QUANTITATIVE CHARACTERS
OF
THE GROWTH AND DEVELOPMENT OF A
PAHROMETABOLOUS INSECT, DIXIPPUS
(CARAUSIUS) MOROSUS BR, ET REDT,

BY

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1. THE LOSS OF WATER IN RELATION TO ECDYSIS

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× The work reported in this paper was carried out at the Department of Zoology, The University of Edinburgh.

LOSS OF WATER IN RELATION TO ECDYSIS

INTRODUCTION

Water economy is a necessary complement of terrestrial life. The limits of terrestrial life are, in part, determined by the organism's ability to utilize and conserve the water of its environment. For small organisms of terrestrial habit, the large surface-to-mass ratio and consequent surface evaporation makes the problem of water conservation particularly acute. It is therefore remarkable that the insects constitute the most successful group of terrestrial animals; and it follows that their success is, in large part, due to their ability to utilize and conserve water in widely divergent environments.

Insects may lose water from the body surface and the alimentary canal. Adaptations for water conservation may therefore be sought at these places. Thus, the integument of most insects is waterproof (Wigglesworth, 1934) and water-loss from the body surface (in dry air) is related to respiration and the resultant opening and closing of the spiracle sphincters. The control exercised here is mechanical and any factor which tends to reduce respiration, such as prolonged starvation (Buxton,1930), will automatically reduce the water-loss

from the body surface and permit the insect to survive for long periods. Water-loss from the alimentary canal is reduced in a large number of insects by reabsorption of water from the contents of the rectum and by the excretion of uric acid which is insoluble and can be eliminated without water (Wigglesworth, 1932). Thus the meal-worm, which is capable of surviving long periods of desiccation (Buxton, 1930), excretes uric acid and releases the faeces as a dry powder, (Wigglesworth, 1932). Babcock (1912) has indicated that grain weevils, clothes moths and other insects living on very dry food-stuffs may retain and use the water of metabolism. Buxton (1932) has summarized the literature on the water relations of terrestrial insects.

The time of moulting in insects constitutes a period in which the organism would appear to be particularly vulnerable to water-loss by evaporation from the body surface. If the moulting fluid were lost at ecdysis, and if the new cuticle remained permeable to water for some time, the insect would suffer severe loss of water. Wigglesworth (1936) alone, has considered the water-loss at this important period. He has shown, that for the imaginal moult of Rhodnius prolixus, the extra loss of water is small due to the almost complete reabsorption of the moulting fluid and the near impermeability to water of the new cuticle before the old cuticle is shed.

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At the cessation of feeding, prior to ecdysis, the insect loses its means of gaining water while evaporation and defaecation (until the gut is almost emptied) continue to eliminate it. Thus it seems that at this important stage of its development the animal must lose considerable amounts of water, to the detriment of its water balance. However, Teissier (1931) has shown that the act of moulting produces no change in the proportion of water in the meal worm. The meal worm may present a special case but it seems probably that terrestrial insects, exhibiting a consistent water economy, will have developed some means of maintaining their water balance at the moult.

Loss of water from the alimentary canal of insects has not been studied quantitatively. Wigglesworth (1932) has shown that it is to some extent controlled. Moreover, large differences in the amount of water lost from the alimentary canal are found in different groups of insects (Wigglesworth 1932) and it appears that changes may be induced in individuals (Wigglesworth 1933a)

The present study presents data for the loss of water by evaporation and with the faeces of Dixippus morosus during its development from the third instar to the adult.

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METHOD

Nymphs of Dixippus morosus were reared in individual cages from the third instar or fourth instar to adults at 23° C. Two sets of three individuals were used, one at relative humidity 40 per cent. over calcium chloride and the other in "saturated" * air over water. The insects were fed on fresh Tradescantia leaves.

Dixippus feeds heavily at night and very little during the day. Accordingly, the animals were taken from the leaves early in the morning, weighed, and placed in clean glass vessels without food or water. After nine hours they were again weighed and then placed on leaves so that they might feed again during the night. This process involves little interference with the normal life of the insect.

The faeces (if any) which were lost during the nine-hour period were carefully collected, dried to constant weight over sulphuric acid and weighed.

All weighings were made on a chainomatic balance accurate to 0.1 milligrams.

* Although a hair hygrometer registered 100 per cent humidity it seems unlikely that the air was really saturated since the glass tank in which the insects were kept was covered with a glass top raised ½ of an inch above the sides. The insects continued to lose some water by evaporation in this atmosphere. Mellanby (1932) has indicated the difficulty of obtaining true saturation.

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The original weight minus the weight at the end of nine hours gives the total loss; and subtracting from this the weight of the dry faeces, gives the loss due to evaporation and water lost with the faeces. Given a value for evaporation it is then possible to calculate the amount of water lost with the faeces.

This reasoning assumes that loss of weight is entirely due to evaporation and defaecation, and that the weights involved in the respiratory exchange may be overlooked. This assumption requires some examination.

Mellanby (1932), Gunn (1933), Koidsumi (1934), Buxton (1930) and Ramsay (1935) have shown from a wide variety of insect material that, provided the insect does not eat or produce faeces, loss of weight may be used as a satisfactory measure of loss of water by evaporation. This method was used by Wigglesworth and Gillett (1936) to estimate the amount of water lost from Rhodnius prolixus at ecdysis. Buxton (1930) has estimated the weight of carbon lost by respiration from starving meal-worms and shown it to be small enough to be neglected in most cases.

Gunn (1933) has discussed the factors involved in the change of weight of a starving insect. If the insect is allowed to take neither food nor water, the change in weight may be expressed as:

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$$W_1 + aO_2 = W_2 + bCO_2 + cH_2O + \text{faeces} \qquad (1.)$$

W_1 and W_2 are the weights of the animal before and after a certain period and a, b, and c are the weights of the respective gases inspired or expired during this period. Expressing the respiratory quotient (R.Q.) in terms of weight the following equation may be derived from (1).

$$W_1 - W_2 = aO_2 \left(\frac{11}{8} R.Q. - 1 \right) + cH_2O + \text{faeces} \qquad (2)$$

The expression $aO_2 \left(\frac{11}{8} R.Q. - 1\right)$ will represent the weight involved in the respiratory exchange. If the respiratory quotient is 0.73 or $\frac{8}{11}$, the value of this expression will be zero and respiration in itself will produce no change in weight. If the value of the respiratory quotient goes as high as 1.0, as it may in Blatta orientalis (Slater, 1927), then Gunn (1933) calculates that the upper limit of the change in weight due to respiration will be 10 per cent. of the total.

Dixippus morosus is a very sluggish insect particularly during the day, when it will remain for long periods quite immobile. It is reasonable to assume therefore that its metabolism is low. In support of this is the observation that even a first-instar nymph will live for more than two weeks at room temperature without food, provided the atmosphere is kept moist.

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Buddenbrock and Rohr (1923) have published data for the respiration of adult Dixippus. From their figures the writer has calculated the respiratory quotient and the actual loss in weight due to respiration. At 23° C. for a period of 9 hours (the temperature and time interval of the experiments reported below) the loss is 0.85 milligrams. Assuming the weight of an adult Dixippus to be 1 gram (a conservative estimate), the loss due to respiration during this period is 0.085 per cent. of the body weight. This is a negligible value. The present study is based therefore on equation (2) above, where a^{O_2} ($\frac{11}{8}$ R.Q. - 1) is negligible, and therefore:

$$W_1 - W_2 = aH_2O + \text{faeces} \quad (3)$$

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LOSS OF WATER FROM THE BODY SURFACE

Water is lost from the body surface of insects mainly by evaporation from the spiracles; but it may also be lost from the general body surface (Ramsay, 1935). Buddenbrock and Rohr (1923) have shown for Dixippus that appreciable amounts of water are lost in this way. We are not here concerned with the site so much as the quantity of water-loss.

If it were possible to weigh the faeces accurately immediately upon release, then the loss in weight due to evaporation could be easily calculated from (3) above, as:

$$W_1 - W_2 - \text{faeces} = cH_2O \quad (4).$$

However, due to the evaporation of water from the wet faeces and the minute weight of a single faecal pellet, this is impracticable. An alternative method is to find a period when no faeces are lost.

Two and sometimes three days before ecdysis the nymphs of Dixippus cease feeding. After one or occasionally two days of fasting the gut is almost emptied and defaecation stops until after the moult. During this period it is possible to get an accurate value for water lost by evaporation, since:

$$W_1 - W_2 = cH_2O. \quad (5).$$

This is the method used by Wigglesworth and Gillett (1936) to estimate the loss of water from Rhodnius prolixus at the last moult.

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Figures 1 and 2 show the absolute values for evaporation at the two humidities and the values relative to body weight. The weight of the exuvium has been subtracted from the loss at ecdysis, so that all values represent water loss only.

There is little or no increase in evaporation before the moult although the old cuticle has been largely digested at this time and must be very thin. This supports the view of Kühnelt (1928) that the waterproof character of the cuticle resides in the epicuticle and is in complete agreement with the work of Wigglesworth and Gillett (1936) who give values for five days preceeding the moult of Rhodnius.

At the time of actual ecdysis there is a sharp increase in evaporation amounting to about four times the "pre-moulting" value. This is true for every instar observed and for both humidities. Moulting occurs at night, and the next day evaporation has fallen to somewhere near the "pre-moulting" level. At the lower humidity, evaporation falls to, or even below, the "pre-moulting" level; at the higher humidity, evaporation falls to a point somewhat higher than the "pre-moulting" level on the day after ecdysis.

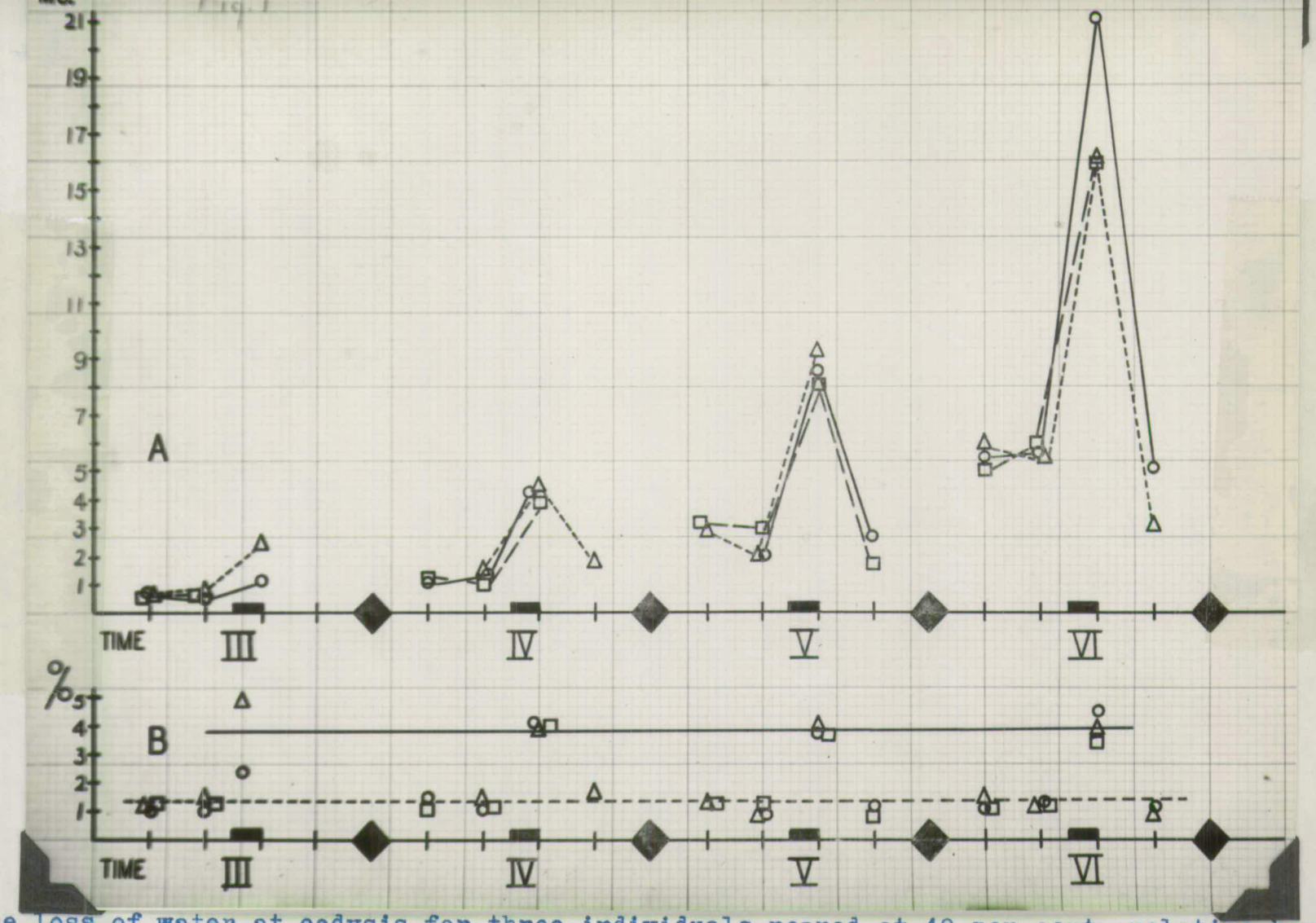

Fig. I - The loss of water at ecdysis for three individuals reared at 40 per cent. relative humidity.
A - The absolute loss. Ordinate: loss of weight in milligrams per nine hours. Abscissa: time-scale. Two intervals are equal to one day. The plotted points represent either the observed loss of weight during nine hours or the loss of weight in fifteen hours reduced to the equivalent value for nine hours. The solid rectangles on the abscissa mark the periods of actual moulting. The solid diamonds represent the periods between moults, and the roman numerals, the instars.
B - The loss relative to body weight. Ordinate: loss of weight expressed as a percentage of body weight. Abscissa: as above. The broken line links the values calculated for the normal loss of weight and the solid line does the same for the increased loss at ecdysis.

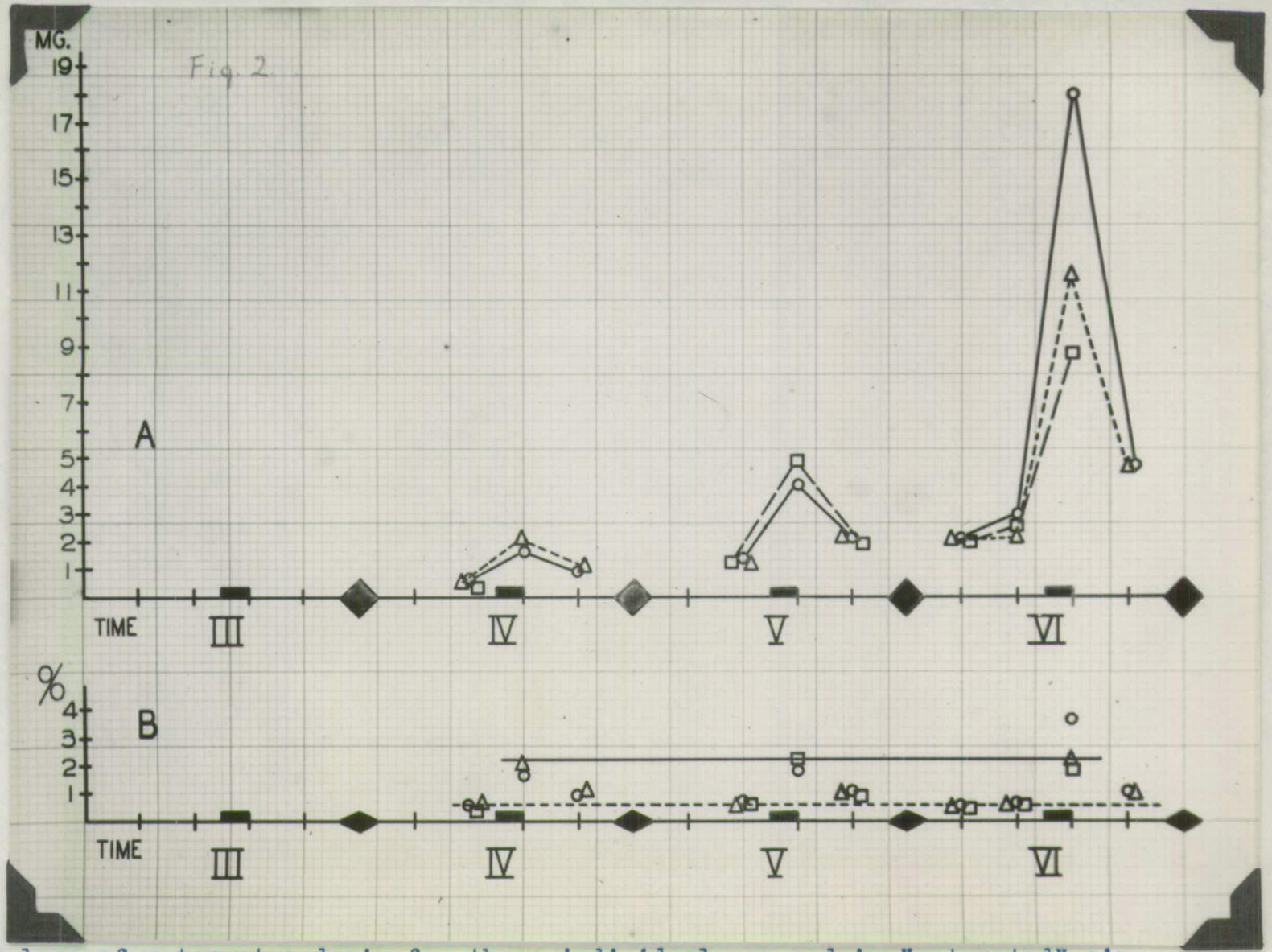

Fig. 2 - The loss of water at ecdysis for three individuals reared in "saturated" air.
A - The absolute loss.
B - The loss relative to body weight.

The ordinates and abscissae are the same as those of Fig. I.

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At the lower humidity evaporation in any given instar is about twice the evaporation at the higher humidity. This relationship holds for both the "pre-moulting" and the "moulting" levels of evaporation. The phenomena are therefore essentially parallel for both humidities.

From moult to moult the water loss at both levels of evaporation increases by 2; and from moult to moult Dixippus increases its weight by approximately 2. At either level therefore evaporation maintains a constant relation to body weight, the loss by evaporation increasing in direct proportion to the increase in mass. In Fig.'s 1B and 2B, the evaporation is expressed as a percentage of the body weight and the plotted points for the various instars fall on two straight lines corresponding to the "pre-moulting" and the higher "moulting" levels of evaporation. There is no increase of body weight at the time of ecdysis. The "moulting" level of evaporation is accomplished therefore by an increased rate of evaporation per unit of mass; but at either level, from moult to moult, evaporation is maintained in linear proportion to mass.

These results will be further considered in the Discussion.

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LOSS OF WATER FROM THE ALIMENTARY CANAL.

Water lost from the alimentary canal may be derived from the Malpighian tubules and the mid-intestine, both of which empty their products into the hind-intestine. Here, the rectal epithelium effects a partial or sometimes, total, absorption of water before the faeces are released. (Wigglesworth, 1932). The amount of water lost from the alimentary canal is, therefore, the amount of water present in the faeces, and this amount will be a function of the absorptive activity of the rectal epithelium. Thus, the rectal epithelium appears to be the seat of an important part of the insect's water-conservation mechanism. Is its activity constant or does it vary with changes in development and environment?

As indicated above, it is impracticable to measure directly the amount of water in the faeces immediately upon their release. An indirect method must therefore be used.

From (3) above,

$$W_1 - W_2 = cH_2O + \text{faeces},$$

where W_1 and W_2 are the weights of the insect before and after a period of 9 hours and cH_2O is the weight of water lost by evaporation. "Faeces" as written here is composed of dry material (Fd) and water (Fw), and we may write:

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$$W_1 - W_2 = cH_2O + Fd + Fw \quad (6).$$

The weights of the insects before and after a period of 9 hours (W_1 and W_2) and the dry weight of the faeces lost during this period (Fd), are known. From (6) then, the actual weight of water lost by evaporation and in the faeces, is:

$$cH_2O + Fw = W_1 - W_2 - Fd. \quad (7).$$

Now, $\frac{W_1 - W_2 - Fd}{W_1 - W_2} \times 100$, will express the percentage of the total loss lost as water.

Fig. 3A shows typical values of this ratio for the last four nymphal instars. It is apparent that most of the loss is due to loss of water. Moreover, the value of the ratio, in any instar, remains more or less constant until just prior to the moult when it is sharply depressed. This means that just before the moult a smaller proportion of the loss in weight is attributable to water, or, conversely, a larger proportion is due to dry material. The water is lost by evaporation and with the faeces. Consequently, one or both of these components must decrease prior to ecdysis to account for the decrease of the ratio at this time. But we have shown above that evaporation increases with increasing body weight and will therefore have a higher value towards the end of the instar than at the beginning.

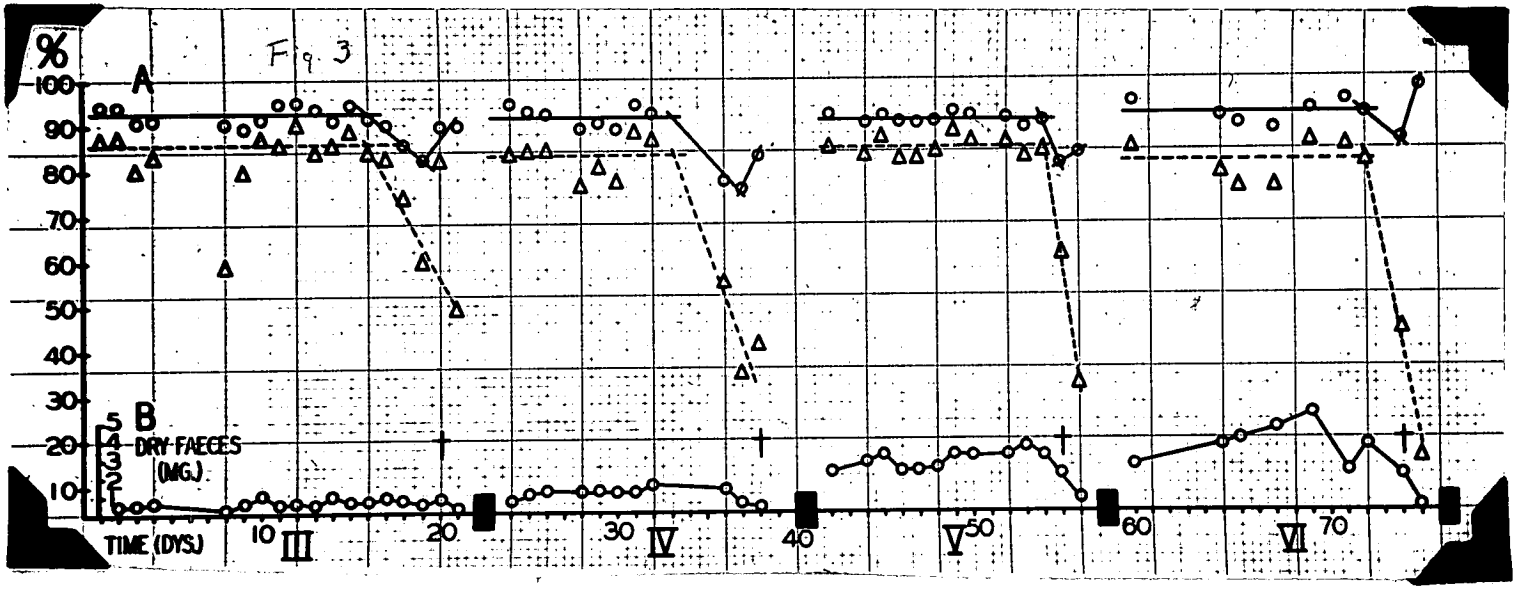

Fig. 3 - Data for a single individual showing typical values.
A - The circles and solid line indicate the values for the percentage of the total loss of weight (during nine hours) lost as water (water of evaporation plus water of faeces). The triangles and broken line indicate the values for the percentage of the total loss of weight lost as water with the faeces.
B - Ordinate: dry weight of faeces lost in nine hours. Ordinate marked in milligrams.

The abscissa is common to both graphs and shows the time in days. The solid rectangles mark the periods of ecdysis and the roman numerals denote the instars. The crosses indicate the cessation of feeding.

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The decrease of the ratio must therefore be due to a smaller amount of water in the faeces. Thus it appears that, prior to the moult, the dry component (dry weight of faeces) of the loss in weight is accompanied by a smaller proportion of water. The insect might be said to prepare for the process of moulting, with its resultant loss of water (Fig's. 1 and 2), by retaining some of the water that is normally lost with the faeces.

The depression of the value of the ratio, "percentage of the total loss lost as water", is in every case followed by a rise in this value. This occurs on the last day of defaecation when the amount of faeces is very small (Fig. 3B), and may be explained as follows. When no faeces are lost, the percentage of the total loss lost as water will be 100 per cent. since evaporation accounts for the entire loss. Similarly, when the amount of faeces is very small, most of the loss will be due to evaporation and the ratio, "percentage of the total loss lost as water" will have a high value.

If now, we exclude that part of the loss due to evaporation and express the ratio in terms of water lost only in the faeces, it should show a steadily decreasing value with no final upward tilt. From (7), the weight of water lost with the faeces will be:

$$Fw = W_1 - W_2 - Fd - cH_2O. \quad (8).$$

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A value for the weight of water lost by evaporation, (cH_2O), is available when defaecation ceases before ecdysis (Fig.'s 1 and 2). W_1. W_2 and Fd are known. Substituting these values in the above equation gives the weight of water lost with the faeces. The ratio, $\frac{W_1 - W_2 - Fd - cH_2O}{W_1 - W_2} \times 100$, will now express the percentage of the total loss lost as water in the faeces.

The values for this ratio are plotted on Fig. 3A (broken line and triangles). The points fluctuate rather widely between 80 per cent. and 90 per cent. until just before ecdysis when there is a sharp, uninterrupted decrease. The very low value on the last day of defaecation is to be expected since at this time a large part of the total loss is due to evaporation, and the small amount of faeces implies a small value for the percentage of the total loss lost as water in the faeces. However, the preceding low value of the ratio is accompanied by no significant change from the normal amount of faeces (Fig. 3B) and we must conclude that just before the moult the faeces are eliminated with less water than at any other time during the instar.

A further consideration of the amount of water in the faeces emphasizes this conclusion. It has been shown above (δ), that the weight of water lost with the faeces may be expressed as:

$$Fw = W_1 - W_2 - Fd - cH_2O$$

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From this, the total weight of the faeces (dry material plus water) will be:

$$Fd + Fw = W_1 - W_2 - cH_2O \quad (9)$$

The ratio, $\frac{W_1 - W_2 - Fd - cH_2O}{W_1 - W_2 - cH_2O} \times 100$ will then express the percentage of water in the faeces.

Fig. 4 presents in graphic form the values of this ratio for the six individuals used in this study. Just before the moult the percentage of water in the faeces is considerably less than at any other time during any instar. The cessation of feeding always occurs after the initial and usually after the final decrease of the ratio, "percentage of water in the faeces". The final decrease is often followed by a slight rise in the value of the ratio and this rise corresponds to the last day of defaecation. There is a tendency for those insects reared at the lower humidity to eliminate drier faeces just before the moult than those reared at the higher humidity.

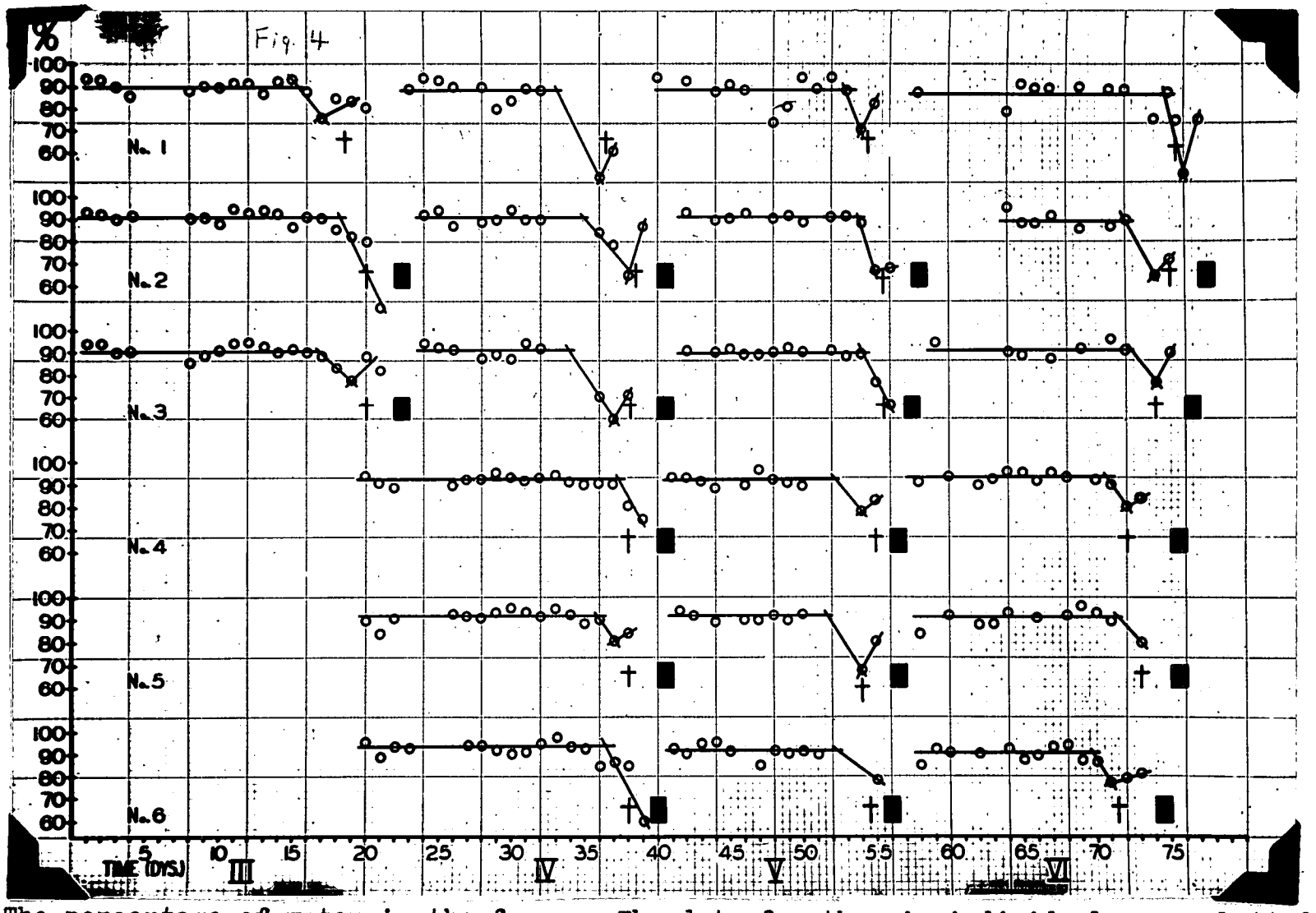

Fig. 4 - The percentage of water in the faeces. The data for the six individuals are plotted on six seperate ordinates, the ordinates showing the percentage of water in the faeces. The abscissa is common to all the data and shows the time in days. The roman numerals indicate the times at which feeding ceases.
Numbers I, 2 and 3 were reared at 40 per cent. relative humidity.
Numbers 4, 5 and 6 were reared in "saturated" air.

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DISCUSSION

Wigglesworth and Gillett (1936) have shown for Rhodnius prolixus that there is no increase in evaporation before the moult; but at the time of moulting evaporation is about doubled. Figures 1A and 2A of the present study indicate that for Dixippus morosus evaporation is increased roughly four times at ecdysis. This is true for all instars examined and at both the high and low humidities. Moreover, the "normal" (i.e. - pre-moulting) amount of water lost by evaporation is greater for Dixippus than for Rhodnius. At 0 per cent. relative humidity and 24° C. Rodnius loses about 1.5 per cent. of the body weight per diem, while at 40 per cent. humidity and 23° C. Dixippus loses about 3.1 per cent. per diem. Both the "normal" loss and the extra loss at moulting are therefore, at a higher level in Dixippus than in Rhodnius. It is suggested that this difference is related to the difference in the normal habit of these two insects, Rhodnius living in an arid environment while Dixippus lives and feeds on more or less lush vegetation.

As in Rhodnius, Dixippus shows no marked increase in evaporation before the moult, although the old cuticle must be very thin at this time. As pointed out by Wigglesworth and Gillett (1936), "That supports the view that

the waterproof properties of the insect cuticle reside in the outermost, non-chitinous, layer - the epicuticle (Kühnelt, 1928)"

Although Rhodnius loses less water than Dixippus during actual ecdysis, this loss remains above the "normal" level for about 5 days, while the evaporation from Dixippus apparently returns to "normal" within about 1 day. In fact, the loss from Rodnius on the day after ecdysis is very little less than the loss on the day of ecdysis, and decreases gradually until a constant level is reached on the fifth day. Feeding occurs after the eighth day. This whole period is greatly abbreviated in Dixippus. Ecdysis, with the associated high loss of water by evaporation, takes place at night. The following day evaporation has fallen to about the "normal" level, and feeding usually begins during the ensuing night. At the higher humidity (Fig. 2A) on the day after ecdysis, evaporation is sharply reduced from the "moulting" level to a value somewhat above the "normal". At the lower humidity (Fig. 1A) the evaporation on the day after ecdysis actually falls below this "normal". Thus it seems that although Dixippus loses more water than Rhodnius during actual ecdysis, this loss is effective for a much shorter period and the total loss associated with moulting will be about the same for both insects.

It follows from the above that, whatever property is

responsible for the reduction of evaporation after the moult, its action is more rapid in Dixippus than in Rhodnius. The literature contains two references pertinent to this property. Observing a gradual decrease in the evaporation from newly-moulted caterpillars, Koidsumi (1934) related this in part to the gradual thickening of the cuticle. From their observations on Rhodnius, Wigglesworth and Gillett (1936) conclude, - "But in view of the fact that the endocuticle is certainly permeable to water, we think it more probable that the progressive loss of permeability in Rhodnius is due to the hardening of the cuticulin in the outer layers of the cuticle. Our main conclusion, however, is that the impermeability of the cuticle is very nearly established before the old skin is shed, so that the extra loss of water associated with moulting is very small." If this is the case we must conclude that the impermeability of the epicuticle of Dixippus is established much more quickly than in the case of Rhodnius. The greater loss during actual ecdysis is thus offset to ensure for Dixippus a minimal loss of water at this critical period.

At the higher humidity (Fig. 2A) the rate of evaporation is greater after ecdysis than before it, while at the lower humidity (Fig. 1A) the rate of evaporation after ecdysis is about the same or slightly less than before. With the spiracles opened by exposure to dry air containing 10 per cent. CO_2 , adults of Rhodnius prolixus, seven days after

ecdysis, lost three times as much water as the preceding nymphs; in dry air alone the adults lost slightly less water seven days after ecdysis than before it. (Wigglesworth and Gillett, 1936). This parallel suggests that moist air and CO_2 have the same effect on evaporation. The effect of CO_2 is to open the spiracles and Mellanby (1932) attributes the same effect to moist air. Hazelhoff (quoted by Jordan, 1927) has demonstrated that the snail, Helix closes the pulmanary aperture most of the time in dry air but opens it in saturated air.

At ecdysis the evaporating surface of the tracheae and the spiracular apertures are enlarged with the increase in general size so that if the spiracles are kept more or less open by exposure to moist air, or CO_2, evaporation will be greater after the moult than before it. This is the explanation advanced by Wigglesworth and Gillett (1936) for Rhodnius in air containing 10 per cent CO_2 and it seems to fit the similar results obtained with Dixippus in moist air equally well. In Dixippus where the observations were made on the day after ecdysis an additional factor may be the permeability of the tracheal cuticle immediately after ecdysis. The ultimate reduction of this permeability with increasing age (Koidsumi, 1934; Wigglesworth and Gillett, 1936) will lead to a lower level of evaporation at the higher humidity than that observed on the day after ecdysis.

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Since their observations were confined to a single moult, Wigglesworth and Gillett (1936) were unable to show the linear relation between evaporation and body weight which has been demonstrated above (Fig's. 1B and 2B) Gunn (1935) presents data which shows that, for a difference of almost 600 mg. in weight, Periplaneta americana and Blatta orientalis exhibit a close proportionality between evaporation and body weight (in dry air at 20° C.). We have seen that, from the third moult (body weight 48 mg.) to the sixth moult (body weight 450 mg.). Dixippus shows a similar proportionality between evaporation and body weight at the time of ecdysis.

Observing that the very small cockroach Blatella germanica loses water at a higher rate than the two larger species, Gunn (1935) states, "This suggests that the water vapour evaporates through the surface of the animal, since if the shape remains constant the smaller the animal the larger the surface area per gram". However, on the basis that surface area is proportional to body weight $^{2/3}$. he calculates the water loss per square centimeter and shows that it is not constant but decreases from the largest to the smallest species. The same calculation gives a similar result for Dixippus, the water loss per square centimeter of body surface increasing from the third moult to the imaginal moult. x

In a developing insect there can be no constant relation between surface area and weight since the surface area

remains more or less the same throughout any instar while the weight increases. It seems possible however, that just before the moult the weight will be in equilibrium with the surface and the relation, Surface Area = K Body Weight $^{2/3}$. will be constant in any instar. An investigation of this point should be of interest.

The work of Ramsay (1935) has shown that the results of experiments on the evaporation from insects must be interpreted with caution. He has shown that the evaporation from Periplaneta americana takes place principally from the tracheal system; but considerable amounts of water are lost from the general body surface. The latter appears to be more complicated than the evaporation from the tracheal system which may be adequately explained on a physical basis. Koidsumi (1934) and Buddenbrock and Rhor (1922) have also shown that a part of the water loss of insects may take place through the general body surface. Koidsumi (1934) has further shown that the evaporation from the body surface of Milionia pupae does not conform with the physical law of evaporation from a simple water surface.

"Evaporation" as considered in this paper includes loss of water from both the general body surface and the tracheal system. It is not likely therefore that we will be able to draw any conclusions as to the site of the loss. However, the observed proportionality between evaporation and body weight at the time of moulting may be

of significance to the study of evaporation from insects.

Wigglesworth and Gillett (1936) have shown that, in dry air containing 10 per cent. CO_2, the spiracles of Rhodnius prolixus are opened and the evaporation is enormously increased. It is apparent then, that considerable quantities of water are lost from the insect when the spiracles are open; and Mellanby (1934) concludes that the greater part of the evaporation from insects takes place in this way. The spiracles are normally opened to supply the needs of the respiratory system (Hazelhoff 1927). Gunn (1933) gives evidence that, under the same humidity conditions and within certain temperature limits, evaporation will be proportional to the degree and extent of opening of the spiracles, and hence to the rate of respiration. * Gunn (1935) adds further weight to this conclusion by showing that in three species of cockroach evaporation is proportional to respiration, 6 mg. of water being lost for every 1 mg. of oxygen consumed.

In the case of Dixippus, evaporation is proportional to the mass of the insect at the time of the third, fourth, fifth and sixth moult (Fig. 1B and 2B). If then, evaporation is proportional to respiration and water is lost only from the

* A small proportion of the respiratory exchange may take place through the integument (Buddenbrock and Rohr, 1922; Fraenkel and Herford, 1938)

tracheal system, we must conclude that from the third instar to the imago respiration is proportional to mass at the time of ecdysis. This appears to be the case in Periplaneta orientalis where, from body weight 200 mg. to body weight 900 mg., respiration remains proportional to mass (Davis and Slater, 1926). However, the work of Bodine (1921) and Butler and Innes (1936) indicates that the respiration of grasshoppers is proportional to a fractional exponent of the body weight. Gunn (1935) shows that the respiration in three species of cockroach is proportional to body weight to the power of 0.75 - 0.8. It seems doubtful therefore, that the observed relation between evaporation and the mass of Dixippus is a simple function of the respiration. Insofar as evaporation takes place from the tracheal system it is probably proportional, at constant humidity, to respiration. In addition, some evaporation takes place from the body surface and this is complicated by an hydrophilous film and the possibility that water is supplied to the surface at a limited rate (Ramsay, 1935). There is also the possibility that the permeability of the cuticle changes in a developing insect. We must therefore conclude that evaporation from insects is a heterogeneous physcial system having two components, evaporation from the tracheae and evaporation from the general body surface. In Dixippus these two components are so combined that the total evaporation is proportional to the weight of the insect at ecdysis.

LOSS OF WATER IN RELATION TO ECDYSIS

The "moulting" level of evaporation from Dixippus offers further difficulties of interpretation. For if the increased loss of water at ecdysis is due to increased permeability of the cuticle, as suggested by Wigglesworth and Gillett (1936), it should be proportional to the surface area of the insect (body weight $^{2/3}$). Fig's. 1B and 2B however, indicate that the "moulting" level of evaporation exhibits the same linear proportionality to body weight as does the lower "pre-moulting" level. This suggests that during ecdysis water is lost in essentially the same manner as before, although at a higher rate. In other words, if the permeability of the cuticle is actually increased at this time, then the amount of water lost from the tracheaemust be proportionately increased; and, since all the available evidence indicates that the greater part of the water loss from insects takes place from the tracheaeand not through the cuticle, this will also be true at the time of ecdysis.

The insect undoubtedly performs considerable work in freeing itself from the old cuticle. The resultant increased metabolism, necessitating a higher rate of respiration, will produce a proportional increase in evaporation. Furthermore, if the new cuticle is more or less permeable to water immediately after ecdysis, then the cuticle of the tracheaewill be likewise permeable. *

* Wigglesworth (1938) has shown that the tracheaeof the mosquito, Aedes aegypti remain permeable to water for

a few minutes after moulting, during which time water is actually absorbed from them and is replaced by air via the respiratory siphon.

Under similar conditions of humidity therefore, the insect will have a much higher rate of evaporation at ecdysis due to increased respiration and permeability of the cuticle; but this evaporation will have the same relation to respiration and evaporation through the body surface as before ecdysis.

The second part of this paper deals with the loss of water from the alimentary canal, and from the results presented we were led to conclude that immediately prior to ecdysis the faeces were eliminated with less water than at any other time during the instar. In the absence of comparable work the discussion will be limited.

The main assumption involved in the calculations supporting this conclusion is that the evaporation is constant in any instar and the value available when no faeces are lost, preceding ecdysis, may be applied throughout the instar. In the preceding discussion it has been shown that evaporation in Dixippus is related to body weight at ecdysis. If this relation holds, not only at ecdysis, but during the instar, then evaporation will not be constant throughout the instar but will increase with increasing weight. However, in the absence of data for evaporation throughout the instar we have used the value available at

the end of each instar.

The part played by evaporation is, in any case, small. When no faeces are lost and the total loss in weight is due to evaporation, this loss represents, at the most, a little more than 1 per cent. of the body weight; but when defaecation occurs the loss of water may be as much as 20 per cent. of the body weight. Moreover, if the evaporation was large and increased with increasing weight there should be an increase in the percentage of the total loss lost as water instead of the observed decrease at the end of any instar, (Fig. 3). It appears therefore, that the faeces account for the greater part of the loss in weight and any decrease of the percentage of water in this loss will be due to less water in the faeces, since the only other component of the loss is evaporation, which is small and will tend to increase throughout the instar.

It is of interest to consider how a reduction of the percentage of water in the faeces might be accomplished. There is no indication that it is related to the cessation of feeding before ecdysis since feeding stops after the initial, and usually after the final, decrease of the percentage of water in the faeces. (Fig. 4) During the period when no food is taken (and hence, no water is gained)

the insect continues to lose water by evaporation and some by defaecation. The reduction in the amount of water lost with the faeces might, therefore, be regarded as an adaptation to offset the loss of water associated with ecdysis. Any undue loss of water at this time would be deleterious to moulting, since water is lost mainly from the blood (Mellanby, 1939) and the maintenance of blood volume is necessary for the mechanical operation of rupturing and escaping from the old cuticle. Mellanby (1939) has pointed out that in insects exposed to desication for some time the blood is reduced in volume; and since the blood volume has important mechanical functions to perform in hatching and moulting these processes are adversely effected. Thus, tsetse pupae, though completely developed, may be unable to escape from the puparium.

Wigglesworth (1932) has shown that the rectal epithelium or "rectal glands" absorb water from the faecal matter in the rectum before the faeces are released; and, as we have stated above, the amount of water lost with the faeces will, therefore, be a function of the absorptive activity of the rectal epithelium. It follows then, that any decrease of the amount of water in the faeces will be due to any increased absorption by the rectal epithelium. We therefore conclude that, just before the moult, the

rectal epithelium absorbs more water from the faeces than at any other time during the instar.

This conclusion suggests an interesting terrestrial parallel to a similar phenomenon reported by Maluf (1939) for certain marine crustaceans. Evidence from blood studies indicates that preceding the moult these crustaceans actively absorb water from their environment, and Maluf states, "The imbibition of water increases the blood pressure and thus aids ecdysis and the expansion of the new integument." The increased absorption of water by Dixippus may not be sufficient to increase the blood volume but it may well serve to protect it against undue loss by evaporation in the absence of feeding, and thus to aid ecdysis.

The increased absorption of water from the faeces is associated with moulting; and moulting is controlled by a hormone (Wigglesworth, 1934). We might consider then, that the increased absorptive activity of the rectal epithelium is due to an accessory action of the moulting hormone. The moulting hormone is no doubt responsible for the secretion of the moulting fluid and its ultimate reabsorption. Wigglesworth (1933b) has shown that 86 per cent. of the abdominal cuticle of Rhodnius prolixus is digested by the moulting fluid and reabsorbed through the general body surface. The cuticle of the proctodaeum will be digested and absorbed in a similar manner. The

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digestion of the lower layers of the proctodaeumal cuticle by the moulting fluid will increase it s osmotic pressure and tend to increase the absorption of free water from the contents of rectum. At the same time considerable osmotic forces must be exercised to effect the reabsorption of the moulting fluid and the products of its digestion. It is suggested that these forces, acting secondarily on the contents of the rectum, will effect an increased absorption of water and thus account for the observed decrease of the percentage of water in the faeces just before ecdysis. If further, the impermeability of the new cuticle is almost established before ecdysis, as suggested by Wigglesworth and Gillett (1936), then absorption will be reduced and this may account for the increase of the percentage of water in the faeces frequently observed on the last day that faeces are lost before ecdysis. (Fig. 4.)

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- SUMMARY -

1. Data are presented for the loss of water by evaporation and with the faeces of Dixippus morosus during its development from the third or fourth instar to the adult. Water lost by evaporation is measured directly during the period of ecdysis; water lost with the faeces is estimated by an indirect method.

2. The evaporation remains relatively constant before ecdysis; but during ecdysis it is increased roughly four times. On the day after moulting, evaporation decreases to about the "pre-moulting" level.

3. These results are compared with the results of a similar study by Wigglesworth and Gillett (1936) using the insect Rhodnius prolixus. The results are similar in form but different in detail.

4. The rate of loss of water is greater at the lower humidity but essentially parallel to the loss at the higher humidity. There is some evidence that the spiracles are opened more in moist air than in dry air.

5. From moult to moult, the normal evaporation, as measured preceeding ecdysis, is maintained in linear proportion to the body weight. This proportionality remains unexplained, but the - - -

respiration is indicated as the chief agent by which it is effected.

6. From moult to moult, the extra evaporation associated with actual ecdysis is also maintained in linear proportion to body weight. It is suggested therefore, that evaporation is accomplished at a higher rate but in the same way at ecdysis as before ecdysis. A plausible explanation, involving increased metabolism and increased permeability of the tracheal cuticula at ecdysis, is outlined.

7. When faeces are lost, the greater part of the loss in weight is due to water lost with the faeces.

8. In any instar and at both the high and low relative humidities, the percentage of water lost with the faeces remains practically constant until just before the moult when it is sharply decreased.

9. The decrease of the percentage of water in the faeces is ascribed to an increased absorption of water by the rectal epithelium; and this in turn may be related as an accessory action to the absorption of the moulting fluid and the products of its digestion before ecdysis.

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THE TABLES

The data on which this paper is based are given in tabular form below.

The meanings of the symbols used in these tables are:

W_I - The original weight of the insect.

W_2 - The weight of the insect after nine hours.

Fd - The dry weight of the faeces lost during nine hours.

cH_2O - The water lost by evaporation during nine hours, i.e. - the total loss in weight during nine hours when no faeces are lost. The value at the end of an instar is taken as the value throughout that instar.

X - The time of moulting.

Ex - The weight of the exuvium. (a part or all of the exuvium is often eaten.)

Underlined figures - The values, for fifteen hours during the night, reduced to the equivalent values for nine hours.

No. 5.

INSTAR IV

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
0	53.00	52.65	.35	0	.35		.35				
1	51.10	50.35	.75	.05	.70	.45		.40	88.8	93.3	46.7
2	62.60	59.60	3.00	.45	2.55	2.70		2.25	83.4	85.0	60.0
3	72.65	64.65	8.00	.75	7.25	7.70		6.95	90.2	90.6	77.5
4-6											
7	87.40	70.20	17.20	1.20	16.00	16.90		15.70	92.7	93.0	84.2
8	95.45	76.40	19.05	1.55	17.50	18.75		17.20	91.7	91.9	82.2
9	84.50	74.50	10.00	.85	9.15	9.70		8.85	91.2	91.5	80.0
10	95.45	79.50	15.95	1.05	14.90	15.65		14.60	93.2	93.4	85.0
11	96.85	74.75	22.10	1.00	21.10	21.80		20.80	95.4	95.5	89.6
12	106.00	89.50	16.50	1.10	15.40	16.20		15.10	93.2	93.3	84.8
13	108.50	93.35	15.15	1.20	13.95	14.85		13.65	91.9	92.1	82.2
14	97.45	85.15	12.30	.65	11.65	12.00		11.35	94.5	94.7	87.0
15	98.70	86.85	11.85	.90	10.95	11.55		10.65	92.2	92.4	82.3
16	115.45	102.60	12.85	1.60	11.25	12.55		10.95	87.3	87.5	72.7
17	108.70	95.65	13.05	1.25	11.80	12.75		11.50	90.0	90.4	78.7
18	105.80	101.20	4.60	.80	3.80	4.30		3.50	81.4	82.6	58.7
19	117.65	112.40	5.25	.80	4.45	4.95		4.15	83.7	84.7	63.8
20	108.35	107.85	.50	.02	.48	.18		.16	88.8	96.0	32.0
21	106.80	106.50	.30	0	.30		.30				
22	117.00*	112.55	4.45	.60	3.85						

No. 5

INSTAR V.

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
22	117.0	112.55	4.45	.60	3.85	3.20		2.60	81.2	86.5	58.4
23	172.35	129.20	43.15	2.35	40.80	41.90		39.55	94.3	94.5	91.7
24	173.20	146.00	27.20	2.10	25.10	25.95		23.85	91.9	92.2	87.8
25	187.10	165.80	21.30	2.30	19.00	20.05		17.75	88.6	89.2	83.3
26											
27	171.35	149.60	21.75	2.10	19.65	20.50		18.40	89.8	90.3	84.7
28	189.55	168.05	21.50	2.10	19.40	20.25		18.15	89.6	90.2	84.4
29	206.25	164.35	41.90	2.90	39.00	40.65		37.75	92.8	93.2	90.2
30	209.40	180.40	29.00	2.60	26.40	27.75		25.15	90.6	91.0	86.7
31	222.10	183.80	38.30	2.90	35.40	37.05		34.15	92.2	92.4	89.1
32-34											
35	240.45	236.00	4.45	1.05	3.40	3.20		2.15	67.2	76.4	48.3
36	230.35	228.40	1.95	.13	1.82	.70		.57	81.4	93.3	29.2
37	226.45	225.20	1.25	0	1.25		1.25				
				Ex.1.85			4.80				
38	215.35	213.40	1.95	0			1.95				

No. 1.

INSTAR III

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ / $\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of water in faeces (%)	Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
0	24.8	24.55	0.25	0	0.25		.25				
1	40.25	34.25	6.00	.35	5.65	5.30		4.95	93.4	94.1	82.5
2	38.40	32.80	5.60	.35	5.25	4.90		4.55	92.8	93.7	81.3
3	41.40	35.80	5.60	.50	5.10	4.90		4.40	89.7	91.1	78.6
4	37.45	34.65	2.80	.30	2.50	2.10		1.80	85.6	89.3	64.3
5-6-7 8	41.10	37;80	3.30	.30	3.00	2.60		2.30	88.5	90.9	69.6
9	42.40	39.60	2.80	.20	2.60	2.10		1.90	90.5	92.8	67.8
10	46.60	40.90	5.70	.50	5.20	5.00		4.50	90.0	91.2	78.9
11	44.30	39.60	4.70	.30	4.40	4.00		3.70	92.5	93.6	78.7
12.	43.60	39.05	4.55	.32	4.23	3.85		3.53	91.6	93.0	77.6
13	43.70	40.10	3.60	.40	3.20	2.90		2.50	86.2	88.8	69.4
14	52.10	43.25	8.85	.60	8.25	8.15		7.55	92.6	93.2	85.3
15	52.90	43.85	9.05	.55	8.50	8.35		7.80	93.4	93.9	86.2
16	52.40	46.55	5.85	.65	5.20	5.15		4.50	87.4	88.8	76.8
17	48.75	46.20	2.55	.45	2.10	1.85		1.40	75.7	82.3	54.8
18	55.90	52.15	3.75	.45	3.30	3.05		2.60	85.3	88.0	69.3
19	63.00	57.70	5.30	.75	4.55	4.60		3.85	8316	85.8	72.6
20	52.73	51.50	1.23	.10	1.13	.53		.43	81.1	95.9	35.0
21	50.75	50.05	.75 .70	0 0	.75 .70		.56 .70				
22	46.80	46.05	3.25 .75	Ex.0 .15	3.25 .60		2.44				

No. 2

INSTAR V

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by Evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
41	92.10	90.85	2.25	.15	2.10				?	93.3	?
42	177.35	138.00	39.35	2.40	36.95	36.45		34.05	93.4	93.9	86.7
43											
44	174.50	151.30	23.20	2.20	21.00	20.30		18.10	89.2	90.5	78.1
45	179.95	153.45	26.50	2.20	24.30	23.60		21.40	90.6	91.7	80.8
46	167.75	147.55	20.20	1.20	19.00	17.30		16.10	93.0	94.0	79.7
47	189.30	162.25	27.05			24.15					
48	178.75	153.80	24.95	1.85	23.10	22.05		20.20	91.5	92.6	81.0
49	201.00	162.10	38.90	2.90	36.00	36.00		33.10	91.9	92.6	85.2
50	191.50	164.50	27.00	2.70	24.30	24.10		21.40	88.8	90.1	79.3
51											
52	218.25	181.20	37.05	2.90	34.15	34.15		31.25	91.7	92.1	84.4
53	238.10	193.40	44.70	3.20	41.50	41.80		38.60	92.3	92.8	86.3
54	226.90	199.50	27.40	2.90	24.50	24.50		21.60	88.2	89.4	78.8
55	246.30	237.40	8.90	2.00	6.90	6.00		4.00	66.7	77.6	45.0
56	235.85	230.80	5.05	.70	4.35	2.15		1.45	67.4	86.2	28.6
56-57	230.80	225.90	4.90	0	4.90		2.94				
57	225.90	223.00	2.90	0	2.90		2.90				
57-58	223.00	209.40	13.60	0	13.60		8.16				
58	209.40	207.70	1.70	Ex.0	1.70		1.70				

No. 3

INSTAR V.

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt.of wet faeces (Mg.)	cH_2O Water lost by evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
41	95.90	93.95	1.95	.30	1.65					84.6	
42	169.90	142.70	27.20	2.15	25.05	25.20		23.05	91.5	92.1	84.8
43											
44	183.45	156.05	27.40	2.70	24.70	25.40		22.70	89.4	90.1	82.8
45	193.50	153.45	40.05	3.10	36.95	38.05		34.95	91.7	92.2	87.2
46	170.90	147.00	23.90	2.35	21.55	21.90		19.55	89.2	90.2	81.8
47	194.60	169.55	25.05	2.50	22.55	23.05		20.55	88.9	90.0	81.8
48	197.90	169.30	28.60	2.75	25.85	26.60		23.85	89.7	90.4	83.5
49	211.50	164.60	46.90	3.30	43.60	44.90		41.60	92.6	92.9	88.7
50	206.30	169.70	36.60	3.05	33.55	34.60		31.55	91.1	91.7	86.1
51											
52	227.60	190.00	37.60	3.25	34.35	35.60		32.35	90.8	91.3	86.0
53	229.25	196.70	32.55	3.65	28.90	30.55		26.90	88.2	89.0	82.7
54	239.30	207.90	31.40	3.10	28.30	29.40		26.30	89.4	90.2	83.8
55	258.40	248.40	10.00	1.90	8.10	8.00		6.10	76.2	81.0	61.0
56	239.40	235.50	3.90	.65	3.25	1.90		1.25	65.8	83.4	32.1
57	230.20	228.20	2.00	0			2.00				
58	212.60	210.00	15.60 2.60	Ex.50 0			8.50 2.60				

No. 3

INSTAR VI

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
58	212.60 ✱	210.00	2.60	0			2.60				
59	333.20	282.40	50.80	2.60	48.20	45.40		42.80	94.3	94.9	84.3
60-63											
64	353.40	311.00	42.40	3.70	38.70	37.00		33.30	90.0	91.3	78.6
65	402.10	364.00	38.10	3.95	34.15	32.70		28.75	87.9	89.6	75.4
66											
67	423.50	383.60	39.90	4.55	35.35	34.50		29.95	86.8	88.6	75.1
68											
69	486.00	409.00	77.00	5.40	71.60	71.60		66.20	92.4	93.0	86.0
70											
71	456.20	405.40	50.80	2.20	48.60	45.40		43.20	95.2	95.6	85.0
72	485.40	436.90	48.50	3.70	44.80	43.10		39.40	91.4	92.4	81.2
73											
74	512.00	499.40	12.60	1.70	10.90	7.20		5.50	76.4	86.5	43.7
75	482.90	476.40	6.50	0.10	6.40	1.10		1.00	90.8	98.4	15.4
76	466.20	460.70	5.50	0 0 Ex3.65			5.40 5.50 21.10				
77	423.90 ✱	418.90	5.00	0			5.00				

No. 3

INSTAR IV

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
23	45.10*	43.85	1.25	.20	1.05				?	84.0	?
24	65.00	54.70	10.30	.55	9.75	9.10		8.55	94.0	94.6	83.0
25	72.30	59.75	12.55	.90	11.65	11.35		10.45	92.1	92.8	83.3
26	77.50	63.15	14.35	1.10	13.25	13.15		12.05	91.6	92.3	84.0
27											
28	74.75	65.70	9.05	1.00	8.05	7.85		6.85	87.3	88.9	75.7
29	78.90	67.40	11.50	1.10	10.40	10.30		9.20	89.3	90.4	80.0
30	79.25	68.85	10.40	1.15	9.25	9.20		8.05	87.5	88.9	77.3
31	96.15	76.45	19.70	1.05	18.65	18.50		17.45	94.3	94.6	88.6
32	98.50	77.30	21.20	1.55	19.65	20.00		18.45	92.2	92.6	87.0
33-35											
36	102.40	96.90	5.50	1.25	4.25	4.30		3.05	70.0	77.3	54.8
37	104.80	101.90	2.90	.70	2.20	1.70		1.00	58.8	75.8	34.5
38	114.40	111.50	2.90	.50	2.40	1.70		1.20	70.7	82.7	41.4
39	107.95	106.75	1.20	0			1.20				
			1.55	0			1.20				
40	105.20	104.00	1.20	0	1.20		1.20				
			8.10	0			4.30				
41	95.90*	93.95	1.95	.30	1.65						

No. 4

INSTAR IV

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
0	50.40	49.65	.75	.15	.60	.15				80.0	
1	75.45	63.80	11.65	.70	10.95	11.20		10.50	93.7	94.0	84.2
2	66.55	62.40	4.15	.35	3.80	3.70		3.35	90.5	91.5	72.3
3	66.15	63.25	2.90	.30	2.60	2.45		2.15	87.7	89.6	63.8
4-6											
7	85.45	74.30	11.15	1.15	10.00	10.70		9.55	89.2	89.7	75.3
8	82.00	69.90	12.10	.90	11.20	11.65		10.75	92.2	92.5	81.4
9	96.00	84.65	11.35	.85	10.50	10.90		10.05	92.2	92.5	81.1
10	94.60	76.85	17.75	.75	17.00	17.30		16.55	95.5	95.7	89.0
11	97.35	78.35	19.00	1.22	17.78	18.55		17.33	93.5	93.6	84.8
12	84.45	75.90	8.55	.65	7.90	8.10		7.45	92.0	92.4	79.6
13	98.00	82.45	15.55	1.05	14.50	15.10		14.05	93.0	93.2	83.6
14	98.15	81.45	16.70	.95	15.75	16.25		15.30	94.1	94.3	85.9
15	109.70	93.20	16.50	1.45	15.05	16.05		14.60	90.9	91.3	79.7
16	109.20	93.60	15.60	1.60	14.00	15.15		13.55	89.4	89.7	76.6
17	97.85	87.15	10.70	1.00	9.70	10.25		9.25	90.2	90.6	77.1
18	119.10	103.10	16.00	1.60	14.40	15.55		13.95	89.6	90.0	77.2
19	122.50	114.70	7.80	1.35	6.45	7.35		6.00	81.6	82.7	59.6
20	110.60	108.80	1.80	.35	1.45	1.35		1.00	74.1	80.5	36.0
21	107.65	107.20	.45	0	.45		.45				
22	104.20 ≠	103.35	.85	Ex.0 0	.85		1.60 .85				

No. 4

INSTAR V.

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
22	104.20 ✗	103.35	.85	0			.85				
23	168.30	132.40	35.90	2.20	33.70	34.55		32.35	93.6	93.8	90.1
24	149.80	127.30	22.50	1.75	20.75	21.15		19.40	91.7	92.1	86.2
25	151.25	133.40	17.85	2.00	15.85	16.50		14.50	87.8	88.8	81.2
26											
27	189.65	159.50	30.15	3.35	26.80	28.80		25.45	88.4	89.0	84.6
28	183.30	148.00	35.30	1.15	34.15	33.95		32.80	96.6	96.7	92.9
29	216.60	174.65	41.95	2.90	39.05	40.60		37.70	92.8	93.2	89.9
30	183.90	156.30	27.60	2.30	25.30	26.25		23.95	91.1	91.7	86.7
31	183.80	161.20	22.60	2.40	20.20	21.25		18.85	88.6	89.3	83.3
32-34											
35	240.45	230.20	10.25	1.95	8.30	8.90		6.95	78.1	81.0	67.8
36	243.65	238.10	5.55	.70	4.85	4.20		3.50	83.3	87.4	63.1
37	233.90	232.55	1.35	.30 0	1.35		1.35				
38	225.75 ✗	223.60	2.15	Ex 0.1 0	2.15		4.02 2.15				

No. 4

INSTAR VI

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
38	225.75	223.60	2.15	0			2.15				
39	280.85	256.05	24.80	2.05	22.75	22.70		20.65	91.0	91.7	83.3
40											
41	417.25	317.70	99.55	6.40	93.15	97.45		91.05	93.3	93.5	91.4
42											
43	405.30	363.25	42.05	4.50	37.55	39.95		35.45	88.8	89.2	84.2
44	448.10	365.00	83.10	5.90	77.20	81.00		75.10	92.7	92.9	90.3
45	424.80	346.25	78.55	3.40	75.15	76.45		73.05	95.6	95.7	93.1
46	435.80	357.65	78.15	3.25	74.90	76.05		72.80	95.6	95.7	93.2
47	497.60	441.00	56.60	4.35	52.25	54.50		50.15	92.0	92.3	88.5
48	457.00	372.40	84.60	4.00	80.60	82.50		78.50	95.1	95.2	92.7
49	443.20	391.60	51.60	3.40	48.20	49.50		46.10	93.2	93.4	89.4
50											
51	479.00	417.30	61.70	4.70	57.00	59.60		54.90	92.0	92.3	88.8
52	479.80	445.70	34.10	3.40	30.70	32.00		28.60	89.4	90.0	83.9
53	571.20	552.70	18.50	3.00	15.50	16.40		13.40	81.7	83.7	72.4
54	524.00	516.30	7.70	.90	6.80	5.60		4.70	83.9	88.3	61.1
55	512.50	510.40	2.10	0			2.10				
56	506.10	503.20	2.90	0			2.42				
				Ex6.25			2.90				
57	460.60	456.00	4.60	0			18.10				
							4.60				

No. 2

INSTAR III

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
0	23.3	22.9	0.40	0	0.40		.40				
1	33.9	31.15	2.75	.15	2.60	2.15		2.00	93.0	94.5	72.7
2	40.0	33.5	6.50	.45	6.05	5.90		5.45	92.3	93.1	83.8
3	36.95	32.6	4.35	.40	3.95	3.75		3.35	89.3	90.8	77.0
4	35.05	29.3	5.75	.45	5.30	5.15		4.70	91.2	92.2	81.7
5-7											
8	41.3	34.9	6.40	.58	5.82	5.80		5.22	90.0	90.9	81.5
9	37.4	34.0	3.40	.25	3.15	2.80		2.55	91.1	92.6	75.0
10	42.1	36.35	5.75	.68	5.07	5.15		4.47	86.8	88.2	77.7
11	44.8	36.75	8.05	.35	7.70	7.45		7.10	95.3	95.6	88.2
12	43.15	35.55	7.60	.50	7.10	7.00		6.50	92.8	93.4	85.5
13	41.6	35.65	5.95	.30	5.65	5.35		5.05	94.4	94.9	84.8
14	46.75	39.45	7.30	.50	6.80	6.70		6.20	92.5	93.1	85.0
15	42.1	38.65	3.45	.40	3.05	2.85		2.45	86.0	88.4	71.0
16	49.9	41.5	8.40	.70	7.70	7.80		7.10	91.0	91.6	84.5
17	48.6	40.9	7.70	.70	7.00	7.10		6.40	90.1	90.9	83.1
18	46.95	43.0	3.95	.50	3.45	3.35		2.85	85.1	87.3	72.2
19	56.1	50.65	5.45	.85	4.60	4.85		4.00	82.4	84.4	73.4
20	53.2	50.05	3.15	.50	2.65	2.55		2.05	80.4	84.1	65.1
21	48.05	47.25	.80	.10	.70	0.20		.10	50.0	87.5	12,5
22	46.55	45.95	.70 .60	0 0	.70 .60		.57 .60				
23	44.65	42.90	1.30 1.75	Ex.10	1.20		1.10				

No. 1

INSTAR V

Dys	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by Evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
38	104.15 ≠	102.45	1.70	0			1.70				
39	181.65	148.05	33.60	1.90	31.70	30.80		28.90	93.9	94.3	86.1
40											
41	197.85	152.50	45.35	3.05	42.30	42.55		39.50	92.7	93.3	87.2
42											
43	181.40	163.10	18.30	1.95	16.35	15.50		13.55	87.2	89.3	74.1
44	207.20	174.80	32.40	2.65	29.75	29.60		26.95	91.0	91.7	83.2
45	185.40	166.80	18.60	1.85	16.75	15.80		13.95	88.3	90.1	75.0
46											
47	218.90	202.20	16.70	3.85	12.85	13.90		10.05	72.3	77.0	60.2
48											
49	215.40	188.80	26.60	4.30	22.30	23.80		19.50	81.9	83.8	73.2
50	241.80	190.50	51.30	2.95	48.35	48.50		45.55	94.0	94.2	88.9
51	227.10	205.30	21.80	2.15	19.65	19.00		16.85	88.7	90.1	77.3
52	217.20	197.90	19.30	1.05	18.25	16.50		15.45	93.6	94.5	80.0
53											
54	238.60	215.00	23.60	2.35	21.25	20.80		18.45	88.7	90.0	78.1
55	269.70	257.80	11.90	2.65	9.25	9.10		6.45	70.8	77.6	54.2
56	239.10	234.0	5.10	.40	4.70	2.30		1.90	82.6	92.1	37.2
57	230.00	228.0		0			2.80				
				Ex1.0			2.00				
	211.70 ≠	207.6					9.18				

No. 1

INSTAR IV

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
22	46.80	46.05	.75	.15	.60				?	80.00	?
23	74.90	64.20	10.70	1.00	9.70	9.25		8.25	89.2	90.6	77.1
24	88.90	66.80	22.10	1.25	20.85	20.65		19.40	93.3	94.3	87.8
25	86.95	67.90	19.05	1.25	17.80	17.60		16.35	92.9	93.4	85.8
26	79.70	68.05	11.65	1.05	10.60	10.20		9.15	89.6	91.0	78.5
27											
28	84.05	71.75	12.30	1.05	11.25	10.85		9.80	90.3	91.4	79.6
29	87.05	79.55	7.50	1.20	6.30	6.05		4.85	80.2	84.0	64.7
30	87.80	79.80	8.00	1.10	6.90	6.55		5.45	83.2	86.2	68.1
31	93.35	82.00	11.35	1.25	10.10	10.90		9.65	88.5	89.0	85.0
32	93.45	81.35	12.10	1.30	10.80	10.65		9.35	87.8	89.3	77.2
33-35											
36	110.30	107.60	2.70	.65	2.05	1.25		.60	48.0	75.9	22.2
37	118.00	115.65	2.35	.35	2.00	.90		.55	61.2	85.1	23.4
38	113.60	112.15	1.45	0	1.45		1.45				
			8.00	Ex.80			4.32				
39	104.15	102.45	1.70	0	1.70		1.70				

No. 2

INSTAR IV

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
24	65.30	55.05	10.25	.70	9.55	9.05		8.35	92.2	93.2	81.4
25	76.70	58.35	18.35	1.05	17.30	17.15		16.10	93.8	94.3	87.7
26	76.10	64.90	11.20	1.40	9.80	10.00		8.60	86.0	87.5	76.6
27											
28	75.00	65.95	9.05	.95	8.10	7.85		6.90	87.9	89.5	76.2
29	73.85	63.30	10.55	1.00	9.55	9.35		8.35	89.2	90.5	79.0
30	89.60	70.85	18.75	1.00	17.75	17.55		16.55	94.3	94.6	88.2
31	70.90	66.00	4.90	.40	4.50	3.70		3.30	89.2	91.8	67.3
32	89.30	74.55	14.75	1.45	13.30	13.55		12.10	89.3	90.2	82.1
33-35											
36	97.70	90.20	7.50	1.05	6.45	6.30		5.25	83.3	86.0	70.0
37	109.05	102.85	6.20	1.10	5.10	5.00		3.90	78.0	82.2	62.9
38	109.40	106.65	2.75	.55	2.20	1.55		1.00	64.5	80.0	36.4
38:9	106.65	104.10	2.55	.35	2.20					86.2	
39	104.10	102.75	1.35	.02	1.33	.15		.13	86.5	98.5	9.6
39:40	102.75	101.00	1.75	0	1.75		1.15			100.0	
40	101.00	99.80	1.20	0	1.20		1.20			100.0	
40:41	99.80	92.10	7.70	0	7.70		4.08			100.0	
41	92.10*	90.85	2.25	.15	2.10						

No. 6

INSTAR V

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
21	102.35	101.30	1.05	0	1.05		1.05				
22	138.80	123.80	15.00	1.05	13.95	13.75		12.70	92.3	93.0	84.7
23	176.20	148.75	27.45	2.40	25.05	26.20		23.80	90.8	91.2	86.7
24	187.00	141.60	45.40	2.10	43.30	44.15		42.05	95.2	95.4	92.6
25	192.80	138.95	53.85	2.55	51.30	52.60		50.05	95.2	95.2	93.1
26	163.10	142.50	20.60	1.75	18.85	19.35		17.60	91.0	91.5	85.4
27											
28	179.70	163.35	16.35	2.35	14.00	15.10		12.75	84.4	85.6	78.0
29	203.50	166.60	36.90	2.80	34.10	35.65		32.85	92.1	92.4	89.1
30	205.30	168.65	36.65	3.10	33.55	35.40		32.30	91.3	91.5	88.1
31	220.45	192.05	28.40	2.25	26.15	27.10		24.85	91.8	92.1	87.5
32	240.55	207.25	33.30	3.20	30.10	32.05		28.85	89.9	90.3	86.7
33											
34											
35	232.50	222.00	10.50	1.90	8.60	9.25		7.35	79.5	82.0	70.0
36	236.65	235.40	1.25	0	1.25		1.25				
37	236.35	234.10		0			2.25				

No. 6

INSTAR VI

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
37	236.35*	234.10	2.25	0	2.25		2.25				
38	302.70	282.25	20.45	2.65	17.80	18.35		15.70	85.5	87.1	76.7
39	380.75	313.55	67.20	4.70	62.50	65.10		60.40	92.7	93.0	89.8
40	404.40	350.00	54.40	4.45	49.95	52.30		47.85	91.5	91.8	88.0
41											
42	416.45	342.00	74.45	6.95	67.50	72.35		65.40	90.4	90.7	87.8
43											
44	437.65	363.00	74.65	5.35	69.30	72.55		67.20	92.7	92.8	90.0
45	499.80	439.00	60.80	7.65	53.15	58.70		51.05	87.0	87.4	84.0
46	408.55	369.60	38.95	3.95	35.00	36.85		32.90	89.2	89.9	84.5
47	522.60	436.20	86.40	5.90	80.50	84.30		78.40	92.9	93.2	90.7
48	534.50	423.40	111.10	6.50	104.60	109.00		102.50	94.1	94.2	92.3
49	495.70	465.10	30.60	3.80	26.80	28.50		24.70	86.7	87.6	80.7
50	510.00	444.20	65.80	5.95	59.85	63.70		57.75	90.6	90.8	87.8
51	493.70	477.90	15.80	3.20	12.60	13.70		10.50	76.7	79.7	66.5
52	592.90	573.60	19.30	3.60	15.70	17.20		13.60	79.1	81.2	70.4
53	558.70	552.20	6.50	.80	5.70	4.40		3.60	81.8	87.7	55.4
54	548.90	546.70	3.30 2.20	0 0	2.20		2.10 2.20				
55	503.10*	498.50	4.60	Ex.18.0 0	4.60		11.52 4.60				

No. 5

INSTAR VI

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by Evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
39	215.35 ✱	213.40	1.95	0			1.95				
40	317.60	300.30	17.30	2.15	15.15	13.15		11.00	83.7	87.6	63.6
41											
42	400.80	317.00	83.80	5.50	78.30	76.30		70.80	92.7	93.4	84.5
43											
44	417.00	371.90	45.10	4.80	40.30	38.30		33.50	87.5	89.4	74.3
45	413.00	373.70	39.30	4.20	35.10	33.10		28.90	87.4	89.3	73.6
46	462.00	409.30	52.70	3.20	49.50	47.50		44.30	93.2	93.9	84.0
47											
48	493.50	410.80	82.70	6.40	76.30	74.30		67.90	91.3	92.2	82.0
49	544.00	469.80	74.20	5.30	68.90	66.90		61.60	92.2	92.7	83.1
50	532.00	421.30	110.70	4.10	106.60	104.60		100.50	96.1	96.3	90.8
51	519.00	438.80	80.20	4.90	75.30	73.30		68.40	93.2	93.8	85.2
52	527.70	468.90	58.80	5.50	53.30	51.30		45.80	89.2	90.6	77.8
53	576.90	556.10	20.80	3.10	17.70	15.70		12.60	80.2	85.1	60.6
54	537.00	535.00	2.00	0			2.00				
			3.60				2.02				
55	531.60	529.10	2.50	0			2.50				
	✱		17.10	Ex.1.80			8.60				
56	512.00	507.40									
57											

No. 1

INSTAR VI

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
57	211.70	207.60	4.10	0			4.10				
58	328.70	296.90	31.80	3.25	28.55	26.00		22.75	87.5	89,8	71.5
59-63											
64	317.10	295.50	21.60	3.40	18.20	15.80		12.40	78.6	84.3	57.4
65	422.50	376.60	45.90	3.10	42.80	40.10		37.00	92.2	93.2	80.7
66	419.20	375.60	43.60	3.60	40.00	37.80		34.20	90.4	91.8	78.4
67	449.50	393.80	55.70	5.30	50.40	49.90		44.60	89.4	90.4	80.0
68											
69	474.30	412.30	62.00	5.25	56.75	56.20		50.95	90.5	91.6	82.0
70											
71	447.20	387.70	59.50	5.90	53.60	53.70		47.80	89.0	90.1	80.4
72	451.10	418.70	32.40	2.90	29.50	26.60		23.70	89.1	91.1	73.2
73											
74	449.10	429.70	19.40	3.35	16.05	13.60		10.25	75.3	82.7	52.8
75	545.90	492.20	53.70	6.05	47.65	47.90		41.85	87.3	88.7	77.8
			26.00	5.25	20.75	20.20		14.95	74.0	79.7	57.5
76	466.20		20.00	2.95	17.05	5.90		2.95	50.0	85.2	14.8
77	446.20	437.40	8.80	.70	8.10	3.00		2.30	76.6	92.0	26.1
			12.90	0			5.80				
78	424.50	419.10	5.40	0			5.40				
	≠		34.00	Ex.6.50			16.10				
79	385.10	382.30	2.80	0			2.80				

No. 6

INSTAR IV

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by Evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
0	50.60	49.80	.80	0	.80		.80				
1	66.75	58.10	8.65	.40	8.25	8.25		7.85	95.1	95.3	86.1
2	77.80	70.05	7.75	.85	6.90	7.35		6.50	88.4	89.0	72.9
3	81.00	67.25	13.75	.90	12.85	13.35		12.45	93.2	93.4	84.0
4	73.85	64.70	9.15	.65	8.50	8.75		8.10	92.5	92.9	81.4
5											
6											
7											
8	90.30	74.10	16.20	11.00	15.20	15.80		14.80	93.7	93.8	85.2
9	79.90	72.75	7.15	.45	6.70	6.75		6.30	93.2	93.7	81.8
10	102.25	81.55	20.70	1.65	19.05	20.30		18.65	91.8	92.0	82.1
11	95.50	84.25	11.25	1.05	10.20	10.85		9.80	90.2	90.6	77.8
12	89.80	82.25	7.55	.65	6.90	7.15		6.50	90.9	91.4	77.5
13	88.90	79.95	8.95	.45	8.50	8.55		8.10	94.7	95.0	85.4
14	92.30	83.20	9.10	.25	8.85	8.70		8.45	97.0	97.2	90.1
15	107.00	91.30	15.70	1.00	14.70	15.30		14.30	93.5	93.6	84.7
16	118.30	97.00	21.30	1.55	19.75	20.90		19.35	92.5	92.7	83.6
17	107.85	98.15	9.70	1.45	8.25	9.30		7.85	84.4	85.1	66.0
18	113.80	107.00	6.80	.85	5.95	6.40		5.55	86.6	87.5	69.1
19	113.50	110.05	3.45	.45	3.00	3.05		2.60	85.2	86.9	62.3
20	107.60	107.20	.40	0	.40		.40				
21	102.35	101.30	1.05	Ex..55 0	1.05		2.00 1.05				

No. 2

INSTAR VI

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by Evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
58	209.40	207.70	1.70	0	1.70		1.70				
59-63											
64	389.80	326.20	63.60	2.80	60.80	57.90		55.10	95.3	95.6	86.6
65	364.00	335.10	28.90	2.90	26.00	23.20		20.30	87.5	90.0	70.2
66	386.60	354.70	31.90	3.30	28.60	26.20		22.90	87.4	89.7	71.8
67	423.90	373.50	50.40	3.85	46.55	44.70		40.85	91.2	92.4	81.1
68											
69	371.70	345.70	26.00	3.00	23.00	20.30		17.30	85.2	88.4	66.6
70											
71	433.00	395.40	37.60	4.40	33.20	31.90		27.50	86.2	88.3	73.2
72	464.40	422.50	41.90	3.80	38.10	36.20		32.40	89.4	91.0	77.4
73											
74	512.00	497.70	14.30	3.05	11.25	8.60		5.55	64.6	78.7	38.8
75	495.40	484.50	10.90	1.45	9.45	5.20		3.75	72.1	86.6	34.4
76-77	470.30	457.50	12.80	0			5.00				
77	457.50	451.80	5.70	0	5.70		5.70				
77-78	451.80	415.50	36.30	Ex.65 0	35.65		16.00				

No. 3

INSTAR III

Dys.	W_1 Original Wt. (Mg.)	W_2 Wt. after 9 hrs. (Mg.)	W_1-W_2 Total loss of wt. (Mg.)	Fd Wt. of dry faeces (Mg.)	W_1-W_2-Fd Total loss of water (Mg.)	$W_1-W_2-cH_2O$ Wt. of wet faeces (Mg.)	cH_2O Water lost by evaporation (Mg.)	$W_1-W_2-cH_2O-Fd$ Water lost with faeces (Mg.)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2-cH_2O}$ Percent of water in faeces (%)	$\frac{W_1-W_2-Fd}{W_1-W_2}$ Percent of total loss lost as water (%)	$\frac{W_1-W_2-cH_2O-Fd}{W_1-W_2}$ Percent of total loss lost as water in faeces (%)
0	23.8	23.55	0.25	0	0.25		.25				
1	39.8	33.60	6.20	.35	5.85	5.70		5.35	93.8	94.4	86.2
2	40.3	33.4	6.90	.40	6.50	6.40		6.00	93.7	94.2	86.8
3	37.9	33.2	4.70	.45	4.25	4.20		3.75	89.3	90.4	79.7
4	40.8	34.5	6.30	.55	5.75	5.80		5.25	90.5	91.2	83.3
-6-7											
8	34.7	33.2	1.50	.15	1.35	1.00		0.85	85.0	90.0	56.6
9	42.2	37.1	5.10	.55	4.55	4.60		4.05	88.0	89.2	79.3
10	49.3	38.15	11.15	.95	10.20	10.65		9.70	91.2	91.4	86.9
11	43.65	38.05	5.60	.30	5.30	5.10		4.80	94.0	94.6	85.6
12	47.80	37.80	10.00	.50	9.50	9.50		9.00	94.7	95.0	90.0
13	42.10	37.25	4.85	.30	4.55	4.35		4.05	93.0	93.8	83.5
14	54.75	45.85	8.90	.80	8.10	8.40		7.60	90.4	91.0	85.3
15	50.50	41.50	9.00	.50	8.50	8.50		8.00	94.0	94.4	88.8
16	46.90	40.65	6.25	.55	5.70	5.75		5.20	90.4	91.2	83.1
17	52.00	44.95	7.05	.75	6.30	6.55		5.80	88.5	89.4	82.2
18	49.30	45.10	4.20	.60	3.60	3.70		3.10	83.7	85.7	73.7
19	50.05	47.80	2.25	.40	1.85	1.75		1.35	77.1	82.2	59.9
20	60.85	53.85	7.00	.75	6.25	6.50		5.75	88.4	89.3	82.1
21	50.55	49.32	1.23	.13	1.10	.73		.60	82.2	89.4	48.7
22	48.65	48.15	.67 .50 3.05	0 0 Ex1.00	.67 .50 2.05		.55 .50 1.10				
23	45.10	43.85	1.25	.20	1.05						

QUANTITATIVE CHARACTERS OF THE GROWTH AND DEVELOPMENT OF A PAUROMETABOLOUS INSECT, DIXIPPUS (CARAUSIUS) MOROSUS BR. ET REDT.

II. INCREASE IN CELL NUMBER AND GROWTH IN SIZE

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INCREASE IN CELL NUMBER AND GROWTH IN SIZE

INTRODUCTION.

The cyclic character of growth phenomena is nowhere so clearly defined as in the post-embryonic growth of insects, or indeed, of arthropods in general, where the moult sharply delimits one stanza of development from the next. The significance of the moult, in terms of internal physiology, is to be found in the cells of the organism where ecdysis is preceeded by a period of active growth and followed by a period of cellular quiescence. The anatomy of insects is of a simple type, the principal tissues being composed of single layers of cells so that the growth of single categories may be followed with comparative ease and accuracy. The insect therefore provides favourable material for the study of growth.

The weight and size increments of insects and their relation to the moult has attracted the attention of a number of workers. Of these the most provocative is the work of Przibram and Megusar (1912) which has led to a general hypothesis of insect growth. Observing that the orthopteran, Sphodromantis bioculata doubled its weight from moult to moult, they concluded that this

regular geometric progression from moult to moult could be accounted for by assuming one division of each cell in the body between two successive moults. Attempting to apply this concept to the data of Luciani and Lo Monaco (1897) for the increase in weight of silkworms, they found that the progression factor was, not 2, but $2, 2^4, 2^3, 2^2, 2^2, 2^1$, from the first to the last instar respectively. This led them to formulate a general statement for insect growth, i.e., that growth in weight follows a regular geometric progression whose exponent is 2 or 2^n while growth in length follows a similar progression with an exponent of $\sqrt[3]{2}$ or $(\sqrt[3]{2})^n$, and that this regular progression is the expression of the division of the body cells. From measurements of entire insects or parts of insects, Teissier (1928) Sztern (1914) and Alpatov (1929) have been able to support this hypothesis. Bodenheimer (1927 and 1933) has extended its application to several orders of insects.

The hypothesis has been criticized by a number of writers on account of the inconstancy of observed progression ratios. (Titshack, 1930; Yagi, 1926; Calvert, 1929; Ludwig, 1932, 1934; Key, 1936; Abercrombie, 1936; Hodge, 1933; Woodruff, 1938). The insects studied grew at a different rate from that

demanded by Przibram's ratio and the ratio did not remain constant but decreased from moult to moult.

The most pertinent criticism of Przibram's hypothesis has come from the attempt to correlate the increase in the number of cells with the progression factor from moult to moult. Abercrombie (1936) found that the increase in size of Japanese beetle larvae is largely due to an increase in cell size rather than in cell number. There was no correlation between the progression factors for the growth of the entire insect and the increase in the number of cells of the mid-intestine and the brain. Abercrombie concluded that, "Bodenheimer's method of calculating cell divisions seems to have no factual basis". Trager (1935) found that the entire larval growth of Lucilia sericata could be accounted for by the increase in the size, not the number, of the body cells. In larvae of Bombyx mori certain types of cells increase in size during an instar while others remain the same size and must therefore increase by cell multiplication in that instar. Trager concludes that, "Solely on the basis of measurements of the growth of entire insects no conclusions whatever can be drawn as to the significance, in terms of internal physiology, of Przibram's growth factor".

It is known that many holometabolous larvae grow entirely or in part by increase in cell size. Weismann (1864) observed that the muscle nuclei and the cells of the fat body increase in size during the larval development of Sarcophaga. Pantel (1898) discovered that the oenocytes of dipterous larvae increased in size from moult to moult. The nuclei of the mid-intestinal epithelium of Acanthoscelides obtectus (Coleoptera) almost double their volume from one larval instar to the next (Bushnell, 1936). The larval alimentary canal of Vanessa urticae (Lepidoptera) grows by an increase in the size of its constituent cells (Hensen, 1929). Perez (1902), Tiegs (1922), Mansour (1927), Berger ( ) and others have made similar observations. Increase of the size of cells appears to be accomplished by an increase of chromatic material. Polyploid cells in insect tissues have been reported by Eilers (1925), Frolowa (1926), Ravetta (1931), Sanderson (1933) and Berger ( ). Reduction to the normal chromosome number takes place at the pupal moult. Trager (1935) has advanced the hypothesis that larval tissues destined to be histolysed during the pupal stage tend to grow by increase in the size of their cells while tissues which persist to the imago tend to

grow by cell multiplication; and this hypothesis is supported by the work of Murray and Tiegs (1935) on the metamorphosis of Calandra oryzae.

Now, all the tissues of non-metabolic insects "persist" to the imago; and their growth seems always to be accomplished by cell multiplication. It is somewhat surprising therefore, that attempts to demonstrate a correlation between Przibram's ratio and the number of divisions of the body cells have been made with holometabolous insects (Trager, 1935; Abercrombie, 1936) where growth is often accomplished by an increase in cell size. Przibram's hypothesis is based on a study of the growth of Sphodromantis bioculata, a paurometabolous insect, and only extended to holometabolous insects through the doubtful concept of "latent divisions" to account for ratios greater than 2 between moults. Bodenheimer (1933) found Przibram's rule to hold best with paurometabolous insects. Sztern (1914) supported Przibram's hypothesis with the observation that there are an equal number of hypodermal cells per unit area of the cuticle in every instar of Sphodromantis.

While the work on holometabolous forms has invalidated Przibram's hypothesis as a generalization on insect growth, it is nevertheless apparent that there

has been no adequate investigation to determine whether Przibram's growth factor indicates the number of cell divisions in paurometabolous forms.

In the present study the increase in the number of cells is related to the increase in size throughout the post-embryonic development of the paurometabolous insect, Dixippus morosus.

The region of the proctadaeum and in particular, the rectal glands, have been chosen for the study of cellular growth. The development of the rectal glands has been studied in holometabolous forms (Perez, 1910; Evenius, 1933) but not in paurometabolous forms so far as I have been able to find. The rectal gland cells are large and may be counted with ease and accuracy. It was conceived as an interesting problem to determine whether the rectal gland cells grew at the same rate as the hypodermis of which they are a specialized continuation. Moreover, it was thought that a study of the histological changes taking place in the proctodaeum just before moulting might lead to an explanation of the observation that the faeces contain a smaller percentage of water just before ecdysis than at any other time during an instar (Smallman, 1940).

The purpose of this paper is therefore:

(1.) To describe the growth and development of the rectal glands throughout their post-embryonic

development.

(2.) To describe this growth in terms of cell numbers and to relate increase in cell number to increase in physical size from moult to moult.

(3.) To discuss histological features of the rectal glands having a bearing on their function, particularly at the time of ecdysis.

- ACKNOWLEDGEMENTS -

I wish to acknowledge my gratitude to Professor James Ritchie of the University of Edinburgh for laboratory facilities and for much encouragement during the course of this work. I have also to thank Professor N. J. Berrill for research facilities at McGill University where this work was completed.

METHOD.

The material for this study consists of microscopical preparations made from 8 individuals of Dixippus morosus from each of the six nymphal instars and four adults. Within each instar four nymphs were decapitated and fixed in alcohol-Bouin two or three days after moulting and four more were similarly fixed shortly before the next moult. The adults were killed and fixed three days after the imaginal moult.

Sections of entire insects were cut at 10 μ in the region of the proctodaeum. The material was stained with "Azan" triple stain or Harris haemotoxylin and light green.

The cell counts were made by selecting a section in the middle of the rectal gland region and counting the cells in five sections on either side of it. Measurements of cells and of the body diameter were made with the assistance of a Zeiss ocular screw-micrometer.

The body lengths of the insects were measured with a vernier caliper before they were killed.

OBSERVATIONS

General Description of the "Rectal Glands"[1]

The proctodaeum is divisable into an anterior intestine and a posterior intestine. The anterior intestine is lined with squamous epithelium and covered internally with a cuticular intima. At the point where the anterior intestine enters the posterior intestine, the epithelium is thrown up into six folds to form a rectal valve (fig. 1, Pl. 1)

The posterior intestine consists of a rectal sac and a short rectum proper which communicates directly with the anus. The epithelium of the rectal sac is of two types; the large, high columnar cells of the rectal glands and the small, squamous cells of the transitional epithelium. The latter connects the rectal glands and maintains the continuity of the rectal sac epithelium between the rectal glands (fig. 2, Pl. 1). There are six rectal glands throughout development. They account for almost the entire inner surface of the rectal sac, the transitional epithelium dipping down between the rectal glands (fig. 3, Pl. 1). Straightening of the transitional epithelium allows for considerable distension of the rectal sac (fig. 5, Pl. 1). The

[1] The "rectal glands" have been called the "rectal pads" and "rectal organs" by some writers to avoid committment as to their function.

rectal glands are supplied with tracheae. Tracheales may be seen between the basement membrane of the rectal gland cells and the muscular tunic of the rectal sac.

The epithelium of the rectal sac is everywhere seperated from the lumen by a thin cuticular intima, 3u - 6u thick. The musculature of the rectal sac consists of internal circular fibres and external longitudinal fibers. The longitudinal fibers are disposed in six triangular groups beneath the six points where two rectal glands are joined by the transitional epithelium. (fig's 2 and 3, Pl. 1) Muscle attachment to the rectal glands themselves is afforded by small areas of exocuticula in the cuticular intima. The cuticular intima is composed everywhere else of endocuticula only. But at both sides of each rectal gland there is a small spot (cross-section of a longitudinal ridge) of exocuticula and from these muscles run diagonally to join the muscularis of the rectal sac. (fig. 1, Pl. 2). The whole musculature provides an excellent mechanism for keeping the contents of the rectal sac under constant and uniform pressure.

The rectal gland cells are large and columnar, 45u - 100u in height and 20u - 25u in width. The cytoplasm is sparsely granular. The ends of the cells sometimes appear to be constricted or tapered

just beneath the cuticula (fig's. 5 and 6, Pl. 1). This appearance has been described for the rectal gland cells of Periplaneta Anstralasiae (Abbott, 1926) and of the cricket and other insects (Cholodkovsky, quoted by Abbott, '26) The nuclei are typically round, measuring 20u x 20u, and contain two or three large granules. They lie halfway between the bases and the ends of the rectal gland cells.

This description agrees in general with the descriptions of Wigglesworth (1933) and Hodge (1936, '39, '40) for the rectal glands of other paurometabolous insects.

Immediately beneath the basement membrane of the rectal gland cells there is a layer of deeply-staining cells which extends around the sides of the rectal glands where they are especially prominent (fig's. 2, 3, 4, 5, 6, Pl. 1) The nuclei are small, densely granular and elongated. The cytoplasm is densely granular and strongly basophilic. Cell borders cannot be seen. This layer becomes very active immediately before ecdysis at which time it appears to be continuous with the transitional epithelium (fig's. 7 and 8, Pl. 1). The region is well supplied with tracheoles.

The Growth of the Rectal Glands.

The growth of the rectal glands of Dixippus takes place only at the time of ecdysis. At this time the cells of the preceding instar are suddenly and completely replaced by the larger number of cells characteristic of the next instar. This method of growth is the same as the "total and periodic regeneration" reported by Tchang-Yung-Tai (1928, 1929) for the mid-gut of Galleria and Achroea. Regeneration of this type was recognized before only at the metamorphosis of holometabolous insects.

In Dixippus the moult is preceded by rapid and striking changes in the hind-gut. The lumen contains less faecal material and is greatly reduced in diameter. The deeply-staining layer beneath the rectal gland cells becomes considerably larger, particularly at the sides of the rectal glands (fig. 4, Pl. 1). Occasional mitoses may be seen in this layer. No cell borders are visible, and since this layer appears to be responsible for the regeneration of the rectal gland cells, we shall refer to it as a regeneration syncytium.

During this period the old rectal gland cells show signs of disintegration. They frequently become vacuolated; the chromatin of the nucleus becomes clumped and the nuclear membrane may disintegrate releasing chromatin into the cell plasm (fig. 2, Pl. 2).

At the same time the cells become detached at their bases before the invading nuclei of the regenerative syncytium. These latter form elongated deeply-staining cells. Occasionally an old rectal gland cell may be seen completely surrounded by these new cells (fig. 3, Pl. 2). Apparently the old rectal gland cells are not cast into the lumen but are reabsorbed by the new cells. The old cuticle is left greatly elevated above the rectal glands (fig. 7, Pl. 1); when the new rectal gland cells have produced a cuticle, the old cuticle becomes detached from the transitional epithelium and is thrown into the lumen (fig. 8, Pl. 1) The regenerative syncytium becomes seperated from the new rectal gland cells but remains prominent until ecdysis (fig. 7 and 8, Pl. 1)

The newly formed cells are narrow and elongated with the nuclei lying at different levels and the glands frequently crowded into "humps" (fig. 4, Pl. 2; fig. 8, Pl. 1) At this stage the number of rectal gland cells characteristic of the next instar may be counted. For instance, there are typically 36 rectal gland cells per cross-section in the second instar and 50 in the third; but in second-instar nymphs killed shortly before ecdysis, 50 elongated, deeply-staining cells may be counted. When feeding has begun after ecdysis and the rectum becomes distended, the cells of the now-enlarged

glands become alined and increase in width to form the normal columnar cells with sparsely granular cytoplasm and round nuclei. (fig.s 3, 4, 5, 6, Pl. 1)

The time required for this total replacement of the rectal gland cells must be very short. The hypodermis has already begun to secrete the new cuticle, preparatory to ecdysis, before the first signs of replacement (a thickening of the regeneration syncytium) appears.

Regeneration of the "partial and continuous" type described by Tchang-Yung-Tai (1928) also appears to occur in the rectal gland cells of Dixippus. Occasional cells may be seen pushing up between the formed cells from the basement membrane (fig. 5, Pl. 2). These regeneration cells are probably formed from the small scattered nuclei which may be seen at the base of the rectal gland cells. This type of regeneration occurs in the mid-gut of many insects (Snodgrass, 1935) and is regarded as a maintenance mechanism whereby injured or exhausted cells are replaced.

Increase in Cell Number and Growth in Size.

The growth of the rectal glands is accomplished by an increase in the number of cells from moult to moult. There is a slight increase in the height of the cells

from the first to the third instar but the nuclei remain the same size throughout post-embryonic development. Each of the six rectal glands is composed of an approximately equal number of cells. However, in order to minimize error in cell counts, the total number of rectal gland cells in ten sections were counted and the average number of cells per section was calculated.

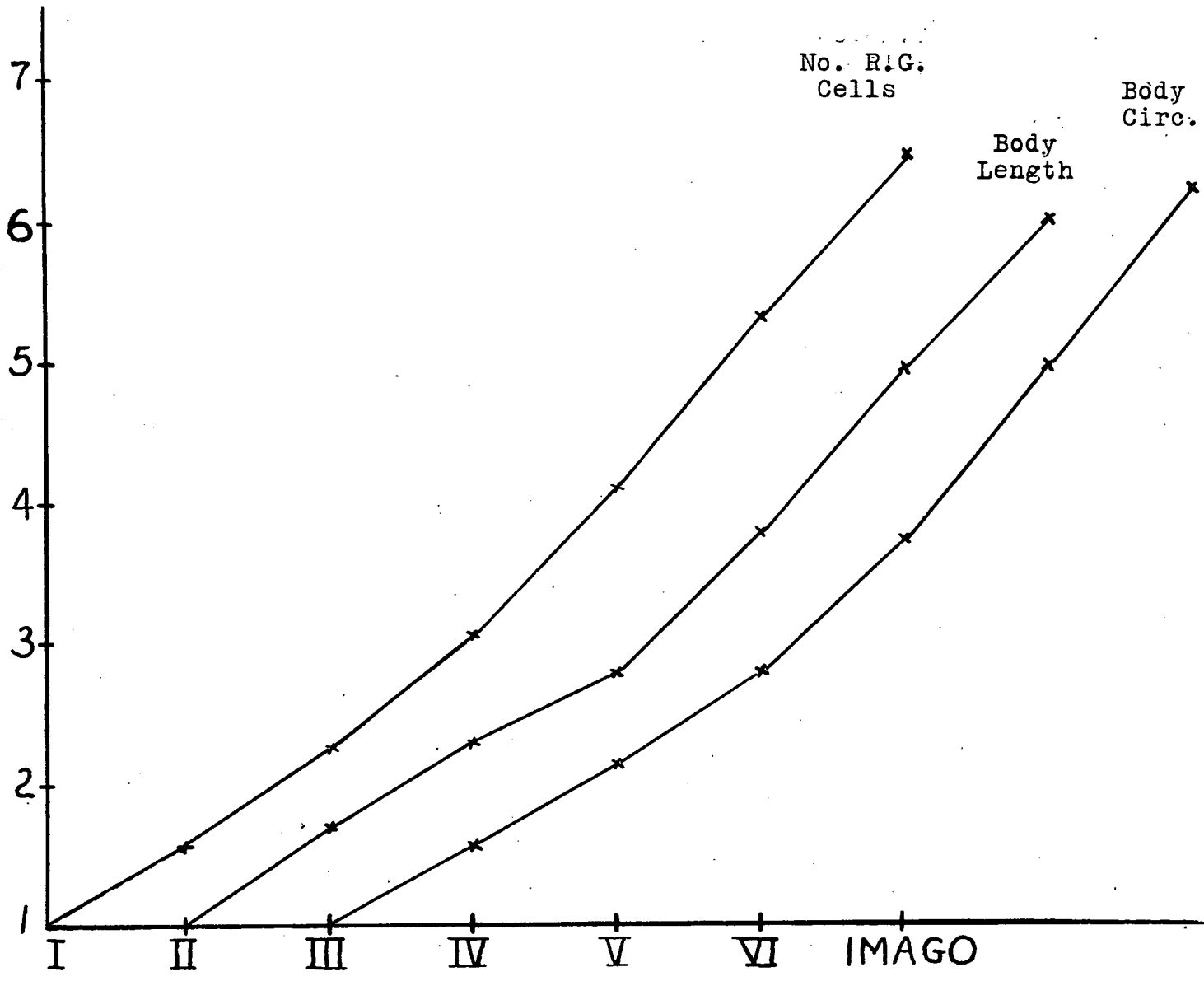
The number of cells, the body length and the body circumference for every nymphal instar and for the imago are presented in Table I, along with the progression factors for each of these categories. The progression factors are calculated by dividing the number of cells or the dimensions of one instar into that of the next instar. They are indices for the comparison of rates of growth.

From Table I it is immediately apparent that the growth of Dixippus does not conform with the rule of Przibram and Megusar (1912). The growth rates for cell multiplication and for size do not remain constant as in a regular geometric progression but diminish progressively from instar to instar. The rectal glands s_{Λ}^{cells} do not double in number from moult to moult.

The rate of increase in the number of rectal gland cells is closely paralleled by the rate of increase in body length and body circumference (Text-fig. I). Body size in insects is a direct consequence of the size of the hypodermis. As stated by Trager

Instar	Rectal Gland Cells	Body Length (mm.)	Body Circum. (mm.)	Progression factors: Cells	Length	Circumference
I	23 ± 1.40	12.6 ± 0.57	1.2 ± 0.34	1.56	1.67	1.58
II	36 ± 1.45	21.1 ± 1.62	1.9 ± 0.38	1.44	1.36	1.37
III	52 ± 1.66	28.8 ± 1.66	2.6 ± 0.51	1.36	1.27	1.31
IV	70 ± 1.70	36.6 ± 2.66	3.4 ± 0.58	1.34	1.31	1.32
V	94 ± 3.25	48.0 ± 3.87	4.5 ± 0.63	1.31	1.30	1.33
VI	123 ± 3.40	62.5 ± 5.24	6.0 ± 0.94	1.21	1.21	1.25
Imago.	149 ± 7.39	76.0 ± 5.52	7.5 ± 1.29			

~~Text Figure~~ Table I - The average number of rectal gland cells in ten consecutive cross-sections from each of four individuals; the average body length and body circumference of eight individuals, four from the first and four from the last of each instar. The progression factors are calculated by dividing the value for one instar by that of the preceding instar.



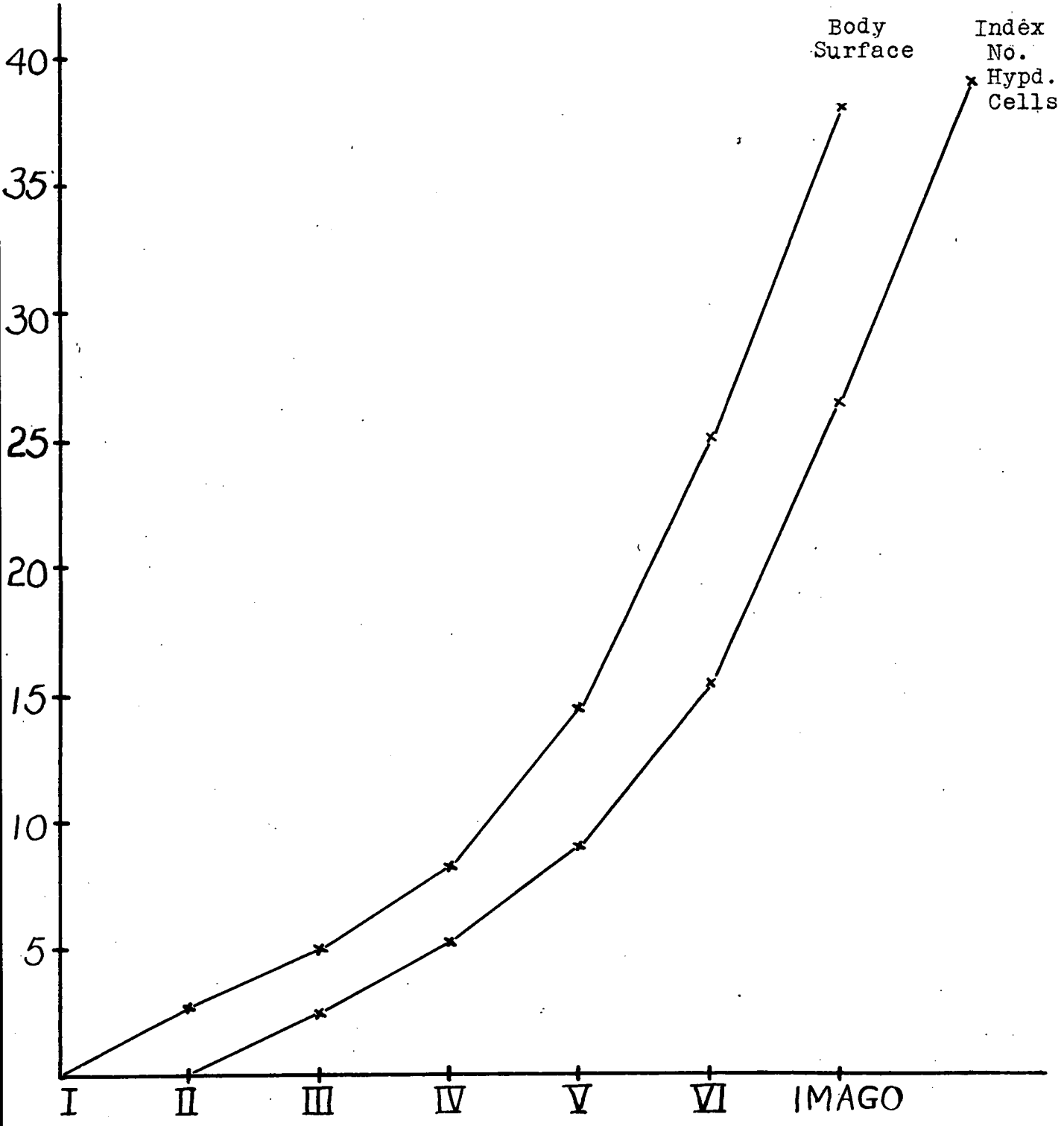
Text-figure I - The increase in the number of rectal gland cells compared with the increase in body length and body circumference from instar to instar. Ordinate: a common scale of relative units calculated by dividing the number of cells or the size in every instar by the corresponding value in the first instar. Abscissa: the instars. Each curve is displaced one instar to the right.

(1935), the hypodermis is, "a tissue which by its very nature must have a length proportional to the length of the body". In Lucilia, where the hypodermal cells increase in size from moult to moult, Trager found a close proportionality between body length and the size of the hypodermal cells. In Dixippus therefore, where the hypodermis grows by increase in cell number, the close proportionality between body size and the number of rectal gland cells in every instar indicates that the hypodermal cells increase at the same rate as do the rectal gland cells.

The proportionality in every instar between the number of rectal gland cells and the body circumference indicates that the number of rectal gland cells may be used as an index of the number of hypodermal cells in an annulus one cell in width. The form of Dixippus is roughly that of a cylinder. Multiplying the length by the circumference will therefore give an approximate measure of the body surface; and multiplying the number of rectal gland cells by the body length will give an index of the total number of hypodermal cells. This data is presented in Table II along with the progression factors. Text-figure 2 presents the data in graphic form.

Instar	Body surface (sq. mm.)	Index for number of Hypodermal Cells	Progression Factors.	
			Body Surface	Number of Hypodermal Cells.
I	15.1	290	2.66	2.62
II	40.1	760	1.87	1.97
III	75.0	1500	1.66	1.70
IV	124.4	2560	1.74	1.76
V	216.0	4512	1.73	1.70
VI	375.0	7688	1.52	1.47
Imago	570.0	11324		

~~Text figure~~ Table II - The body surface is calculated by multiplying the body length by the body circumference. The index for the number of hypodermal cells is calculated by multiplying the number of rectal gland cells by the body length. The progression factors are calculated by dividing the value for one instar by the corresponding value for the preceding instar.



Text-figure II - The increase in the body surface compared with t the increase of an index for the total number of hypodermal cells from instar to instar. Ordinate: a common scale of relative units calculated by dividing the body surface or the index in every instar by the corresponding value in the first instar. Abscissa: the instars. The second curve is displaced one instar to the right.

According to Przibram's hypothesis, the progression factor for surface area is 1.41 or $\sqrt{2}$. and remains constant from moult to moult. The progression factors for the surface area of Dixippus are always greater than 1.41 and decrease progressively from moult to moult. There is however, a close agreement between the rate of increase of the surface area and the rate of increase of the index for the total number of hypodermal cells. To this extent therefore, Przibram's hypothesis appears to have some basis in fact, - that the rate of increase in size is indicative of the rate of increase of the cells of one tissue, the hypodermis.

The Function of the Rectal Glands.

Wigglesworth (1933) has recorded considerable evidence from all the principal orders of insects that the function of the rectal glands is the reabsorption of water from the faecal material in the rectal sac. Smallman (1940) found for Dixippus that the faeces released immediately before ecdysis contained a smaller percentage of water than at any other time during an instar. The histology of the rectal glands shows an interesting relation to these observations.

As we have seen, the musculature of the rectal sac keeps its contents under constant and uniform pressure. At the same time the transitional

epithelium allows considerable distension of the rectal sac and this means that the faecal material can be retained in contact with the rectal glands for some time. This arrangement may reasonably be considered a device to facilitate the reabsorption of water from the faeces, as pointed out by Chun (1876)

The rectal gland cells are obviously highly specialized cells of the rectal epithelium. They have lost the "embryonic" power to divide, in contrast to other tissues of ectodermal origin in which this "embryonic" character is retained to a remarkable degree (Wigglesworth, 1937). The cells are large with large nuclei, an indication of some active function. A narrow granular zone at the ends of the rectal gland cells just beneath the intima is often apparent. Such an appearance has been described by Abbott (1926) for the rectal glands of Periplaneta. Abbott considered this zone to be associated with secretion but Wigglesworth (1933) has pointed out that such an appearance might equally well be associated with absorption. That the intima of the rectum is permeable to water has been shown by Eidmann (1922) and Abbott (1926) These facts point to an absorptive function for the rectal glands.

The pre-ecdysial changes taking place in the rectal glands and described above may possibly be related to the observed decrease in the percentage of

water lost with the faeces at this time. The first of the pre-ecdysial changes observable is the active proliferation of the regeneration syncytium. This growth activity and the expansion of the resultant cells are processes requiring water. The densely granular appearance of these cells suggests a high osmotic pressure by means of which water could be obtained for growth processes from the faeces and across the permeable intima.

Preceding ecdysis the intima is seperated from the rectal glands so that there is an enclosed space into which solid material may not enter (fig. 7, Pl. 1) This condition has been described by Hodge (1940) as the normal condition in the grasshopper, Rodenotatum carinatum, and he believes it to be an adaptation for the reabsorption of water from the faeces. To quote him, "It seems probable that the extreme overdevelopment of the intima of the rectal sac of this species is a special adaptation for the reabsorption of every possible bit of moisture from the very dry food". It may be significant therefore, that in Dixippus the intima is seperated from the rectal glands only preceding ecdysis and at this time there is a reduction in the percentage of water lost with the faeces.

It is not clear whether the cuticular intima of the rectal sac is partially digested and loosened from the rectal glands by the action of moulting fluid, or whether the intima is merely left behind when the old rectal gland cells are absorbed by the new cells. However, either of these processes would tend towards an increase in osmotic pressure and hence an increased absorption of free water from the faeces.

DISCUSSION.

The growth of insects is accomplished by an increase in the size of the cells, an increase in their number or an increase in both the size and the number of cells. Growth by increase in cell size appears to occur only in holometabolous forms (Weismann, 1864; Pantel, 1898; Tiegs, 1922; Mansour, 1927; Trager, 1935; Abercrombie, 1936, Bushnell, 1936; and others); while growth by increase in cell number appears to be the method of growth in paurometabolous forms (Sztern, 1914; Wigglesworth, 1933a). Both methods of growth are known to occur in some holometabolous insects (Trager, 1936; Murray and Tiegs, 1936). These changes in the size or number of cells appear to have a definite relation to moulting and metamorphosis since these periods are preceded by a period of cellular activity and followed by a period of cellular quiescance (Wigglesworth, 1940) The ultimate physical expression of these changes in cell size or cell number is a change in the weight and size of the insect.

Growth by increase in cell number may be accomplished in two ways. The cells may multiply by mitotic division, as in the hypodermal cells of Rhodnius (Wigglesworth, 1933a); or there may be a complete replacement of existing cells by a larger

number of new cells. This latter method of growth is reported in the present paper for the rectal glands of Dixippus. Tchang-Yung-Tai (1928) reports the same type of growth to occur in the mid-gut of Galleria and Achroea at the time of moulting. This type of regeneration is known to occur at the metamorphosis of holometabolous insects, but Tchang-Yung-Tai (1929) believes it occurs very widely in the epithelial structures of insects, and indeed, of arthropods in general. The observation that total and periodic regeneration occurs in the rectal glands of Dixippus extends his "theorie de l'activite cellulaire periodique" to a paurometabolous insect. It is probably significant that total replacement takes place in the rectal glands and the mid-gut where the cells are large and highly specialized and have lost the power to divide by normal mitosis. In these places there are two types of cells: the "functional" cells which have lost the power of reproducing themselves, and the "embryonic" cells which replace the former, partially and continuously for maintenance against exhaustion and injury of cells, and totally and periodically for purposes of growth.

The abrupt change in size at the time of moulting in arthropods has attracted the attention of

numerous writers, and several attempts have been made to place the growth rate on a mathematical basis. Brooks (1886) found for the early stages of stomatopod Crustacea that each stage increased by a fixed percentage of its length. Dyar (1890) found that the growth of the head capsule of lepidopterous larvae could be expressed in the form of a regular geometric progression. Przibram and Megusar (1912) extended this concept of a constant growth rate in insects and gave it a biological basis in their hypothesis of a constant rate of cell multiplication.

It would be surprising to find that in the insects the rule, that the rate of cell multiplication decreases with increasing age, so universal among other groups of animals, should be replaced by a constant rate. The concept of a constant growth rate in insects has been criticized by many writers (Ripley, 1923; Titshack, 1930; Yagi, 1926; Peterson and Haeussler, 1928; Calvert, 1929; Taylor, 1931; Ludwig, 1932, 1934; Gaines and Campbell, 1935; Abercrombie, 1936; Woodruff, 1938; and others). In general the findings of these authors agree that the growth rate of insects decreases with increasing age and that different insects exhibit different growth rates. Woodruff (1938) has suggested that the

growth rate as well as the absolute size of insects is genetically determined. The present work shows that the rate of growth in size of Dixippus is in general greater than the rate of growth reported by Przibram and Megusar for Sphodromantis and that it decreases from instar to instar.

The hypothesis of Przibram and Megusar is based on average growth factors calculated for the growth increments of Sphodromantis. For length these growth factors varied from 1.13 to 1.38 with an average of 1.26. Their error appears to lie in assigning constancy to this average value and in assuming it to be an indication of the rate of multiplication of all the body cells.

The discontinuous nature of insect growth is imposed by the firm cuticula which in turn is an expression of the activity of a single layer of cells, the hypodermis. It should be clearly recognized then, that progression factors such as those of Brooks, Dyar and Przibram based on uni-dimensional measurements of entire insects will give a direct index of growth for only one tissue of the insectan organism, namely, the hypodermis. We have seen in the present paper that growth in the size of Dixippus is closely approximated

by an index for the increase in number of the hypodermal cells. Sztern (1914) has shown that there are an equal number of hypodermal cells per unit of area in every stage of Sphodromantis. Trager (1935) has reported a striking agreement between the growth in size of Lucilia and the growth in size of the hypodermal cells. It appears therefore, that measurements of the size of the insects may be used as an index for the increase in the number or size of the units of a single growth category - the hypodermis. The continuous increase in weight between the moults of insects probably indicates an increase in the size of the muscle and fat tissues at least. Measurements of the size of the animal from moult to moult give no direct measurement of these changes but are direct measurements of changes in the hypodermis only.

The growth of size in insects is probably best represented as the growth of a sheet of cells, the hypodermis, whose size and possibly, proportions change in a discontinuous manner from instar to instar. The expression of size in insects is therefore the result of changes in the size or

number of the cells in this "sheet of cells", the hypodermis.

SUMMARY.

(1.) The histology of the rectal sac of the stick-insect, Dixippus morosus is described. The rectal epithelium is of two types: the large columnar epithelium of the six rectal glands, and the squamous epithelium of the "transitional epithelium" connecting the rectal glands. Immediately beneath the rectal glands there is a deeply-staining layer which becomes active just before ecdysis. It appears to be a regenerative layer.

(2.) The growth of the rectal glands is accomplished just before ecdysis by the total replacement of existing cells by cells derived from the regenerative layer. The existing cells are replaced by a larger number of cells. This type of regeneration appears to occur where the cells are highly specialized and have lost the ability to reproduce themselves.

(3.) The rate of increase of the rectal gland cells closely approximates the rate of increase of the length and circumference of Dixippus. Since growth in size of insects is necessarily a

result of the growth of the hypodermis, it is concluded that the cells of the rectal glands grow at the same rate as the cells of the hypodermis of which they are a specialized continuation. An index of the total number of hypodermal cells closely approximates the calculated surface area of Dixippus throughout post-embryonic development.

(4.) It is concluded that measurements of the size of insects may be used as a direct index of the growth of only one tissue, - the hypodermis.

(5.) The histology of the rectal glands indicates that they are suited for the function of reabsorption of water from the faeces. There is some evidence that a reduction in the percentage of water in the faeces just before ecdysis is related to histological changes in the rectal glands at this time.

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DESCRIPTION OF FIGURES. PLATE I.

Figure I - X I40. Cross-section of an entire insect in the region of the rectal valve. Note the elevated intima, characteristic of the period immediately before ecdysis.

Figure 2 - X 360. Showing the transitional epithelium joining two rectal glands, and the group of longitudinal muscles beneath it.

Figure 3 - X I40. Entire cross-section of a second-instar insect, showing the six rectal glands.

Figure 4 - X I40. Showing the rectal glands of a third-instar insect.

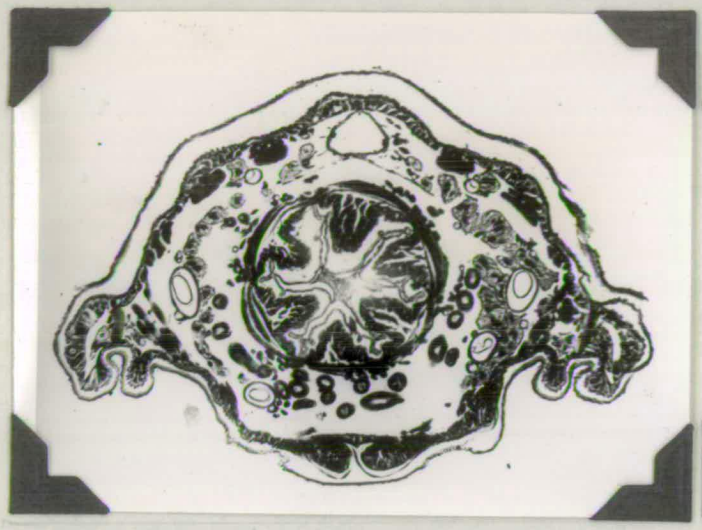
Figure 5 - X I40. Showing the rectal glands of a fourth-instar insect.

Figure 6 - X I40. Showing a single rectal gland of an adult.

Figure 7 - X I40. Showing the densely granular replacement cells of the rectal glands and the elevated intima just before ecdysis.

Fegure 8 - X I40. Showing the densely granular replacement cells of the rectal glands and the old intima being cast into the lumen just before ecdysis. The regeneration layer, immediately below the rectal glands, is quite distinct in this photograph.

PLATE I



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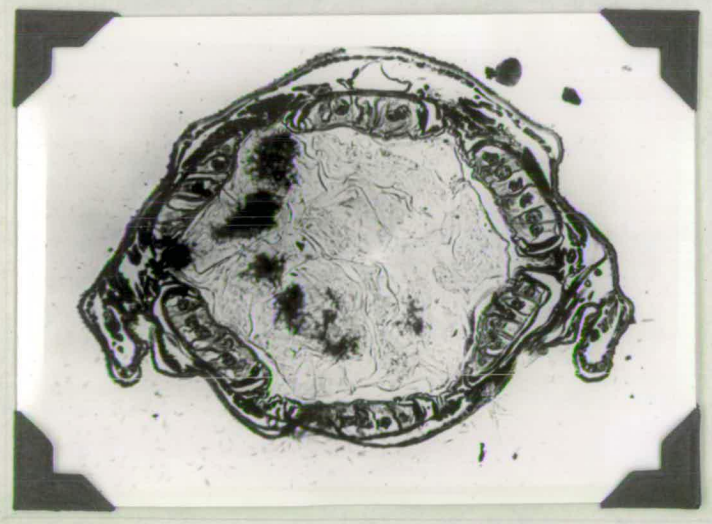

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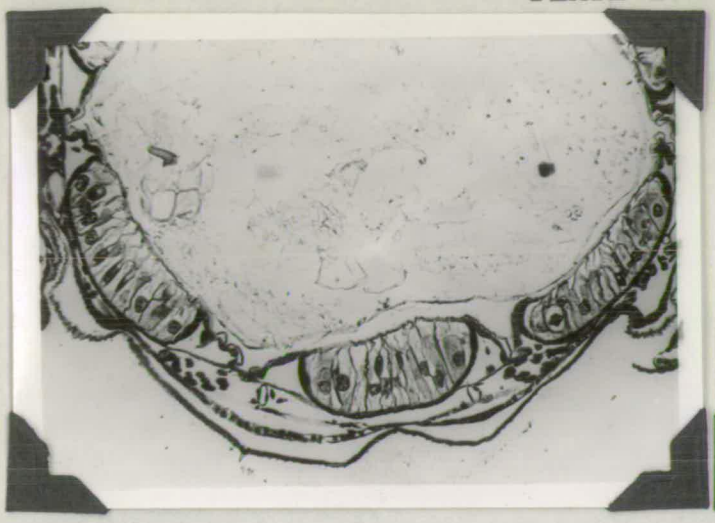

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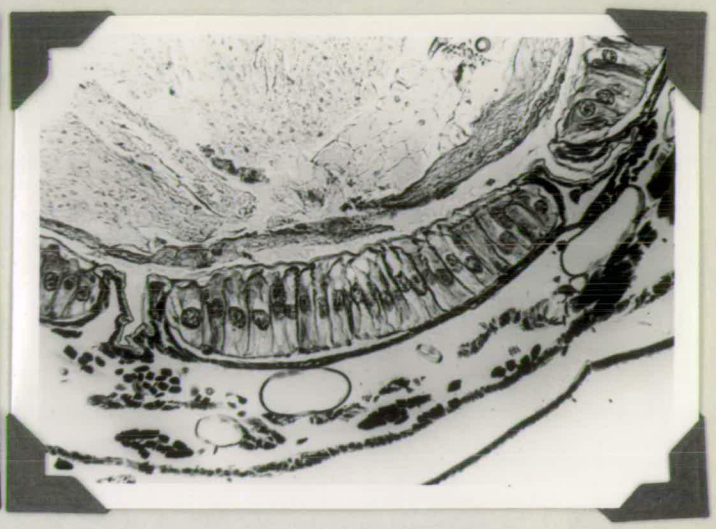

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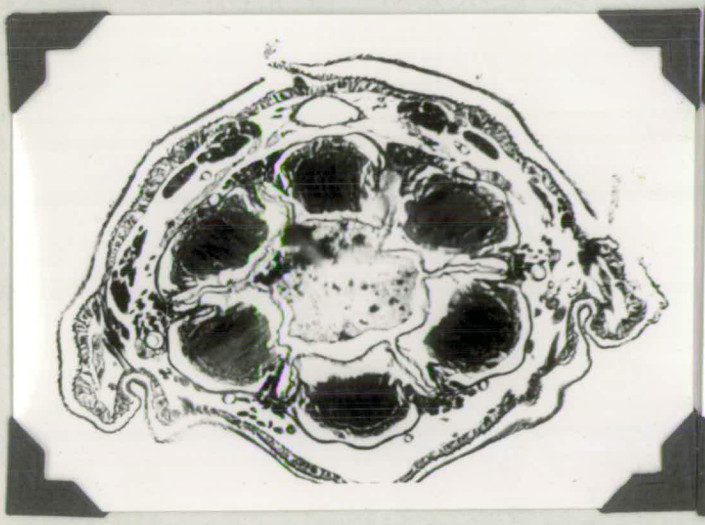

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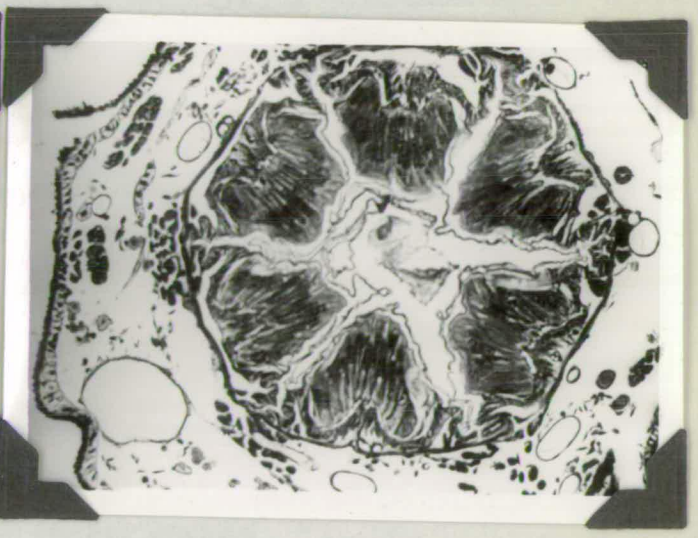

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DESCRIPTION OF FIGURES. PLATE 2.

Abbreviations: Ch - chromatin; Ep - transitional epithelium; Ex - exocuticular spot; I - intima; I' - old intima; Mc - circular muscle; Ml - longitudinal muscles; Ms - muscle strand; Nc - new cell; Oc - old cell; Rc - replacement cell; Rgc - rectal gland cell; Rs - regeneration syncitium; Tr - tracheole; Va - vacuole.

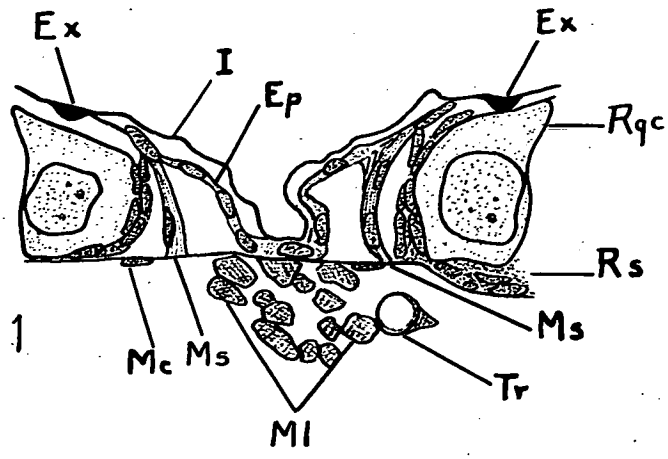
Figure I - Showing the exocuticular spots in the intima and the muscle strands running from them to join the muscularis of the rectal sac between two rectal glands.

Figure 2 - Showing disintegration processes in the old rectal gland cells prior to complete replacement. Vacuolation of the cells, disappearance of a cell boundary between the two nuclei, clumped chromatin and nuclear membrane disintegration with release of chromatin, are all evident.

Figure 3 - Showing an old rectal gland cell with new cells pushing up on either side of it.

Figure 4 - Showing the narrow, elongated, dense cells of the rectal glands after replacement and just before ecdysis.

Figure 5 - Showing a single replacement cell pushing up between formed rectal gland cells.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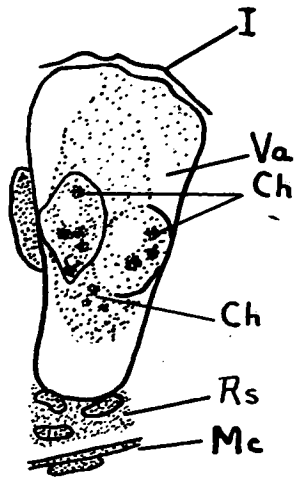
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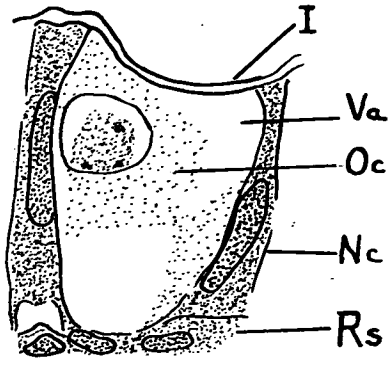
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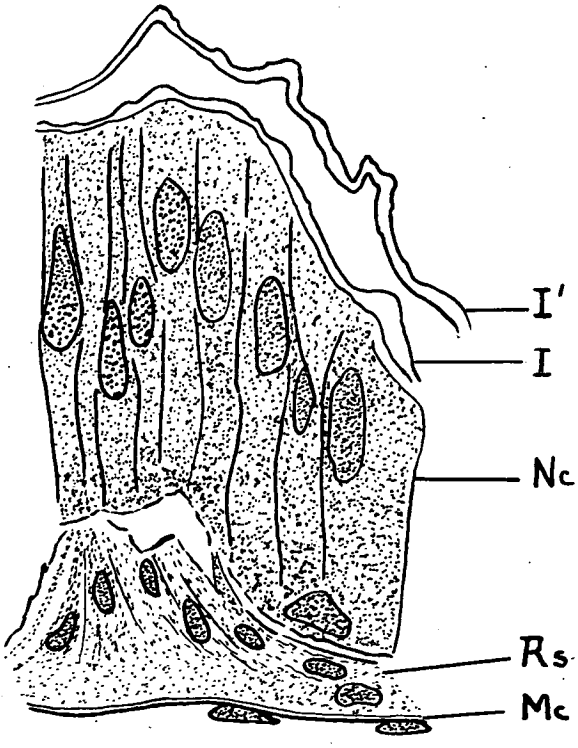
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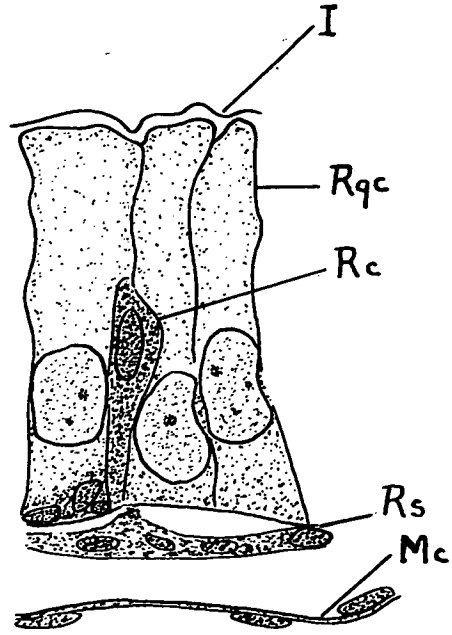
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5

A SIMPLE APPARATUS FOR THE DIRECT DETERMINATION OF THE VOLUME OF SMALL IRREGULAR OBJECTS.

BY

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* The work reported in this paper was carried out at the Department of Zoology, the University of Edinburgh.

APPARATUS FOR THE DETERMINATION OF THE VOLUME OF SMALL OBJECTS

The apparatus described was developed in connection with a study of the growth and development of the stick insect, Dixippus morosus. The principle is that of simple displacement and consistent results are obtained with objects as small as 0.007 milliliters.

The apparatus is shown in Fig. 1. It consists of two vacuum stop-cocks joined by a tube which serves as a specimen chamber. The top stop-cock is of the 3-way type and has a side-tube (C) of 1 millimeter bore fused to the upper part of the wall. The lower stop-cock serves merely to drain the vessel. A side-arm is fused to the specimen chamber and a graduated pipette is attached to this by means of heavy rubber tubing. The pipette is continuous with a rubber tube and funnel supported by the arm of a unicam. The stop-cocks were lubricated with Apiezon "L" stop-cock grease.

Mercury is introduced into the funnel and rises into the pipette until it reaches the level of the side-arm of the specimen chamber. With the stop-cock in position "A", water is introduced into the specimen chamber until it

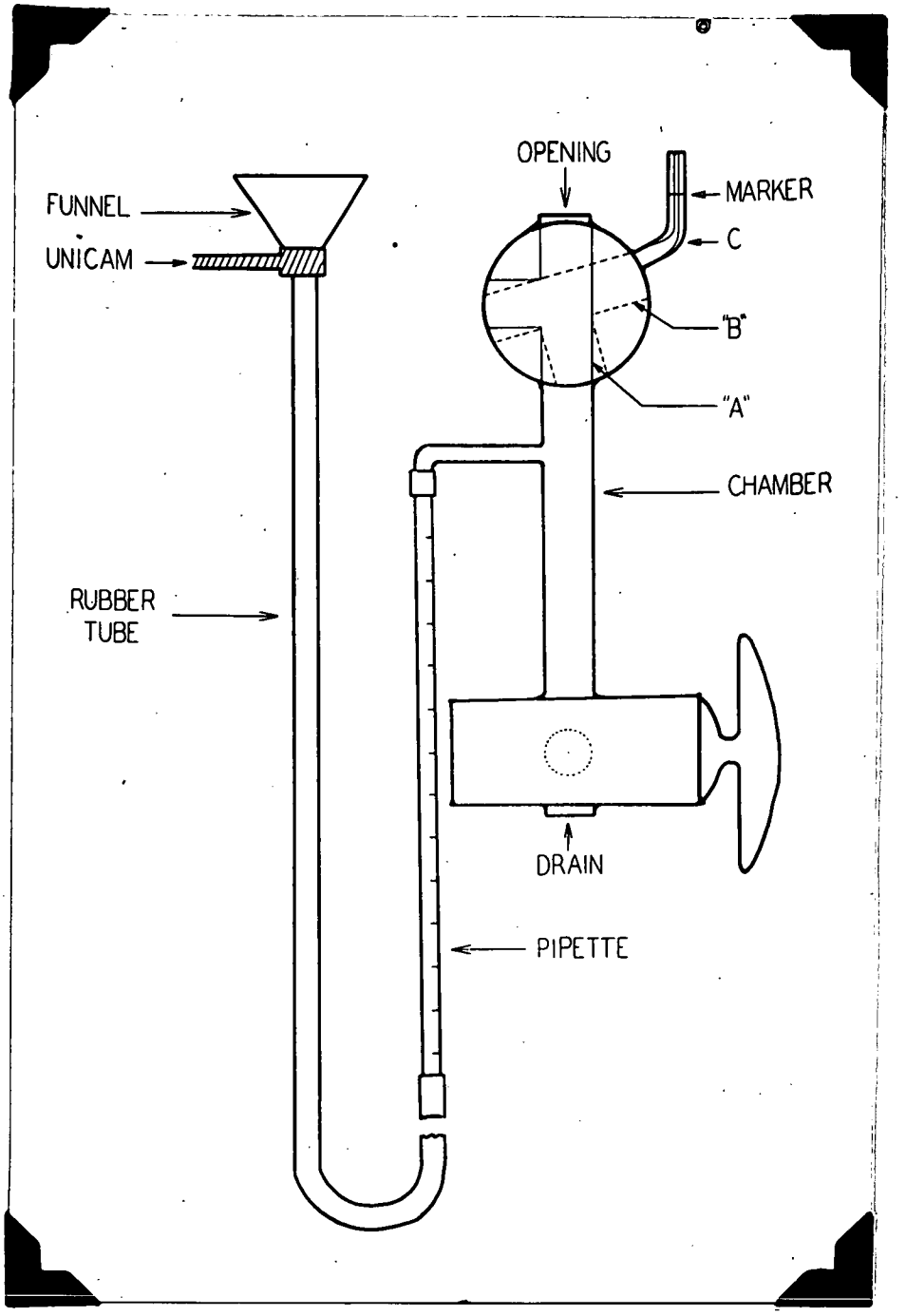

Fig. I - The apparatus. The solid line "A" indicates position "A" referred to in the text; the dotted line "B" indicates position "B".

APPARATUS FOR THE DETERMINATION OF THE VOLUME OF SMALL OBJECTS

flows into the side arm and comes into direct contact with the mercury. More water is added until it rises into the bore of the stop-cock. The mercury column is then lowered until the mercury-water portion is near the lower end of the pipette. The stop-cock is now turned to position "B".

The vessel now forms a continuous fluid system and the only opening is the tube C. Raising the mercury in the pipette forces the water into the tube C where it is brought to coincide with the reference level marker. The height of the mercury in the pipette is read and this reading is the zero for the subsequent measurement.

The mercury column is now lowered until the water level falls from tube C into the bore of the stop-cock where an air-space is formed. This air-space should be somewhat larger than the size of the object to be measured. The stop-cock is now returned to position "A" and the air-space rises in the bore so that the level of the water is somewhat below the opening. The object is now inserted at the opening and sinks into the specimen chamber. The stop-cock is again turned to position "B" and the mercury column raised until the water level once more coincides with the reference level. The pipette is

now read and the difference between this reading and the former reading gives the volume directly.

The volumes of small strips of copper of known weight were calculated from the specific gravity. Their volume was then measured with the described apparatus using a pipette graduated in intervals of 0.002 milliliters. The following results were obtained. For convenience, the results are expressed in cubic millimeters.

Calculated Volume cu. mm.	Observed Volume (Mean of 10 measurements) cu. mm.	Standard Deviation of the Mean	Coefficient of Variation
7.0	6.75	± 0.52	7.7%
10.5	10.40	± 0.46	4.6%
28.7	27.80	± 0.74	2.7%
61.7	59.90	± 1.10	1.8%

SUMMARY

An apparatus for the direct determination of the volume of small irregular objects is described. The principle is that of simple displacement. Results are presented

which indicate that consistent results may be obtained with objects as small as 0.007 millililters.

This work was carried out at the Department of Zoology, the University of Edinburgh. The writer is grateful to Professor James Ritchie and Dr. F. Gross for much help and encouragement.