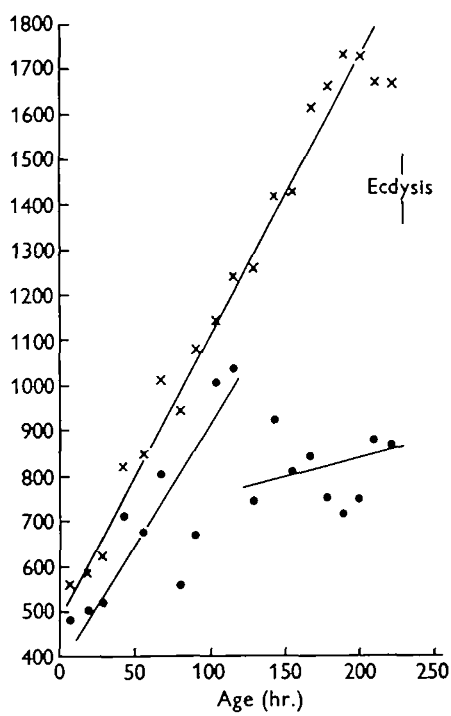

Fig. 8

Fig. 7. Change of weight and oxygen consumption during the fifth instar. Group A. × = weight (mg.), ● = oxygen consumption (mm^3/hr.). Values for the formula $y=a+b\ (x-x')$, for weight $a=1312\cdot6$, $b=6\cdot0804$; for oxygen consumption $a=894\cdot1$, $b=1\cdot6305$, for both $x'=151\cdot58$.

Fig. 8. Change of weight and oxygen consumption during the fifth instar. Group B. × = weight (mg.), ● = oxygen consumption ($mm.^3$/hr.). Values for the formula $y=a+b\ (x-x')$, for weight throughout the stadium $a=1170\cdot7$, $b=5\cdot5698$, $x'=111\cdot50$; for oxygen consumption Part 1, $a=711\cdot8$, $b=5\cdot7148$, $x'=61\cdot58$; Part 2, $a=829\cdot7$, $b=1\cdot2551$, $x'=175\cdot55$.

Individuals of the fifth instar can be divided into two groups with regard to their trends in oxygen consumption, although in the weight trend both groups are similar. Group A contains two individuals in which the trend is adequately represented by a single straight line (Fig. 7). Group B has four members in which the slope of the line changes after about half the stadium has elapsed so that two straight lines are required to fit the observations (Fig. 8). This second group shows that a change in the trend of oxygen consumption can occur whilst the weight of the animal is steadily increasing and at a time not coinciding with an ecdysis.

The co-relationship between trend lines for weight and oxygen consumption is estimated graphically (Tippet, 1952). In the first instar there is clearly a high degree of co-relationship between the trend lines This gradually lessens in successive instars until in the growing period of the adult stadium it is zero. It is important to realize that this only applies to series of measurements made on the same individual.

(c) *The changes occurring during the growing period of the locust's life*

In the previous sections the basis for linking measurements together has been that they were made on the same individual. This link is absent here since different individuals are used in successive instars. The trend lines of individuals from successive instars are linked together to form a life curve of weight and oxygen consumption on the basis that their values for '*a*' for weight and oxygen consumption fall on a straight line on a log-log plot. This follows the observation by Butler & Innes (1936) and by Zeuthen (1947) that the oxygen consumption is proportional to a power of the body weight. The curve (Fig. 9) is constructed by taking the values of '*b*' and the duration of each stadium of these selected individuals and applying them in the correct order to an animal whose weight on hatching is 12 mg. and whose oxygen consumption is 15 mm.^{3}/hr. The curve shows clearly the close correlation between weight and oxygen consumption in the young animal, its absence in the older locust, and its reappearance in adult life in the steady period when the trends for both are horizontal.

DISCUSSION

The discussion will be confined to the relationship between the changes in weight and oxygen consumption which occur in periods of short duration. From the facts given above it is possible to understand the relationships between these two qualities in the intact organism. This can be aided by setting up models of two extremes, the first in which a locust feeds for short though not necessarily regular periods and the second where the meals are separated by long time intervals.

In the first model we start with a locust whose weight and oxygen consumption are at the mid-point of the fluctuations they can undergo and which has a moderate amount of food in its gut. If in this animal the intervals between the meals are short, less than the time taken to empty the crop, which according to Voskresenskaja (1936) is 2 hr., then the midgut can always be kept full by food passing to it from the crop. The digestion and absorption of food from the midgut may be expected to remain constant so that there will be no fluctuation in metabolism on this account. The oxygen consumption will not fluctuate, and when the trend line is removed will appear as a horizontal straight line. Thus there is a method by which the intermittent increase of weight can be smoothed so that its effect on oxygen consumption

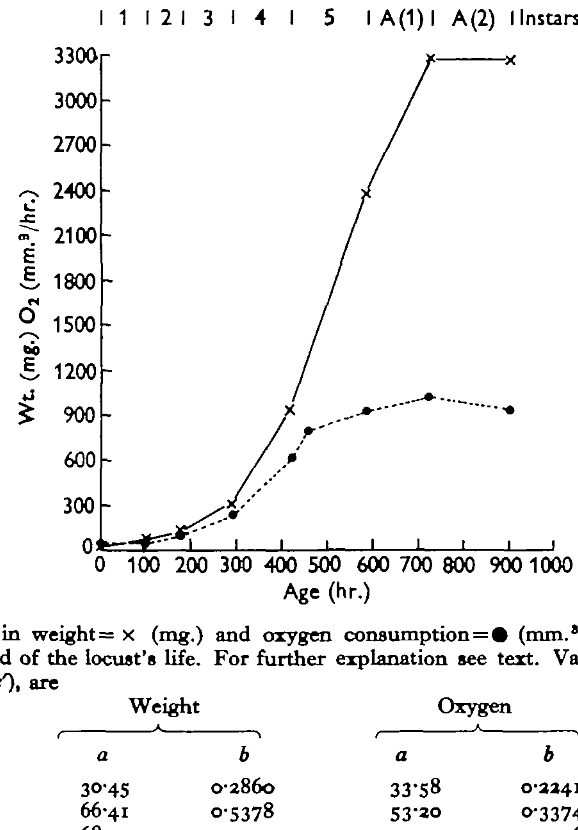

Fig. 9. Changes in weight = × (mg.) and oxygen consumption = ● (mm.³/hr.) throughout the growing period of the locust's life. For further explanation see text. Values for the formula $y = a + b(x - x')$, are

	Weight		Oxygen		
Instar	*a*	*b*	*a*	*b*	*x'*
1	30·45	0·2860	33·58	0·2241	58·35
2	66·41	0·5378	53·20	0·3374	53·45
3	168·27	1·3447	132·50	1·0326	63·36
4	441·5	2·4619	269·5	1·2177	83·30
5	1314·0	6·1977	—	—	109·16
5 Group A	—	—	690·5	4·3814	43·68
5 Group B	—	—	935·6	1·0702	151·58
A(1)	2036·5	5·9886	1134·3	0·8985	156·00
A(2)	2801·6	−0·0655	1070·8	−1·2430	358·45

is eliminated and the changes in the latter can be represented by a smooth curve. This will only be true for absolute values of oxygen consumption, since, if oxygen consumption is expressed as a function of weight, the intermittent increase in weight will affect the oxygen consumption curve. It is assumed here that the animal must feed at intervals of less than 2 hr., but, providing the meals are more frequent than the time taken for the food from one meal to be depleted to the state where oxygen consumption is affected, the actual time interval is probably not important.

In the second model an animal is considered whose gut is empty and whose oxygen consumption has sunk to the lowest level for a fasting period. When this animal feeds it will fill up its gut and crop, eating the largest meal which it is capable of holding. According to Voskresenskaja the crop and gut can be filled in less than 1 hr. As has been shown above, this meal will not cause an increase in oxygen consumption for some 4–5 hr., when oxygen consumption will rise sharply. As the food becomes used up the metabolism and oxygen consumption will fall again to their previous low level (unless the animal feeds), to be stimulated again by the arrival of another large meal. Voskresenskaja (1936) gives 9 hr. as the time taken for the locust to empty its gut. So unless the animal feeds at intervals shorter than this time both weight and oxygen consumption can be expected to show their maximum fluctuations.

The first of these models shows that a smooth oxygen consumption curve for a growing animal is theoretically possible and can be obtained providing the animal eats little and often.

SUMMARY

The weight and oxygen consumption of individual locusts, six from each instar, have been measured at 12-hourly intervals throughout each stadium.

An analysis of short duration changes shows that fluctuations in live weight are unavoidable in the growing animal, and that they are accompanied by fluctuations in oxygen consumption. In time the latter lag behind the former.

As the fluctuations of these two variables are out of phase, there is a considerable range of oxygen consumption which must be regarded as normal for any given weight. Calculations are made to find the limits of these fluctuations compatible with normal growth.

The changes that occur during a stadium can be represented by a straight trend line for both weight and oxygen consumption. Exceptions occur in the case of weight of the adult where two straight lines are necessary, one for the growing phase and one for the steady phase of this instar. In certain cases in the fifth instar two straight lines are necessary to express the trend in oxygen consumption which may show an abrupt change in the middle of the stadium.

A high degree of correlation is found between the trend lines for weight and oxygen consumption in the early instars. A low degree is found in latter instars where the weight increases and the oxygen consumption remains nearly constant.

A curve representing the changes of weight and oxygen consumption that occur during the growth of the locust has been constructed.

REFERENCES

BUTLER, G. B. & INNES, J. M. (1936). A comparison of the rate of metabolic activity in the solitary and migratory phases of *Locusta migratoria*. *Proc. Roy. Soc.* B, **119**, 296–304.

CALVERT, P. P. (1929). The different rates of growth among animals with especial reference to Odonata. *Proc. Amer. Phil. Soc.* **68**, 227–74.

DAVENPORT, C. B. (1931). Individual *v.* mass studies in child growth. *Proc. Amer. Phil. Soc.* **70**, 381–9.

DIRSH, V. M. (1950). A practical table for the determination of sexes of nymphs of *Locusta migratoria migratorioides* (R. & F.). *Proc. R. Ent. Soc. Lond.* B, **19**, 136–8.

DIXON, M. (1943). *Manometric Methods.*

FAURE, J. C. (1932). The phases of locusts in South Africa. *Bull. Ent. Res.* **23**, 293–424.

TEISSIER, G. (1931). Recherches Morphologiques et Physiologiques sur la Croissance des Insectes. *Trav. Sta. biol. Roscoff*, **9**, 29–238.

TIPPETT, L. H. C. (1952). *The Methods of Statistics.*

VOSKRESENSKAJA, A. (1936). In Uvarov, B. P. (1947). Recent advances in acridology: anatomy and physiology of Acrididae. *Anti-Locust Bull.* no. 1.

WEYMOUTH, F. W. *et alii* (1944). Total and tissue respiration in relation to body weight, a comparison of the kelp crab with other crustaceans and with mammals. *Physiol. Zoöl.* **17**, 50–71.

YAGI, N. (1926). Analysis of the growth curves of insect larvae. *Mem. Coll. Agric. Kyoto*, no. 1.

ZEUTHEN, E. (1947). Body size and metabolic rate in the animal kingdom with special reference to marine microfauna. *C.R. Lab. Carlberg*, Ser. Chim, **26**, 17–161.