

OXYGEN CONSUMPTION AND CELL SIZE. A COMPARISON OF THE RATE OF OXYGEN CONSUMPTION OF DIPLOID AND TRIPLOID PREPUPAE OF *DROSOPHILA MELANOGASTER* MEIGEN

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INTRODUCTION

The generalization that metabolic rate, per unit of weight, decreases with the size of the animal is certainly valid for birds and mammals; it seems fairly clear that it is also true of poikilotherms (see Weymouth, Crismon, Hall, Belding & Field, 1944; Zeuthen, 1947; Hemmingsen, 1950; and Ellenby, 1951). Apparently the rate of metabolism usually varies as the 0.75 power of the body weight (Kleiber, 1947); the difference of this value from $2/3$ has led to the conclusion that metabolic rate is not proportional to the surface-area, and that, in fact, the surface-law is not valid. There are cases, however (Ellenby, 1945*b*, 1951), where it has been shown that, although metabolic rate is not proportional to a $2/3$ power, it nevertheless is proportional to the surface area. Now, while there is general agreement about the variation in metabolic rate with increasing body size, the reason for this variation remains obscure. On many occasions it has been suggested that the extent of the surface area of the cells was the causal factor; but the fallacy of this view has been pointed out just as frequently. Rubner (1913), for example, by estimating the surface of the cells showed that such a hypothesis was impossible. Clearly such a factor can only be significant if cells increase in size, but not in number, with increasing body size; this, in general, is not so. But as I have already pointed out (Ellenby, 1945*a*), there are cases in insects in which it is known that all size increase is due to increase in cell size (Trager, 1935; Abercrombie, 1936) and other cases in which some of the increase is due to increase in cell size (Trager, 1935). Of particular relevance is the work of Alpatov (1930), who showed that the size of the cells in *Drosophila melanogaster* could be influenced by rearing the animals on different diets. It was therefore of interest to discover whether the differences in rate of oxygen consumption shown to exist between *Drosophila* prepupae of different sizes (Ellenby, 1938, 1945*a*) could be due to differences in cell size; a line giving triploids provided ideal material for such a study, for, as is well known (Boveri, 1905) nuclear size generally corresponds to chromosome content, and nuclear size, in turn, is related to cell size.

Gowen (1930*a*, *b*) examined the duration of life and the carbon dioxide production of male, female, triploid females, and sex intergrade imagines of *Drosophila*. On the whole the results were in agreement with Rubner's view (1908) that the

total energy transformed during the whole life of an animal is an approximate constant. He found no direct correlation between cell size and metabolic rate of the different classes of flies examined. But this work is unsatisfactory in a number of ways: (1) The carbon dioxide production of a number of flies was measured while they were free to move in a stoppered vial; differences of activity of the different classes examined would therefore affect the results. (2) Allowance was not made for the different body sizes of the various types of animals examined and results were calculated per unit of body weight. Body size influences the rate of oxygen consumption of prepupae of *D. melanogaster* (Ellenby, 1937, 1945*a*), and, possibly, the rate for imagines. On the other hand, the size of an animal may vary independently of the size of its cells; for example a triploid animal with large cells may be the same size as a diploid with smaller cells (Fankhauser, 1945; Beatty & Fischberg, 1951). It is therefore necessary to consider the size of the animals used, as well as the size of their cells. The techniques developed for the study of prepupal oxygen consumption in relation to surface area suggested that examination of the problem would be of interest.

MATERIALS AND METHODS

The progeny of a triploid female mated to a normal diploid male consist of eight different types, due to variations in the ratio between the number of *X*-chromosomes and the number of autosomes present in the cells of a given individual (Bridges, 1921, 1922). Externally, they can only be identified in the imaginal stage: if one wishes to compare the prepupal oxygen consumption of certain of these different forms it is therefore necessary to work with individual prepupae of unknown type rather than with groups, and to identify their type subsequently. The present investigation was restricted to an examination of triploid and diploid females and diploid males, that is, to the forms with 'balanced' chromosomes and very largely to a comparison of the first two forms. The main interest was to compare individuals as similar as possible in all but cell size, as there is good reason for believing that the other categories with 'unbalanced' chromosomes differ in other ways too. The triploid females which it was most desired to examine make up only 3.9% of the progeny (Bridges & Anderson, 1925; Dobzhansky, 1929), but, fortunately, to discover them is not quite as difficult as it sounds, for Dobzhansky (1930) has shown that there are sharp differences in the time of development between the forms with 'balanced' chromosomes and the forms with 'unbalanced' chromosomes. At 27° C., the mean developmental period for diploid females is 199.24 hr., for males it is 205.20 hr., and for triploid females it is 203.83 hr.; on the other hand, the other forms have times ranging from 255 to 272 hr. These times, of course, are for the whole developmental period; but, nevertheless, there seemed little doubt that similar differences would exist in the length of the larval period so that it would be possible to reduce the size of the population which had to be examined for triploid females.

The stock was the same as that used by Dr Sarah Pipkin in her work on triploids (1942); it was homozygous for the three recessive *X*-chromosome mutants *yellow₂*,

vermilion, *forked*, and, at the time the experiments were carried out, had been inbred for about a year. It was maintained at 25° C. in half-pint milk bottles on the maize-meal-agar-molasses medium in use in the laboratory at University College, London.

Breeding techniques were essentially similar to those already described (Ellenby, 1938, 1945*a*). Triploid females, already mated for 4 days, were allowed to lay eggs for 4 hr. in culture bottles yeasted the previous night; they were then transferred to sets of other bottles in turn. As before, it was found possible to obtain prepupae differing greatly in size by varying the number of egg-laying females per bottle.

As the usual paper towelling was not added the larvae crawled up the sides of the bottle to pupate. For reasons already given, it was important to collect prepupae for the respiration experiments as soon as they began to form. As soon as this began to happen all prepupae already formed were removed from the walls of a set of bottles, and the bottles were set aside in the incubator for an hour; prepupae removed at the end of this time were used in the respiration experiments. They were washed, adhering food removed, and their surfaces were sterilized by rinsing with alcohol. Animals showing the slightest movement during these manipulations were rejected. At this stage it was also possible to reject undesired males, their testes being visible through the body wall (Kerkis, 1931) since the prime interest was the comparison of diploid females with triploid females. The remaining prepupae were placed in the respirometers which were then transferred to a water-bath. The respirometers and water-bath were maintained in a constant temperature room so that, despite the large volume of air in the apparatus, an equilibration period was unnecessary; nevertheless, a 15 min. period was allowed so that the first reading was taken after about 1 hr. from the end of the period of collection. Subsequent readings were taken, generally at intervals of an hour, until the experiment had lasted 5 hr.

The constant temperature room had been subjected to prolonged test and had shown itself apparently capable of maintaining very accurate control. Unfortunately, during the course of the experiments the ambient temperature went up considerably and the room proved to be less satisfactory than had at first appeared. Throughout the course of the experiments the mean temperature was 26.0 ± 0.5 ; however, on any particular day, the temperature of the water-bath varied by less than ± 0.1 ° C. As the oxygen consumption of a number of prepupae of different sorts was measured simultaneously on any particular day, the fact that the temperature control was less satisfactory than desirable has the effect of increasing the variation but not the validity of any comparison between the different sorts.

As it is only possible to identify triploids in the imaginal stage it was necessary to measure the oxygen consumption of individual prepupae whose constitution could be determined subsequently. Respirometers of a modified Gerard-Hartline type were used (Gerard & Hartline, 1934); these have already been fully described (Ellenby, 1946). Each consists essentially, of two vessels, separated by a drop of manometric fluid; but one vessel, a capillary tube holding the animal and some KOH, is contained within the other, a very large tube, the manometric fluid separating the two. The system is a differential one, but as the volumes of the two

tubes are so different, the movement of the manometric fluid is equal to the actual volume change in the capillary due to the oxygen consumption of the animal. In fact, each large tube contained two of the capillaries, specially adapted for the particular task, and six of the large tubes were mounted so that they could be rotated about a horizontal axis. The oxygen consumption of twelve prepupae could thus be measured simultaneously. Movement of the manometric fluid was measured with a traversing microscope reading from lines etched on the capillaries. 1 cm. corresponded to between 2 and 3 cu.mm. depending on the particular apparatus.

The prepupae were weighed on a Fabergé torsion balance (Fabergé, 1938) at the end of each experiment. Each was then gummed to a microscope slide and kept in a vial with moist filter-paper so that the imago could be examined on emergence. Puparial surface area and cell size were both estimated by methods already described. For the former (Ellenby, 1945 *a*) the puparia left behind on the slides after emergence were used: each was flattened between two microscope slides and the outlined areas of the projected images measured with a planimeter. As triploids can only be identified in the imaginal stage it is impossible to determine their cell size in the prepupal stage. Cell size was therefore conveniently estimated by measuring the size of the epidermal cells of the wing. It was felt that, as a first step, the assumption that the other cells would show the same relationship was justified, in view of the supporting evidence from other animals (Fankhauser, 1945). Accordingly, the right wing of each fly was mounted in Euparal and retained; epidermal cell size was determined, in certain cases, by counting the number of bristles, each of which corresponds to a single cell, in a known area of the wing (Dobzhansky, 1929; Alpatov, 1930).

RESULTS

The culture techniques proved satisfactory and prepupae were obtained ranging in wet weight from 0.72 to 2.15 mg., compared with the range for the earlier work of 0.85–1.70 mg. Moreover, the ranges for the three types overlapped almost completely, being 0.79–1.80 for males, 0.72–2.01 for diploid females, and 1.18–2.15 for the triploid females. Of the 100 prepupae whose oxygen consumption was measured, the results for twenty-five had to be rejected: most of these were cases where the imago failed to emerge, but there were also a few where the flies unfortunately escaped before their type was determined. There was also one intersex. Thirteen males were included, and of the remaining sixty-two, twelve turned out to be triploid females and fifty diploid females. The surface area of forty-eight of the seventy-five was measured successfully, eight of them triploids.

A typical curve for the rate of oxygen consumption per mg. for a single prepupa is presented in Fig. 1: similar curves were obtained in all cases. It follows the expected course for this early stage of the prepupal period (Ellenby, 1938), the rate of oxygen consumption decreasing by between 30 and 40% during the course of the experiment. This agrees well with the previous finding. Of greater interest, however, is the level of oxygen consumption for the different classes of prepupae examined.

As similar curves were obtained in all cases, it is permissible to average, for each experiment, the rate of consumption for the whole experimental period. In Fig. 2 values for mean rate of oxygen consumption per mg. wet weight per hr. are plotted against body weight for all classes examined; Fig. 3 shows the values for oxygen consumption per sq.mm. of puparial surface. (Log. plots, for total oxygen consumption, total surface, and body weight, are shown below, in Fig. 4.) Values, on either standard, are somewhat lower than those already reported for the oxygen consumption of *vestigial* and *wild-type* prepupae (Ellenby, 1945*a*), the earlier mean value for oxygen consumption per sq.mm. of 0.426 comparing with 0.388 for the present series. In fact, the agreement between the series is reasonable, for the present value is based on experiments an hour longer and at a slightly lower temperature.

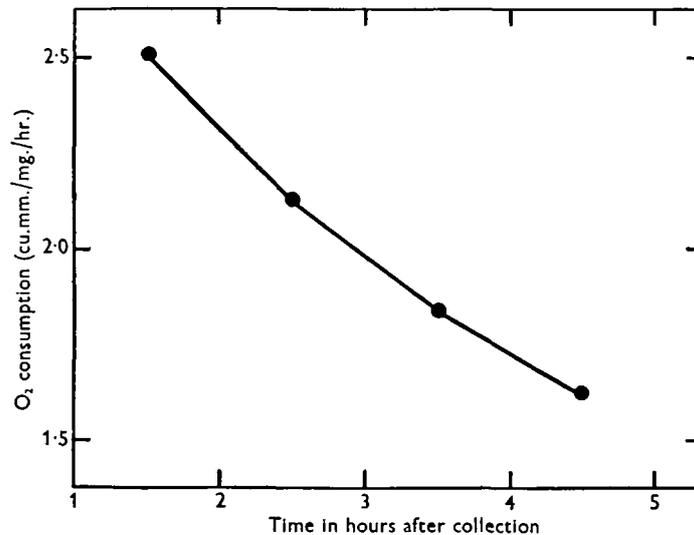


Fig. 1. Oxygen consumption of a single prepupa during the first hours of the pre-pupal period.

Oxygen consumption per unit of body weight falls off steadily with increasing weight; on the other hand, consumption per unit of surface is more constant. But, clearly, despite the variation in the results, and whatever the standard of comparison, there is little difference between triploids and the other classes. On either standard, more of the values for triploids, represented by closed circles, are below the line than above it, more so in the case of the weight standard. But there is little in it, all the triploid values being more or less 'in the thick' of the points for the other forms.

Transformation to a surface basis shows that the regression coefficient for oxygen consumption/sq.mm. and body weight, fitted by the method of least squares, is +0.0236 compared with +0.0141 obtained in the earlier work. Moreover, as the standard error of regression is ± 0.01 the regression coefficient differs significantly from zero (P almost 0.02) showing that, unlike the earlier finding, oxygen

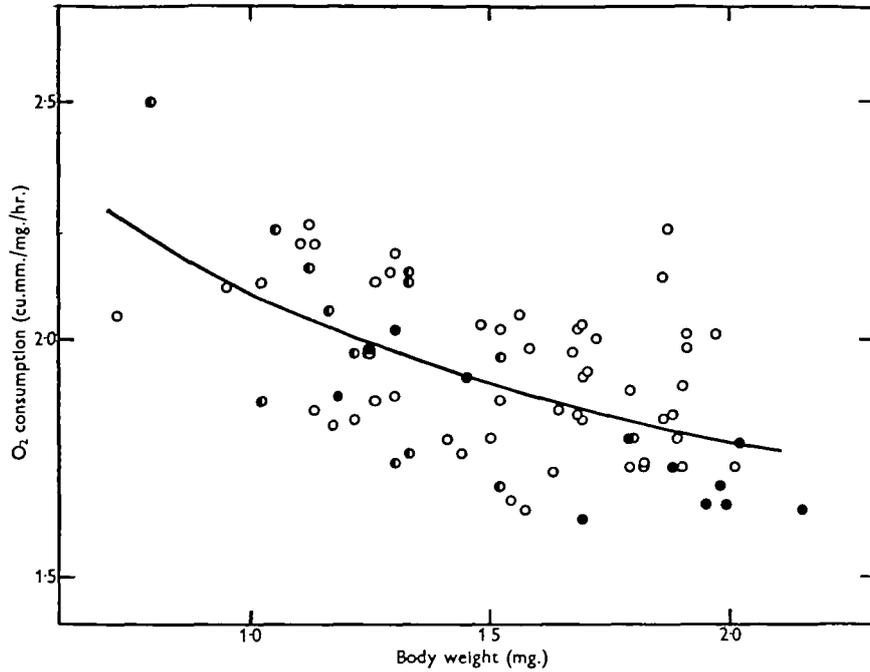


Fig. 2. Oxygen consumption and body weight for three types of prepupae. ○, diploid female; ◐, diploid male; ●, triploid female. Curve fitted by means of a logarithmic transformation.

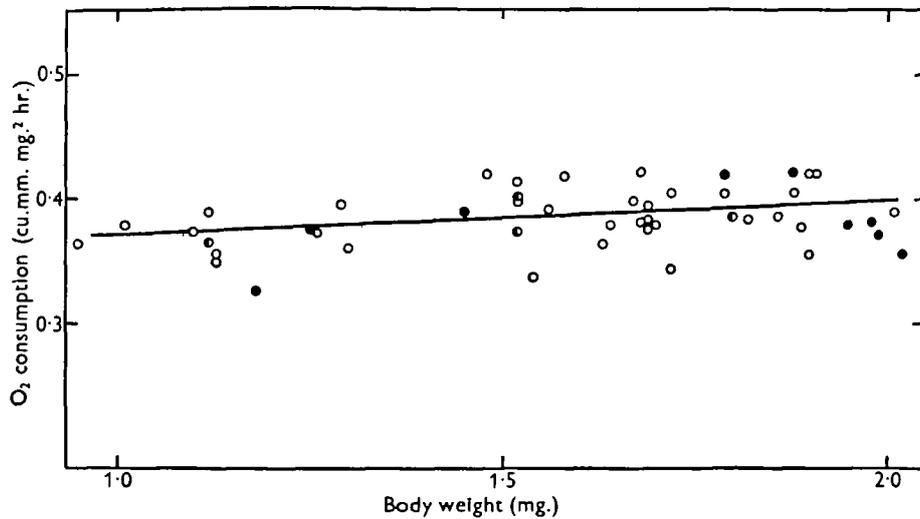


Fig. 3. Oxygen consumption and surface area for three types of prepupae. ○, diploid female; ◐, diploid male; ●, triploid female.

consumption per unit of surface varies significantly with the weight of the animal. The trend with increasing body size is small, amounting to a difference in oxygen consumption per sq.mm. between the extremes of the weight range of about 8%. Nevertheless, a straightforward comparison between mean values for the different classes of prepupae is out of the question. As a covariance analysis was necessary, it seemed desirable to carry it out with the more extensive data for oxygen consumption per unit weight.

The line fitted to the results presented in Fig. 2 was calculated from a logarithmic transformation of both variables. Covariance analysis with the logs. of the values showed that there were no significant differences between the regression coefficients for the different classes of prepupae; for the purposes of the diagram therefore, the curve was drawn from the pooled data. This showed that oxygen consumption per mg. was proportional to the -0.228 power of the body weight. As the weight ranges of the three types overlap almost completely, there is no objection to comparing the rates of oxygen consumption at the mean for all types.

For this the 'within lots' regression coefficient, a coefficient which is a weighted average of those for the separate classes calculated independently, provides the best

Table 1. *Comparison of adjusted means, logs.*

Type	Mean weight (mg.)	Mean O_2 (cu.mm./mg./hr.)	Adjusted mean O_2 (cu.mm./mg./hr.)	s.e. of estimate	Adjusted mean O_2 (cu.mm./mg./hr. antilog.)
Male	0.1113	0.2898	0.2739	± 0.0126	1.879
Female	0.1908	0.2721	0.2737	± 0.0058	1.878
Triploid female	0.2268	0.2491	0.2586	± 0.0126	1.814

estimate of the population regression. In fact, in the present case, its value, at -0.220 , differs little from the value calculated from the pooled data; but it is the correct coefficient to use, and, as a good deal of experimental error is eliminated in this way, its standard error is very much smaller.

Mean values for oxygen consumption and body weight are calculated for each class, the difference of the mean class weight from the grand mean estimated, and then, by means of the 'within lots' regression coefficient, the adjusted mean value for oxygen consumption at the grand mean body weight is estimated; in effect, the class means are 'slid' along the regression line until they correspond to a common value for body weight. It is then possible to estimate whether there are significant differences between the adjusted mean values. The results of such an analysis are shown in Table 1. The test of significance is carried out with log. values as the regression analysis was carried out with a logarithmic transformation, but, for interest, the antilogs. of the adjusted mean values have been included. The agreement in adjusted mean values for male and female is astonishingly good, the two values only differing in the fourth place. Triploid females are slightly lower at 0.2586 (about 4% on the geometric means), but the standard error for difference

from the others is ± 0.0139 showing that the difference is very far from statistical significance. Clearly, the metabolic rates of diploids and triploids must be assumed to be identical.

In view of these findings an extensive investigation of cell size was hardly justified. However, Alpatov (1930) has shown that some slight increase in body size is probably due to increase in cell number: as Dobzhansky (1929) made no allowance for the body size of the animals used in his comparisons of triploid and diploid cell size, it was therefore necessary to ascertain whether, at any particular level of body size, triploid individuals had larger cells than diploids; I have satisfied myself that this is so. Particularly suitable material was provided by two specimens, one a diploid female (no. 49), the other a triploid (no. 76); this is presented in Table 2. The two animals have the same prepupal body weight, and the same puparial surface area. But although their wing dimensions are identical, the cells of the triploid are about one and a half times the size of those of the diploid; nevertheless, their rates of oxygen consumption are the same.

Table 2. *Comparison of diploid and triploid individuals of equal body weight*

Series no.	Type	Body weight (mg.)	Puparial surface (sq.mm.) area	O ₂ consumption (cu.mm./mg./hr.)	Wing length (mm.★)	Wing width (mm.★)	Cells per 0.01 sq.mm.★
49	Diploid ♀	1.25	6.83	1.97	1.65	0.99	58
76	Triploid ♀	1.25	6.60	1.98	1.65	0.99	40

★ Dimensions as defined by Alpatov, 1930.

DISCUSSION

The results clearly show that, after adjustment for differences in body size, there are no differences in the rates of oxygen consumption of triploid female prepupae and either diploid male or female. As triploids have larger cells than diploids of the same body size, cell size has no influence on the general level of metabolism.

The values for oxygen consumption and body weight were used in the analyses which led to the above conclusions rather than values relating oxygen consumption and surface area because they were more extensive; owing to casualties in the measurement of surface area there were only forty-eight values as compared with the seventy-five for oxygen consumption and body weight. However, Fig. 4 shows that the forty-eight surface and oxygen values would have given almost as much information as the seventy-five!

Fig. 4 *a, b* are double logarithmic plots of the values for total oxygen consumption and total surface area (*a*), and total oxygen consumption and body weight (*b*), for the forty-eight animals for which all three values are known: the scales for oxygen consumption are identical, and the scales for the other two variables have been made as equal, relatively, as possible. The agreement with the fitted line is clearly better in the case of the surface relationship but perhaps not so markedly superior as is shown by the regression analyses. After subtracting the variance attributable to

regression, the error mean squares derived are 0.000647 for the oxygen surface relationship, and 0.00084 for the oxygen weight relationship. The error variance of the former is therefore three-quarters of the latter and the forty-eight surface oxygen values give as much information as sixty-four oxygen weight values!

I have already had occasion to point out that a better prediction of the oxygen consumption of the littoral isopod *Ligia oceanica* could be obtained from the square of a fairly inaccurately determined linear dimension than from an accurate determination of body weight (Ellenby, 1951). However, in the present case the animals were reared under very constant culture conditions and spent their entire lives at one temperature; moreover, they were weighed after they had spent 5 hr. in the constant atmosphere of the respirometers. It is therefore most remarkable that, even under such constant conditions, a relatively inaccurate surface-area measurement should enable a better prediction of oxygen consumption to be made than an

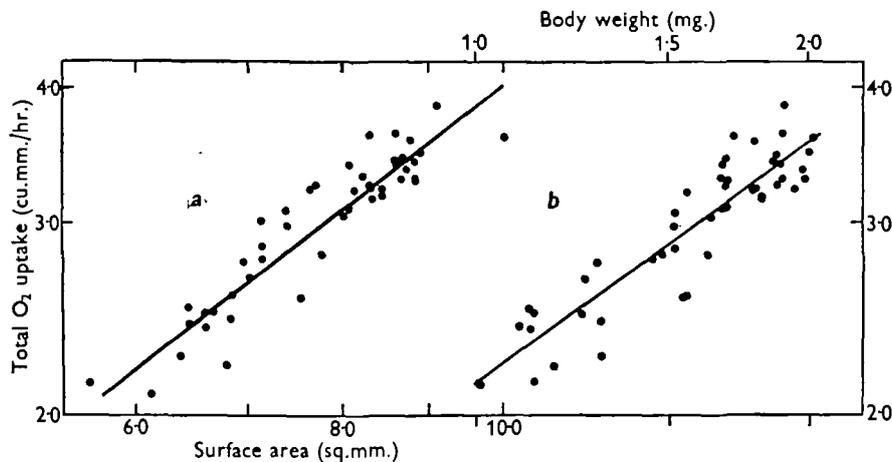


Fig. 4. *a*, total oxygen consumption and total surface area; *b*, total oxygen consumption and body weight. All on double logarithmic grid with comparable scales.

extremely accurate determination of body weight. Hitherto it has been thought that oxygen consumption is proportional to some function of body weight and that the relationship of the former to surface area was due to the relation of surface area itself to body weight; these findings suggest that, in the present case at least, the reverse may be the case; however, a more detailed investigation of this and the *Ligia* material is in progress.

The results of the present investigation differ very markedly from those presented in 1945, for in the present case, the rate of oxygen consumption per unit weight decreases far less rapidly with increasing body size. The two series of values are plotted in the same diagram in Fig. 5, closed circles being used for the earlier series. The series are not exactly comparable; each experiment of the present series lasted longer, reducing the mean value somewhat, and the temperature at which the measurements were carried out was not quite the same. Nevertheless,

these factors would presumably affect the general level of the curve relating rate of oxygen consumption and body weight rather than its slope; in fact, it is clear from the figure that the slopes are markedly different.

The curves presented in Fig. 5 were calculated from the data of each set of results using a logarithmic transformation; they are the lines which fit the two sets of data best if each set is considered to be one population. Each curve depicts the trend of the points very well. The regression coefficients in the two cases for the relationship of oxygen consumption per mg. and body weight are -0.446 and -0.228 for the earlier and present series respectively; total oxygen consumption

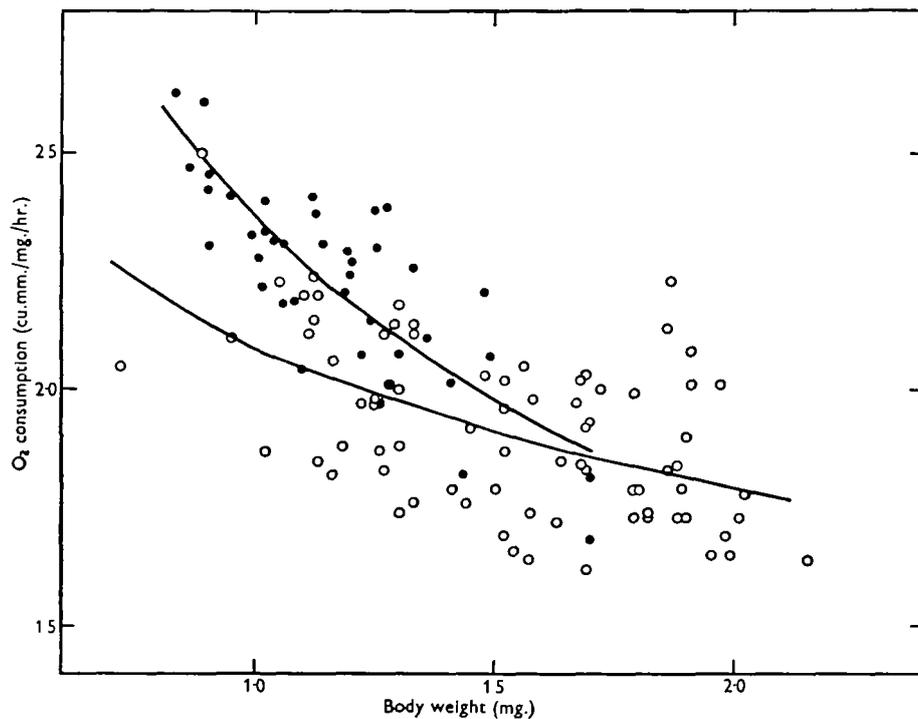


Fig. 5. Oxygen consumption and body weight for two series of experiments. O, present series, all types; ●, earlier series (Ellenby, 1945a), all types. Curves fitted by means of logarithmic transformations.

would then be proportional to the 0.554 and 0.772 power in the two cases. An analysis of variance showed that the difference between the regression coefficients is highly significant the ratio between the appropriate mean squares, being 8.42 giving a value for P , the probability, of 0.01 . However, treating each set of values as one population is not strictly correct; the earlier series includes results for five types of animals, namely *vestigial* male and female, *wild-type* male and female, and the male heterozygote, and the present series is based on *yellow*, *vermillion*, *forked* male, female, and triploid female. Even though the analyses showed that there were no differences between the regression coefficients for each category calculated separately, the best estimate for the population trend for each series is given by the

'within lots' regression coefficients. These were accordingly evaluated; they showed little difference from the values already presented, being -0.475 for the earlier series and -0.220 for the present series compared with -0.446 and -0.228 respectively, slightly increasing the disparity. As the stricter analysis eliminates a good deal of experimental error, the standard error of regression in each case is very greatly reduced, actually to a small fraction of its former size. Whereas the difference between the original regression coefficients was highly significant, with the more precise test it is overwhelmingly so. And all coefficients, precise or less precise, differ significantly from the -0.333 expected on the basis of a $2/3$ power relationship; the value for the earlier series shows that oxygen consumption per unit weight falls off more rapidly, while that for the present series shows it to fall less rapidly than such a relationship would lead one to expect. These, and other relationships are summarized in Table 3.

Table 3

	Present series	Ellenby, 1945 <i>a, b</i>
(1) Regression coeff. of O_2 /mg. on body weight	-0.220	-0.475
(2) S.E. of regression of (1)	± 0.0022	± 0.0044
(3) Relation of total O_2 uptake to body weight	$+0.780$	$+0.525$
(4) Regression coeff. of O_2 uptake/sq.mm. on body weight	$+0.0236$	$+0.0141$
(5) S.E. of regression of (4)	± 0.010	± 0.016
(6) Regression coeff. of surface/mg. on body weight	-0.431	-0.485
(7) S.E. of regression of (6)	± 0.054	± 0.034
(8) Relation of total surface to body weight	$+0.569$	$+0.515$

My earlier paper (Ellenby, 1945 *a*) showed that oxygen consumption was proportional to surface area; surface per mg. bore the most unusual relationship to body weight of the -0.485 power, that is, total surface was proportional to the 0.515 power of body weight (Ellenby, 1945 *b*); the unusual nature of these values is discussed in the original paper and more recently (Ellenby, 1951); although ' $2/3$ ' is seldom obtained, it is rare in the case of either the surface area or oxygen consumption relationship for values to differ from it so much. Nevertheless, the agreement between the regression coefficients for puparial surface area and prepupal oxygen consumption, each in relation to body weight, was very close indeed and the conclusion that oxygen consumption was proportional to surface area gained strength, if anything, from the agreement between two such unusual values. However, the more conventional values obtained in the present case for the oxygen relationship (see Weymouth *et al.* 1945, Kleiber, 1947, Ellenby, 1951), 0.78 for total, and -0.220 for consumption per unit weight, reduces the comfort derived from the mutual support of two atypical values: further inquiry was necessary.

Values for total puparial surface area for the animals examined in the present investigation are presented in Fig. 6: there is reasonable agreement among the data.

Regression analysis showed that surface area per mg. was proportional to the -0.431 power of body weight, or total surface, as presented in the diagram, to the 0.569 power. Agreement with the earlier values of -0.482 and 0.518 is reasonable; as might be expected, the two sets of values do not differ significantly; in fact, an analysis of variance showed that the mean square for error was more than twice as great as the mean square appropriate to the difference between the coefficients. The present value, unlike the earlier one, does not differ significantly from a ' $2/3$ ' relationship, but, of greater interest is the fact that it does differ significantly from the -0.228 power which was found to relate oxygen consumption per unit weight

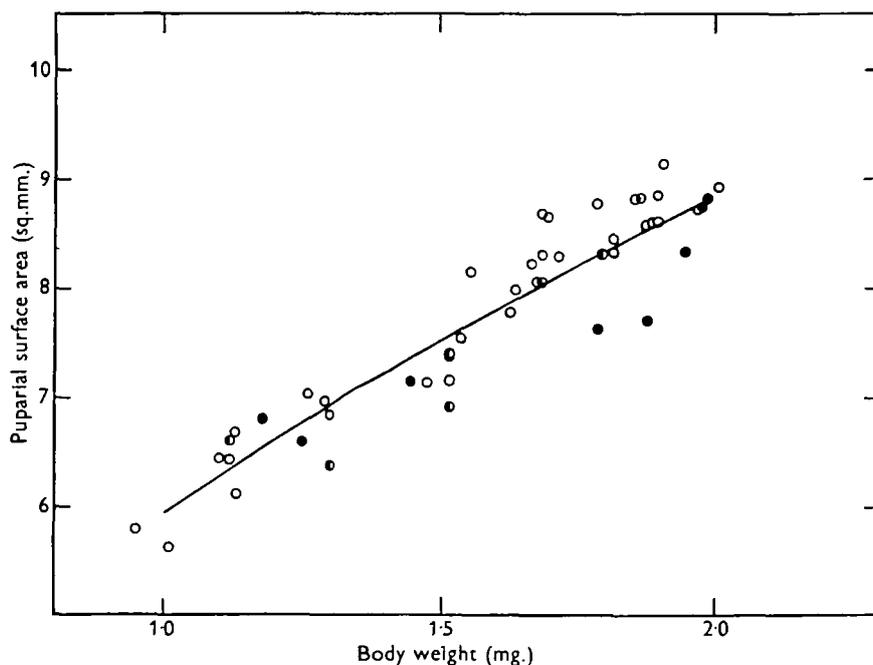


Fig. 6. Puparial surface area and body weight. ○, diploid female; ◐, diploid male; ●, triploid female. Curve fitted by means of logarithmic transformation.

to body weight; with a standard error of regression of ± 0.054 , P is almost 0.001 . The finding that oxygen consumption per unit surface is not constant over the size range (Fig. 3) is thereby confirmed.

The relationship between surface area and body weight is reasonably similar for both sets of determinations; both coefficients relating surface and weight are atypically low and there is no significant difference between them. It is possible, however, that there is some fault in the technique tending to give low surface area measurement for large animals. Measurement of 'surface area' is at best an approximation justifiable only so long as the approximation is similar over the size range. If there were a progressive error of this sort in the method, 'surface' would appear to increase less rapidly than it should, thus giving a low coefficient.

The puparial surface area is measured after the empty puparium is compressed between slides. During the process, a certain amount of distortion takes place, and, clearly, if the relative distortion increases with the size of the animal, a progressive error would be introduced. Now although there is distortion in the compressed puparium, the anterior end is completely undistorted. Part of the anterior end of the puparium is modified into the operculum through which the imago emerges (Strasburger, 1935); it therefore always splits in exactly the same way, the portions previously united to the lateral margins of the operculum being laid out to each side. A comparison of the area of this undistorted portion with that of the whole puparium would therefore be of interest.

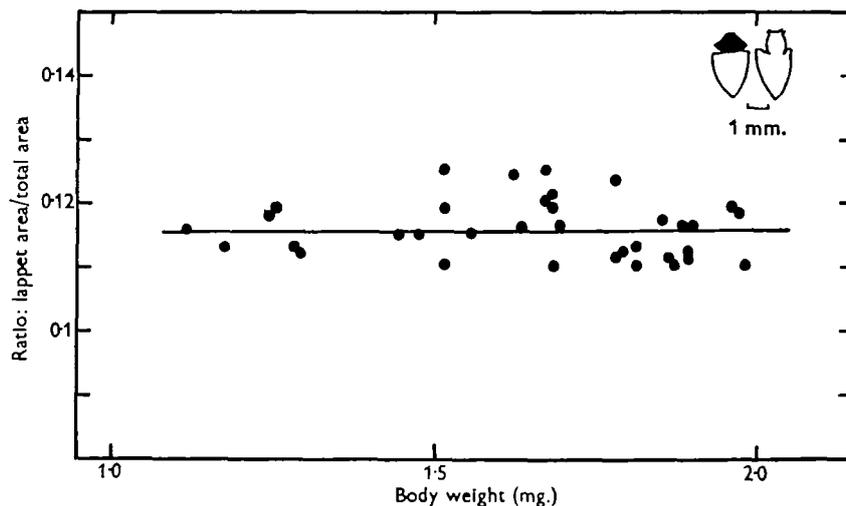


Fig. 7. Inset shows the outline of the projected flattened puparium; black portion is the undistorted anterior, 'lappet', portion. Diagram shows the relationship of the area of this undistorted portion to the area of the whole. The line fitting the data does not differ significantly from the horizontal showing that the degree of distortion produced by the flattening process is approximately constant over the size range.

In Fig. 7 the ratio of the two areas has been plotted against body weight for the thirty-eight cases where the comparison was possible; the areas concerned are the whole area of the inset of Fig. 7 and the black portion alone. Clearly there is no tendency for the values for this relationship to vary with the size of the animal; in fact, the regression coefficient is only $+0.000028$ compared with a mean value of 0.116 . The demonstration is not complete proof that there is no progressive change in the degree of distortion, for the ratio would also be constant if there were an increase in the relative area of the undistorted portion at the same rate as an increase in the distortion of the remainder; but such a coincidence seems unlikely. It seems reasonably certain, therefore, that the comparatively low value for the exponent relating surface area and body weight is a true representation of the relationship and is not due to technical faults.

In both sets of data, then, surface area varies as some power of the body weight

round about 0.55. In the earlier series, oxygen consumption varied at the same rate with increasing body size, while in the present series it was found to vary as the 0.78 power. The discrepancy is most surprising—and most intriguing. Values for the exponent of body weight vary for different animals, but the differences are comparatively slight. In the present case, not only is the difference between the two values large, but it is observed in animals belonging to the same species and, of exactly the same stage. As in the case of the surface area measurements, the simplest explanation would be that the discrepancies were due to faulty experimental procedure; but it is difficult to envisage a technical fault which affected the rate of oxygen consumption progressively with increase in body size. If the low value for the weight exponent had been obtained in the present, rather than the earlier series, one might have thought that a condition of oxygen lack was perhaps built up by the more heavily respiring larger animals in the comparatively restricted volume of the micro-respirometers; there is no basis for such a possibility in the earlier experiments. Moreover, a comparison of the two curves of Fig. 4 would, if anything, add confidence to one's support for the earlier, and atypical, value; the scatter is less and the values follow the trend with considerable fidelity. This is borne out by a comparison of the standard errors of estimate for the regression analyses of log. oxygen consumption per mg. on log. body weight; for the earlier series the value is ± 0.0014 on a mean value of 0.345 compared with the corresponding values in the present series of ± 0.0015 and 0.279. The earlier series at least holds its own.

The earlier experiments were carried out on groups of prepupae and not with single animals as in the present series. As I have already pointed out (Ellenby, 1945*b*), since oxygen consumption is not proportional to weight itself, the total weight of a group of animals will not give a fair estimate of its metabolic potentialities; two groups of the same number of individuals may have the same total weight, but if the size distribution in the two groups is not identical, the total oxygen consumption of the two groups will differ. Moreover, if the distribution is skew in opposite directions for groups of small and large animals, the shape of the curve relating oxygen consumption to body weight will be affected. If groups of small mean weight have an abnormal number of small animals and there is also a tendency for groups of large mean weight to have a non-normal number of large animals, the mean rate of oxygen consumption will be higher than expected for the group of small mean weight, and lower for the group of large mean weight: oxygen consumption per unit weight would then appear to decrease more rapidly than it should. The possibility seemed unlikely, but worthy of consideration in the two series. Examination of the sizes of the puparia making up the groups used in the original surface-area measurements, however, showed them to be reasonably homogeneous and certainly without the progressive change required. Admittedly, these groups are not those used in the original oxygen consumption measurements but they were collected under exactly the same conditions and are undoubtedly comparable, certainly from the present point of view.

But working with groups will have another and, theoretically, more serious

aspect, for it can be shown mathematically* that, if rate of oxygen consumption follows a 'power law', 'mean' oxygen consumption per mg. based on the total consumption of a group of animals will always be less than the true mean based on the values for the individual animals; furthermore, this effect will vary with the absolute size of the animals concerned and so alter the *shape* of the curve relating oxygen consumption and body weight. Fortunately these effects will be small if the groups are reasonably homogeneous, which was almost certainly the case; but it seemed desirable to estimate the possible magnitude of the effect.

In order to test the effect of grouping, the results for the present series of experiments with individual prepupae were arranged in arbitrary groups. For any particular day, the animals would correspond, more or less, with a group used in the earlier series. Groups were kept homogeneous as to type, and animals differing greatly in size were not included in the same group, as such animals undoubtedly did not occur in the groups of the earlier series. Nevertheless, there was a fair variation: for example, the first group contained animals of mean weights 1.97, 1.91, 1.26, 1.69, 1.90, 1.88, and 1.82 mg. The regression coefficient calculated from these synthetic groups with a log. transformation of the values for both mean oxygen consumption and mean weight showed that oxygen consumption per unit weight was proportional to the -0.261 power of the body weight or total consumption to the 0.739 power. These values are little different from those arrived at with the individual items.

There are a number of cases in which the exponent relating oxygen consumption and body weight has been shown to vary for different parts of the weight range (Michal, 1931; Zeuthen, 1947) and for animals raised on different diets (Teissier, 1931). These help little in the present instance for the animals of both series of experiments were raised under identical conditions, and, unlike the other cases, were compared at exactly the same stage of growth. Moreover, Zeuthen's claim that oxygen consumption varies directly with body weight up to 1 mg., only decreasing for animals beyond this size, is also of little help; although the animals of the first series of experiments were, on the whole, of slightly lower weight range than the later series, they are almost all above 1 mg., and, moreover, their oxygen consumption decreases more rapidly than that of the later series. The conclusion that the differences in the exponents obtained in the first and second series are due to differences, not necessarily genetical, in the animals used in the experiments seems inescapable. As, despite their genetical differences, the two sets would have been considered far more uniform than those generally used in metabolic studies, an understanding of the reasons for the different values would probably help to explain why, in fact, metabolic rate does vary with the size of the animal. The results certainly suggest that it is most unwise to assume any particular value for the exponent for a particular uninvestigated case.

* I am very grateful to Dr D. A. Evans for demonstrating this to me.

SUMMARY

1. The oxygen consumption and surface area of individual diploid and triploid prepupae of *Drosophila melanogaster* have been measured, the cells of triploid animals being larger.

2. The mean weights for the types examined are different but their ranges overlap almost completely. By covariance analysis it is shown that, after adjustment for difference in body size, there are no differences in the rates of oxygen consumption. It is concluded that, for these animals, cell size has no influence on the rate of oxygen consumption.

3. The relationships between body weight, surface area, and oxygen consumption have been further investigated. It is shown that, despite the greater inaccuracy of the method by which surface area is determined, oxygen consumption can be predicted more accurately from surface area than from body weight.

4. The results are discussed in relation to an earlier investigation of the oxygen consumption of other genotypes (Ellenby, 1945*a, b*). Possible technical causes of certain differences between the two series of results in the relationship of oxygen consumption and body weight are explored; it is concluded, however, that they are almost certainly due to differences, not necessarily genetical, between the animals used in the two series.

The experiments on which this paper is based were carried out in 1938 in the Department of Zoology, University College, London. I am most grateful to Dr Sarah B. Pipkin for the patience with which she instructed me in the techniques of handling a triploid stock.

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