THE RATE OF THERMAL ACCLIMATION IN THE GARDEN SLUG <u>LIMAX</u> <u>FLAVUS</u> <u>L</u>.

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Approved for the Major Department

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THE RATE OF THERMAL ACCLIMATION IN THE GARDEN SLUG *LIMAX FLAVUS* L.

INTRODUCTION

The classical assumption that physiological activity rates of poikilothermic animals fluctuate according to the thermal environment has undergone a revision during recent years. There has developed during the last three decades an extensive and growing body of evidence in support of the thesis that many poikilothermic animals can, to a considerable degree, regulate their physiological activity rates by homeostatic mechanisms of various kinds. Thus, many poikilotherms have gained a striking degree of independence from variations of the thermal environment by this compensatory process which has become known as thermal acclimation (see definition p. 5).

The experimental evidence of thermal acclimation, both field and laboratory, has been discussed by the comprehensive reviews of Bullock (1955) and Fry (1958) on temperature acclimation, and Prosser (1955), who summarizes evidence not only of temperature compensation, but of adaptation to oxygen tension, osmotic pressure, and drugs as well. A recent and thorough discussion of all aspects of physiological adaptation may be found in Prosser (1958a).

Early investigators in this area of temperature acclimation were interested in determining the existence of acclimation per se and little emphasis was placed on the controlling mechanisms. Most of the recent investigators, however, have been concerned with these mechanisms (Prosser 1958a; Precht 1955).

As the interest in the mechanisms controlling temperature acclimation has increased, the speed with which an animal compensates for variations in external temperature, by altering the rates of internal reactions, has assumed increasing importance. This rate, or time course, serves as a possible guide to the types of controlling mechanisms i.e. nervous, enzymatic, hormonal, or a combination of these (Fisher 1958; Grainger 1958). The adaptation rate has in addition become of increasing importance to the comparative physiologist in determining as Prosser (1958b) states, "Whether certain basic patterns of response are common and whether others are peculiar to particular organisms."

While many workers in the past have failed to determine or report the time course in their temperature acclimation experiments, the rate of acclimation in the case of fish has been fairly well established as being of the order of days (Wells 1935a,b,c,; Sumner and Wells 1935; Sumner and Doudoroff 1938; Doudoroff 1942, 1945; Brett 1956; Meuwis and Heuts 1957). Grainger (1956) has reported the time course of heat tolerance acclimation for certain small crustaceans as being of the order of a few hours. Segal (1956) in a field transplant study of the limpet Acmaea limulata reported acclimation to be 70 percent completed in 14 days. Full compensation to both warm and cold temperatures in 2 weeks was reported by Roberts (1957) for the crab Pachygrapsus crassipes. Ohsawa (1956) reported acclimation of heat tolerance was completed in 48 hours in the periwinkle Nodilittorina granularis.

Time course information is needed particularly among certain groups of organisms, such as the terrestrial poikilotherms, where there is a paucity of information regarding the rate of compensation. In the few clear cases where temperature acclimation of insects has been reported, the time course of change appears to occur in one day or less (Mellanby 1939, 1954; Colhoun 1954). Mellanby (1940) reported the heart beat of an adult amphibian to be fully acclimated in one day to chill immobilization. There have been several reports of thermal acclimation of reptiles in the literature (Gelineo and Gelineo 1955; Dawson and Bartholomew 1956) but in none of these was the time course clearly established. In view of its importance to the physiologist and since many investigators have failed to determine the time course in their temperature acclimation experiments, there is a definite need of additional information in this area.

The purpose of this experimental investigation was twofold. First, to verify the data of Segal (1959) that the garden slug Limax flavus L. shows temperature acclimation of its oxygen consumption over a range of 10° to 30°C. Second, to determine the time course of this acclimation, to both high and low temperatures, over the same temperature range. Such information, it was felt, would add to our present knowledge of thermal acclimation in general and more specifically would provide additional evidence as to rate of acclimation in one species of terrestrial cold blood.

The variety of terms and their meanings which has developed in the area of physiological variation necessitates the definition of any terms used to describe the various phenomena. Prosser (1958c) states:

> Throughout this symposium a reluctance to define physiological adaptation has been evident. This is understandable because "adaptation" like the term "general physiology," has a different meaning for everyone who uses it. We have purposely omitted sensory adaptation, but have included as adaptation any alteration or response of an organism which favors its survival in a changed environment, hence is "useful" in a strictly objective sense. Thus an adaptation can be either morphological or physiological. When the term is applied to a population of organisms or a species, an adaptation may be genetically determined; when applied to individual organisms an adaptation may be environmentally induced. . . . Whether a response or alteration in an organism is "useful," that is favors survival, cannot always be readily decided.

It is with the above meaning that the term adaptation will be used at present. As such it will include the terms "acclimatization" and "acclimation." The literature is burdened with a variety of usages for these two terms including several which make important distinctions between the two forms of the word. Prosser (1955) states:

> Acclimation has been used for phenotypic and acclimatization for genotypic adaptive alterations. Acclimation has been used for changes induced phenotypically in the laboratory by varying a single environmental factor and acclimatization for changes induced under natural conditions where the critical environmental factors are less well known. These distinctions may ultimately be very useful, but in practice it is rarely possible to distinguish phenotypic from genotypic change or to know whether a single factor may be the important environmental variant.

To avoid confusion between the terms "acclimatization" and "acclimation" only the latter term will be used herein and will include all types of compensatory change to a changed environment with the exception of extremely rapid compensation (sensory) measured in minutes.

MATERIALS AND METHODS

Materials

The tawny garden slug *Limax flavus* L. is a terrestrial gastropod mollusc of the family *Limacidae*. It is a native of temperate Europe introduced into this country where it is now a widely spread urban slug. The body of this large slug is yellowish gray above, with many irregular spots of clear yellow on the shield, oval yellow rugai on the body and bluish tentacles (Pilsbry 1948). Several adult specimens collected in Emporia, Kansas had a length of almost 6 inches and weighed 10 to 14 grams but the average adult size is about 3 to 4 inches weighing 6 to 9 grams.

Limax flavus is nocturnal, as are most slugs, and thrives in dark and damp locations, such as those under old decaying boards and logs, beneath damp refuse, and in cellars and wells (Kingsley 1885). Food consists largely of vegetation but it has been reported in Europe (Cooke 1895) as a pest in stored meal and flour. The population maintained in this laboratory has thrived on a diet consisting only of white bread, although they may also have been eating fungi off the fir bark substrate on which they were kept.

Limax flavus was selected as the experimental animal because (1) it was available in large numbers, (2) the animal is subjected to diurnal fluctuations of temperature in its natural environment, and (3) information regarding thermal acclimation in this species was available from previous studies in this laboratory.

The animals selected for this experiment were the descendents of an original population collected in Emporia, Kansas and brought into the laboratory during the summer of 1957. The individuals used in these experiments were hatched during the fall and winter of 1958 and ranged in age from 5 to 7 months and in weight from 0.40 to 1.18 gm. They were maintained from hatching until the beginning of these experiments in two Labline refrigerated incubators at a constant temperature of 10° ± 1.0°C. or 20° ± 1.0°C. under constant light conditions of 11 hours of light and 13 hours dark with 14 watt fluorescent lamps used as a light source. Animals used in the experiments were transferred to constant dark for the duration of the study. Every 2 or 3 days animals were exposed to dim light for the short period of time required to introduce new dried bread and water and to remove uneaten bread.

Respiration rates were measured in 12 modified Wennesland-Scholander, compensated volumetric, plastic-block microrespirometers, which are a closed system type, not affected by changes in barometric pressure. This instrument (Plate 1a) allowed a total volume displacement of approximately 1124 microliters (µl.) divided into 900 units of

Plate 1. Modified Wennesland-Scholander, plastic-block microrespirometer used to determine rate of oxygen consumption. (a) Intact respirometer; (b) Animal chamber; (c) KOH container; (d) Compensation chamber.

1.25 µl. each by a Veeder-Root counter. In lieu of smaller chambers, the 135 ml. volume of the animal chamber (Plate 1b) was reduced to 30 ml. by the addition of paraffin for greater sensitivity of measurement for the small animals utilized in these experiments. Carbon dioxide was absorbed by a small container (Plate 1c) of 10 percent potassium hydroxide placed in the animal chamber. During measurements the respirometers were immersed in a 20 gal. aquarium (Plate 2a) in which the water was maintained at the selected temperature ± 0.2°C. by circulation through a Wilkens-Anderson 40 gallon low temperature water bath (Plate 2b).

Weight of animals to the nearest 0.01 gram was obtained on an Ohaus triple beam balance after the animals had previously been blot-dried with soft cloth.

Methods

It previously had been determined in this laboratory (Segal 1959) that L. flavus shows temperature acclimation of its oxygen consumption over a range of 10°C. to 30°C. While the complete physiological range of this species was unknown, animals of all ages had been maintained in this laboratory at constant temperatures of 10°C. and 30°C. for periods of 6 months and longer. It should be noted, however, that while a constant temperature of 30°C. did not appear to depress the respiratory rate, it did cause the cessation of

Plate 2. Equipment used to maintain the microrespirometers at constant temperature during rate measurements. (a) Forty gallon aquarium with respirometers immersed; (b) Low temperature water bath.

egg laying and a sharp decrease in food consumption. With this information in mind, temperatures of 10°, 20°, and 30°C. were selected as experimental acclimation temperatures and the intermediate temperature of 20°C. was chosen as the rate measurement temperature.

Assuming that L. *flavus*, due to its normally nocturnal habit, would be more quiescent in constant light, all rate measurements were made in constant dim light under 100 percent relative humidity conditions. To avoid possible diurnal rhythm effects all measurements were made during the same period of day, 11 a.m. to 3 p.m. No attempt was made to withhold food from the animals for a period prior to respiratory measurements for it was found that 30°C. animals, which ate very little, did not show less scatter around the rate line. This suggested that the variation in oxygen consumption due to digestive action did not appreciably contribute to the scatter.

In measuring oxygen consumption the respirometers, each containing one animal, were immersed in a constant temperature bath. It was found that 30 minutes sufficed for thermal equilibration with the respirometers open to the atmosphere. A minimum of 1.5 hours was allowed for the animals to become quiescent but on several occasions up to 3 hours was required. At the end of the equilibration period, the manometer openings were closed and the oxygen consumption recorded.

Oxygen consumption measurements were made every 30 minutes or hourly, depending on the consumption rate, for a minimum of 3 hours. During each experiment one respirometer was run empty as a thermobarometer, and any variation, which was normally less than 1 percent of the consumption of a single animal, was added or subtracted from the respirometer readings to give more accurate oxygen consumption of the animals. At the termination of an experiment the animals were removed from the chambers, blot-dried, and weighed to the nearest 0.01 gram.

Respiration rates are given in microliters (µl.) of oxygen consumed per gram of wet weight per hour. Individual rate data were discarded if there was continuous or nearly continuous activity of animals during the experimental period or if very high or very low values were obtained which were later shown to be due to injury or death of the animal.

One hundred and fifty animals of the selected weight range were randomly divided into six groups of 25 animals each at the beginning of the experiments. Four groups were selected from animals with a thermal history of 20°C. and two groups from animals previously maintained at 10°C. The two 10°C. thermal history groups were placed at a holding temperature of 10° ± 1.0°C. while the four 20°C. thermal history groups were divided, two being placed at 20° ± 1.0°C. and the remaining two at 30°C.

All groups were allowed to acclimate to their respective temperatures for a minimum of one week under conditions of 100 percent relative humidity and in constant darkness to avoid any possible photoperiod effect. At the end of one week the oxygen consumption of 20 to 40 individuals from each temperature was measured at 20° ± 0.2°C. To determine if these rates constituted complete acclimation to the various temperatures, respiration rates were remeasured after a period of several days to insure that no further change in the rate level had taken place.

After the respiration rates of animals acclimated to 10°, 20°, and 30°C. were established, a second series of experiments was begun to determine the rate of thermal acclimation. These experiments were divided into three phases. First, a group of animals acclimated to 20°C. were transferred to the 30°C. incubator for periods of 6, 18, 24, and 36 hours. After each acclimation period the respiratory rates of from 20 to 40 animals were measured, in dim light, at 20°C. Next, another group of animals acclimated to 20°C. were transferred to 10°C. for periods of 12 and 24 hours and the respiration rates of a minimum of 20 animals were measured at 20°C. at those times. Lastly, animals acclimated to 10°C. were transferred to 20°C. for periods of 12 and 24 hours with the rate of at least 20 animals measured at 20°C. at those times. The 20°-10°C. and 20°-30°C. transfers were made by transferring the animal boxes between incubators. It was found that the equilibration of the internal temperature of these boxes was completed in 30 to 45 minutes after a transfer was made. The 10°-20°C. transfer animals were acclimated in the respirometers at the water bath temperature (20°C.). Thirty minutes prior to the end of an acclimation period the manometer openings were closed and three 30-minute respiration readings recorded. The average of these three readings was considered the respiratory level after exposure to that particular acclimation period.

Treatment of data

The statistical measures employed were as outlined by Johnson (1949). Weight-regression curves were fitted by the method of least squares. The regression coefficient, (b_{yx}), was calculated to obtain the slope and the standard error of this coefficient (s_b) was utilized as a measure of variation. To test the significance of the weight-regression curves, the experimental results were compared to the t-distribution table (Fisher and Yates 1949) with the t_o value of each curve being obtained as a ratio of the regression coefficient (b_{yx}) to its standard error (s_b). Probability values of $P<.05$ from the t-table were considered statistically significant.

The respiration-weight data were plotted logarithmically so that equal percentage deviation was shown by equal spatial spread from the weight-regression curves. Weight-corrected points representing the oxygen consumption rate at each time-temperature condition were obtained by utilizing the points of intersection of a perpendicular erected at the arbitrarily selected weight of 0.7 gm. on the weight-regression curves.

RESULTS

Effect of thermal acclimation

When the respiration rates were measured at 20°C. it was found that animals from low temperatures had a higher rate of oxygen uptake than high temperature ones. Respiration rates of three groups of animals, acclimated for one week or longer at temperatures of 10°, 20°, and 30°C. respectively, were measured at 20°C. and a comparison of the weight-regression curves of these three groups is shown in Figure 1. Over the weight range measured, the 10°C. animals showed approximately 25 percent higher oxygen uptake than the 20°C. animals and 66 percent higher than the 30°C. group while the animals acclimated to 20°C. had a 33 percent higher rate than the 30°C. individuals.

While the acclimation temperatures affected the position of the weight-regression curves, there appeared to be little difference in the slope of these curves as measured by the regression coefficients. These coefficients (Table I) were -.1458, -.1780, and -.0893 for the 10°, 20°, and 30°C. groups respectively. Considering the variation about these regression lines as shown by the standard errors of .0660, .0596, and .1003 (Table I), these differences in slope were insignificant.

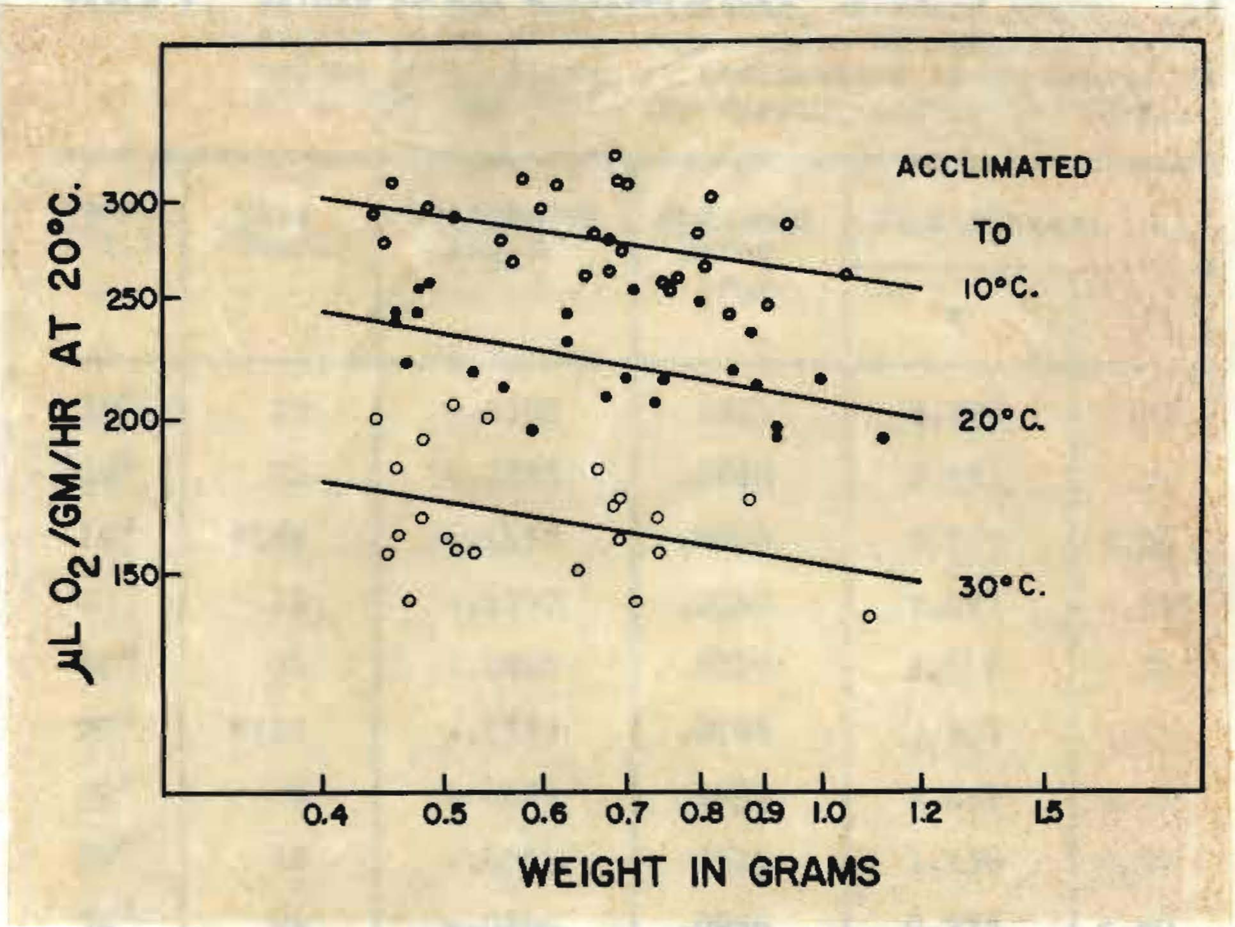

Figure 1. Regression of weight-specific oxygen consumption in *L. flavus* at 20°C. after acclimation to 10° (o), 20° (●), and 30°C. (○).

TABLE I. Values of the b-coefficients, standard errors, and significance of the regressions of respiration on weight in *L. flavus* at acclimation temperatures of 10°, 20°, and 30°C. for varying periods of time.

Temp. ($^\circ$C.)	Time (Hours)	Regression (b_{yx})	Standard Error (s_b)	Significance (b_{yx})	
				t	P
10°	12	-.2303	.0841	2.735	<.02
10°	24	-.1731	.0668	2.591	<.03
10°	*168	-.1458	.0660	2.209	<.03
20°	12	-.1239	.0560	2.211	<.03
20°	24	-.0840	.0719	1.168	<.30
20°	*168	-.1780	.0596	2.986	<.01
30°	6	-.0450	.1033	0.453	<.70
30°	18	-.0594	.0514	1.156	<.30
30°	24	-.0264	.0958	0.275	<.80
30°	36	+.0203	.0773	0.263	<.80
30°	*168	-.0893	.1003	0.894	<.40

*Sufficient for complete acclimation .

Effect of body size on oxygen consumption

Size, as measured by wet weight, appeared to be an important factor affecting the physiological rates in this investigation. Within the weight range of 0.4 to 1.2 gm., larger animals showed consistently slower respiratory rates at all temperatures from 10° to 30°C. (Fig. 1).

The small variations in the weight-regressions and the narrowness of the weight range utilized prevent the use of any single expression to fully describe the effect of weight on rate in this species. In general, however, the smaller (0.4 gm.) animals had approximately 27.5 percent higher oxygen uptake per gram of body weight than did the large (1.2 gm.) animals when fully acclimated to temperatures of 10°, 20°, and 30°C.

In the weight range measured, the weight-regressions of fully acclimated animals (Fig. 1) appeared to be linear. A comparison of the distributions about the regression lines with the *t*-distribution table showed the regression coefficients of animals acclimated to 10° and 20°C. to be significant at the 5-percent level. The regression coefficient for animals acclimated to 30°C. due to considerably greater scatter about the line, was found to be statistically insignificant (Table I). The cause of this greater variation among animals placed at 30°C. is not clear. It is assumed, based on the knowledge that feeding activity is curtailed and that egg laying ceases, that this temperature is at the extreme end of the physiological range for this species and that certain stress mechanisms may be coming into play producing greater individual variation.

Effect of body size on the rate of acclimation

Within the weight range of 0.4 to 1.2 gm. size proved to be an important factor in determining the speed at which L. flavus adapted to a new temperature. Small animals consistently acclimated more rapidly after a temperature change than did large animals whether the new temperature was higher or lower than that to which the animals had previously been adapted.

After establishing the respiratory rate levels for animals fully acclimated to temperatures of 10°, 20°, and 30°C., animals from the first two groups were placed at higher and lower temperatures to determine the time course of acclimation to a new temperature. Because animals maintained at 30°C. had shown a sharp decrease in feeding activity, no attempt was made to use these animals in the time course experiments because of possible starvation effects.

The logarithmically plotted rate-weight curves of 10°C. acclimated animals placed at 20°C. for periods of 12 and 24 hours; of 20°C. animals at 10°C. for 12 and 24 hours;

and of 20°C. animals at 30°C. for 6, 18, 24, and 36 hours are shown in Figure 2A, 2B, and 2C respectively. In general, the more rapid adaptation of the smaller animals was reflected by a change of the weight-regressions during the period of acclimation to the new temperature. When animals fully acclimated to 10°C. were placed at 20°C. (Fig. 2A) the regression of -.1458 (Table I) was reduced to -.1239 after 12 hours at 20°C. and to -.0840 after 24 hours. Similar results were obtained when 20°C. acclimated animals were transferred to 30°C. (Fig. 2C) the slope decreasing from -.1780 to -.0264 after 24 hours and becoming +.0203 after 36 hours. When 20°C. animals were transferred to the lower temperature of 10°C. (Fig. 2B) the more rapid acclimation of the small animals caused an increase in the weight-regression from -.1780 to -.2303 after 12 hours exposure.

While in all three temperature transfers the weight-regressions changed during acclimation to the new temperatures, they were essentially equal when the animals reached a steady respiratory rate i.e. were fully acclimated to the new temperature (Figs. 1 and 2).

With one exception (20°C. animals at 10°C. for 24 hours), the weight-regressions during the periods of acclimation to 10° and 20°C. (Figs. 2A and 2B) were found to be significant at the 5-percent level (Table I) when compared

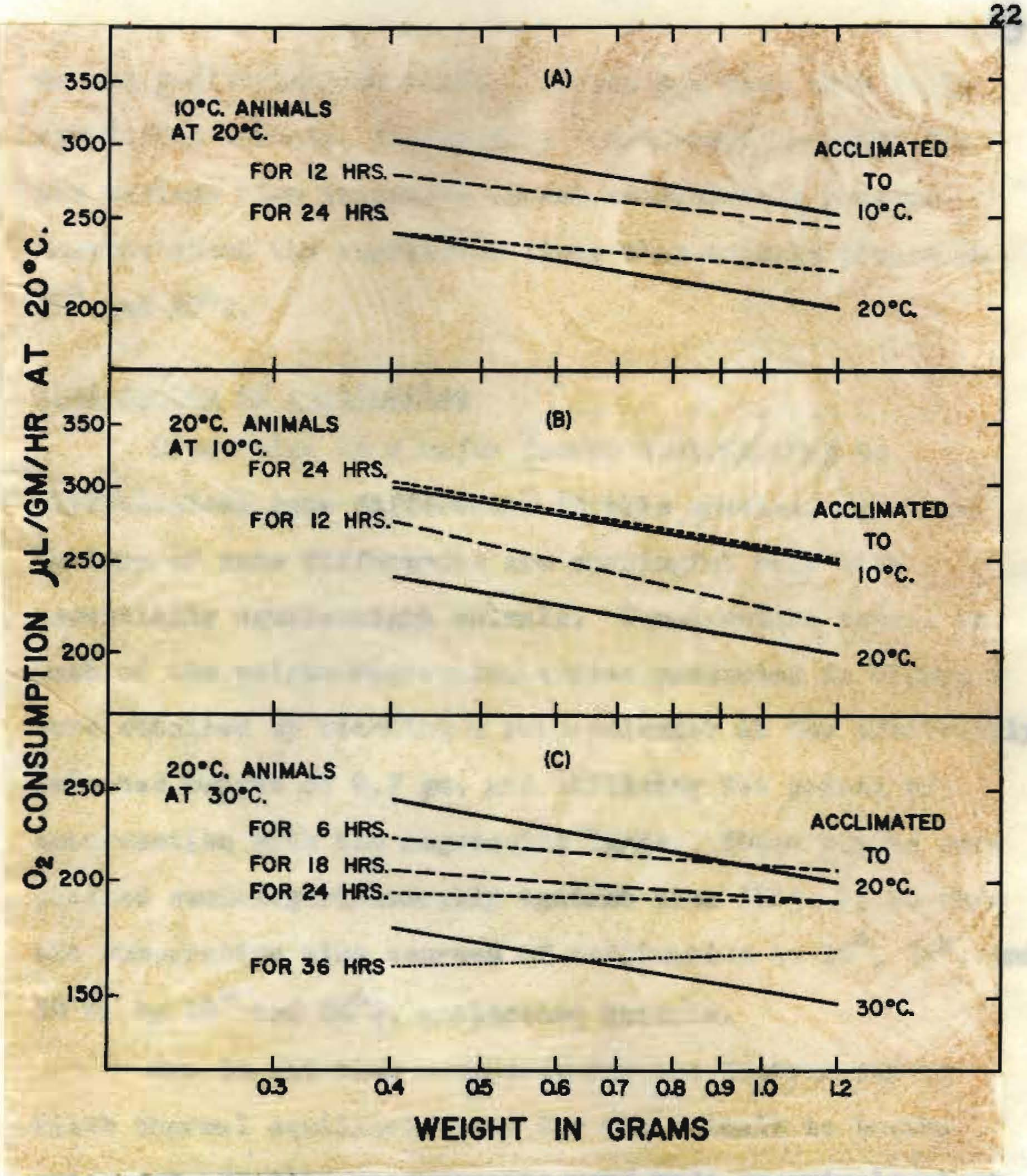

Figure 2. Regressions of weight-specific oxygen consumption at 20°C. for L. flavus: (A) acclimated to 10° and 20°C. and after transfer to 20°C. for 12 and 24 hours; (B) acclimated to 10° and 20°C. and after transfer to 10°C. for 12 and 24 hours; and (C) acclimated to 20° and 30°C. and after transfer to 30°C. for 6, 18, 24, and 36 hours. Each line represents 17 to 31 animals. Solid lines are base lines from Figure 1.

to the *t*-distribution table. As was the case with fully acclimated animals, individuals transferred to 30°C. for the various time intervals showed considerably greater scatter about the regression lines than animals placed at 10° and 20°C.

Time course of acclimation

Since size is a major factor contributing to physiological rate differences in this species, any comparison of rate differences are meaningful only with essentially equal-weight animals. Equal-weight points on each of the weight-regression curves presented in Figure 2 were obtained by erecting a perpendicular at the arbitrarily selected weight of 0.7 gm. and utilizing its points of intersection with the regression lines. These points were plotted semi-logarithmically against time (Fig. 3) to show the comparative time courses of acclimation to 10°, 20°, and 30°C. by 10° and 20°C. acclimated animals.

Due to the time necessary for the instruments to reach thermal equilibrium and for the animals to become quiescent, no rate measurements were made on animals short of 6 hours exposure to a new temperature. Any overshoot phenomenon during the first hours of exposure to a new temperature were thus not disclosed by the techniques used in this investigation.

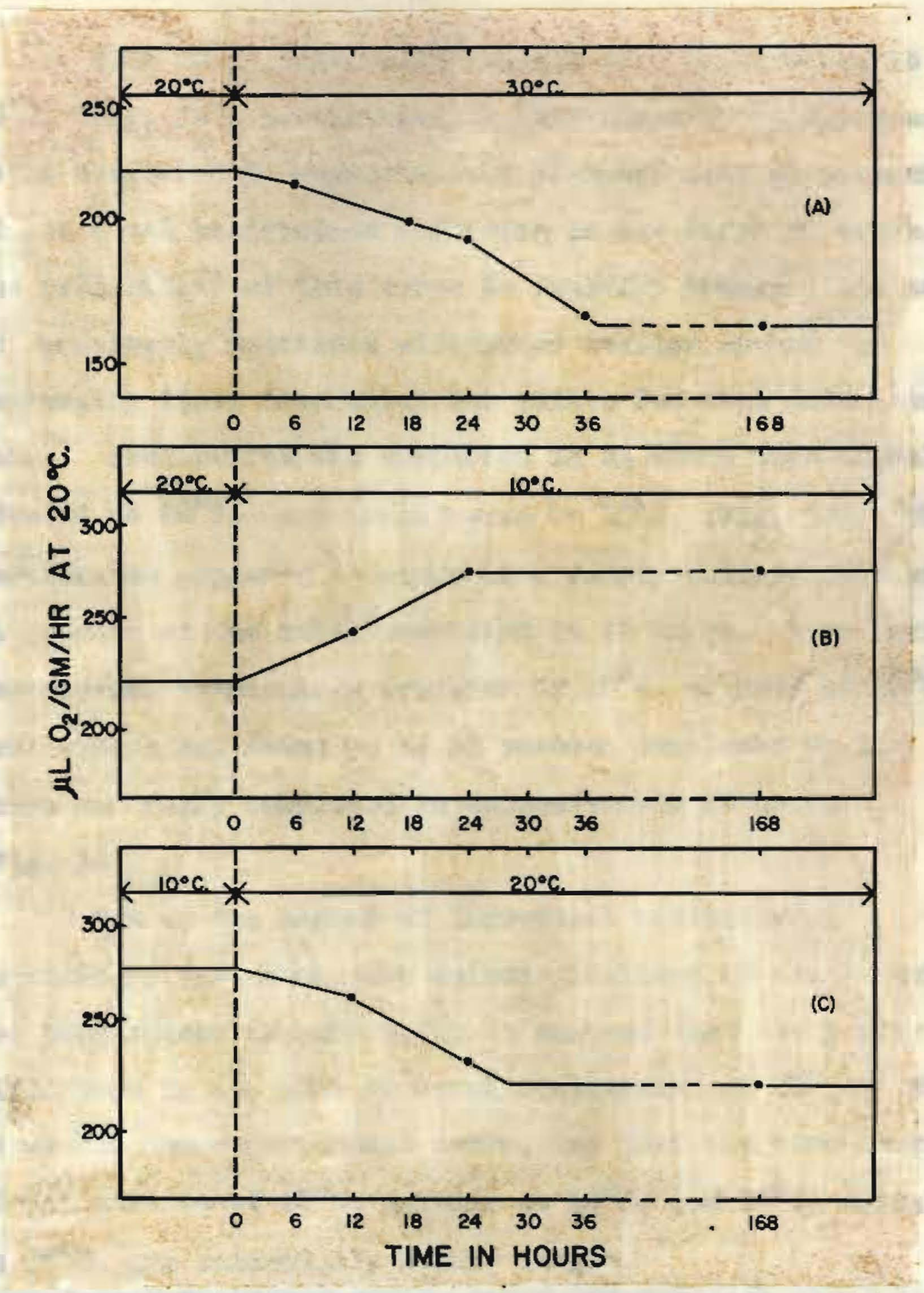

Figure 3. Acclimation of weight-specific oxygen consumption at 20°C. as a function of time for L. flavus transferred: (A) from 20° to 30°C.; (B) from 20° to 10°C.; and (C) from 10° to 20°C. Points represent equal-weight (0.7 gm.) animals taken from the weight-regression curves in Figure 2.

When 20°C. acclimated animals were transferred to 30°C. (Fig. 3A), acclimation to this temperature appeared to be completed in approximately 36 hours with 48 percent of the total acclimation occurring in the first 24 hours. The reliability of this curve is doubtful however, due to the previously mentioned widespread scatter around the regression lines from which the points for this curve were taken. Acclimation was completed in 24 hours when animals adapted to 20°C. were transferred to 10°C. (Fig. 3B). The acclimation appeared to occur at a fairly uniform rate with 44 percent of the total completed in 12 hours. Upon making the reverse temperature transfer of 10°C. animals to 20°C., acclimation was found to be 82 percent completed in 24 hours and fully completed in approximately 28 hours (Fig. 3C).

Due to the amount of individual variation in respiratory rate among the animals involved in the latter two temperature transfers, it is assumed that the 3 hour difference in the time to total acclimation at 10° and 20°C. is within the experimental error, and that the time courses of acclimation of 20°C. animals at 10°C. and 10°C. animals at 20°C. are essentially mirror images.

DISCUSSION

Acclimation of Respiratory Rate

The results of this investigation support the findings of Segal (1959) that the slug L. flavus shows compensatory change of its metabolic rate under changing temperature conditions. Cold-adapted animals show a higher respiration rate at 20°C. than warm-adapted individuals. Such compensatory responses to temperature by poikilotherms have been shown for many widely divergent groups and are well documented in the reviews on this subject by Bullock (1955), Fry (1958), and Prosser (1955, 1958a). This thermal acclimation accomplishes, within limits, the same results that homiothermism accomplishes for the warm bloods in increasing the degree of environmental freedom which the animal enjoys.

Compensations to thermal stress by altering internal rates may go far in explaining the survival and distribution of many cold bloods and are of considerable ecological significance in habitat selection and in establishing range limits. It seems quite probable that temperature acclimation, as shown in this investigation, acting as a homiostatic mechanism would extend the activity range and affect the habitat selection of L. flavus. Whether this is actually the case, however, could be established only by comparing the results of these laboratory findings with field investigations.

To determine if a given example of thermal acclimation is phenotypic or genotypic in nature, it is necessary to show whether the rate character measured is, in time, readily reversible under changing-temperature conditions. If reversibility is shown, we may say that the ability to compensate is acquired during the ontogeny of the individual i.e. is phenotypic in nature. The changing-temperature conditions may be artificially imposed by laboratory acclimation, as was done in this investigation, or by transplantation, as for example, was done by Rao (1953) and Segal (1956).

When cold adapted L. flavus were placed at a higher temperature it was found that the animals acclimated by decreasing their metabolic rates over a 24-hour period to a new lower level (Fig. 3C). This acclimation could be reversed, as was shown by the results given in Figure 3B, by returning the now warm-adapted animals to the colder temperature whereupon their metabolic rate increased to its original level. Thus, we may conclude that in the thermal acclimation of L. flavus we are dealing with the phenomenon of individual (phenotypic) adjustment to temperature change.

Influence of Weight on Respiratory Rate

The regression of metabolism with body weight as shown in Figure 1 is a rather general phenomenon which has been reported for a number of animal groups by numerous authors some of whom have discussed the phenomenon in detail (see Weymouth et al 1944; Ellenby 1951; Zeuthen 1953).

While no attempt has been made herein to establish a single expression to describe the effect of weight on metabolic rate, the data shows a significant decrease in oxygen uptake per gram with increasing weight. These findings add support to the evidence that the dependance of metabolism on body size is an important variable which must be taken into account when comparing the physiological rates of animals differing in weight.

Influence of Weight on Acclimation

Since the weight-regression curves of *L. flavus* fully acclimated to 10°, 20°, and 30°C. were essentially parallel over the weight range measured, it would seem logical that if animals from any one of these groups were transferred to one of the other temperatures the time course of acclimation to the new temperature would be the same for any weight animal. This, however, was not found to be the case as indicated by the results shown in Figure 2 wherein small animals are found to acclimate faster after a 10°C. temperature change.

Several workers, for example Rao and Bullock (1954), have demonstrated cases of changing temperature sensitivity with variation in animal size as indicated by a change in slope in the common rate-temperature curve; but at no place in the literature examined has there been found a report of animal size affecting the time course of acclimation as shown here. Whether this more rapid acclimation of the small animals is necessarily adaptive in nature cannot be stated.

There are several possibilities as to the cause of this phenomenon if we assume that the same controlling mechanisms are working over the entire weight range. First, it seems entirely possible that the difference in time course is due to an increase in the surface-to-volume ratio with a decrease in size. Thus, smaller animals with a greater surface-to-volume ratio would presumably be affected more rapidly by a change in temperature. This could be verified by taking linear measurements of various size animals and correlating the rate of change in surface-to-volume ratio with the rate of change in the time course of acclimation.

A second idea, somewhat related to the first, is suggested. If the receptors triggering the mechanisms controlling acclimation of physiological rates are deep within the animals' tissues, then it would seem plausible that a change in temperature would penetrate the lesser mass of the small animals and trigger the mechanisms of acclimation more rapidly than it would the larger animals. This could be investigated by comparing the rate of change in internal temperature with the rate of change in the time course of acclimation for various size animals subjected to a change in temperature.

While neither of these ideas explain this phenomenon, they do suggest possibilities for future investigations.

Time course of acclimation

In reviewing thermal acclimation in fishes, Brett (1956) reported acclimation to be more rapid for a rising than for a falling temperature. Ohsawa (1956), in his study of the periwinkle Nodilittorina, and Roberts (1957), in his work with the crab Pachygrapsus crassipes, also demonstrated more rapid acclimation to higher than to lower temperature. While the results of this investigation do not refute this trend, neither do they support it. Acclimation to a higher temperature is shown (Fig. 3C) to have as long a time course, even slightly longer, than acclimation to a lower temperature (Fig. 3B). Whether the difference between these results and previous findings may be due to the terrestrial habit of L. flavus must await further investigation of thermal acclimation in terrestrial cold-bloods.

The complete acclimation of 10°C. animals at 20°C. and 20°C. animals at 10°C. in a time course of one day (Fig. 3) is rather rapid when compared with most of the previous findings regarding the time course of acclimation. As mentioned earlier, the time course of acclimation for fish and other aquatic cold-bloods has been reported as being of the order of several days up to 2 weeks with only certain small crustaceans having been shown (Grainger 1958) to acclimate in less than one day. On the other hand, in the relatively few cases reporting the time course of acclimation for terrestrial poikilotherms, acclimation appears to be complete in one day or less. This rapid acclimation was reported for insects by Mellanby (1939, 1954) and Colhoun (1954) and for an adult amphibian by Mellanby (1940) who, noting that the time course of acclimation in both insects and amphibian was one day or less, pointed out the possibility that such a rapid time course may be widespread. In view of the many clear cases of longer time courses among the aquatic cold-bloods, this would not seem very likely. However, with the rather scanty evidence available, including the results of this investigation, there does seem to remain the possibility that rapid thermal acclimation may be widespread among terrestrial poikilotherms. While care must be taken in attempting to designate the usefulness of any physiological adaptation, it would seem that rapid adaptation to a changing temperature would be of greater benefit to a terrestrial than to an aquatic animal due to the more rapid temperature changes to which the terrestrial animal is subjected. Only additional investigation on the time course of thermal acclimation in terrestrial cold-bloods can disclose the extent of this rapid acclimation phenomenon.

As Bullock (1955) points out, a number of investigators have called attention to the time factor in obtaining points for the common rate-temperature curve but this point is still badly neglected in the physiological literature. The rapid acclimation of L. flavus presents a strong case for the importance of the time factor in determining a physiological rate after a temperature change; for even in the range of a few hours, all or part of the adjustment one wished to study may already be completed by the time the temperature change is completed and rate-measurements begun.

A related point, which apparently has not been mentioned in the literature, is the importance of the time factor in determining a physiological rate of an animal subjected to either alternating temperatures in the laboratory or fluctuating temperatures in the field. In a rapidly adapting animal, such as L. flavus, the time spent at any one temperature becomes an important variable because of the acclimation which would take place during any period longer than a few hours duration. Thus, any attempt to determine accurately a physiological rate of such an animal, which has been subjected to alternating or fluctuating temperatures, must necessarily involve a knowledge of the time course of acclimation for that animal and the time spent at each alternating or fluctuating temperature condition.

SUMMARY

1. Oxygen consumption has been studied in the garden slug, Limax flavus, that have been maintained at experimental temperatures of 10°, 20°, and 30°C. for a period of one week and longer.

2. It has been shown that the oxygen consumption of equal weight animals, when measured at 20°C., is higher in individuals that have been maintained at lower temperatures.

3. Within the weight range of 0.4 to 1.2 gm., the oxygen consumption varies inversely with increasing weight.

4. Animals acclimated to 10°, 20°, and 30°C. for one week or longer have been transferred to higher and lower temperatures and the oxygen uptake at 20°C. measured at intervals from 6 to 36 hours to determine the time course of thermal acclimation in this species.

5. It is shown that in the weight range of 0.4 to 1.2 gm., small animals acclimate more rapidly after transfer to higher or lower temperatures.

6. When 20°C. animals are transferred to 10°C. and 10°C. animals to 20°C., it is shown that the rate of acclimation for equal-weight animals is the same with acclimation being completed in approximately 24 hours.

7. The results lead to the interpretation that the difference in oxygen consumption between high and low

temperature animals is an expression of a phenotypic compensatory mechanism operating to increase the environmental freedom which the animal enjoys and could be of ecological significance in habitat selection and in establishing range limits.

8. The importance of weight, as a variable which must be accounted for in comparing physiological rates, is discussed.

9. The data suggests that the time factor is an important variable which must be accounted for in determining a physiological rate after a temperature change and of particular importance in fluctuating temperature studies.

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APPENDIX

s ____________ Date ____________ Animal # ____________

History ____________

at ____ °C ____ hrs.

ined for ____ days.

at ____ °C ____ hrs.

Conditions ____________

ty Conditions ____________

Gross ____ gm.

: Tare ____ gm.

Net ____ gm.

Respiration Rate Measured at:

Temperature ______ °C.

Light ____________

Humidity ____________

Average Oxygen Consumption

μL O_2/hr. ____________

μL O_2/gm/hr. ____________

Reading	Units	μL O_2	μL O_2/hr.	μL O_2/gm./hr.	Remarks