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## Growth of the White-Mouse Gastrocnemius Muscle<sup>1</sup>

### I. In Terrestrial Gravity

CHARLES C. WUNDER AND JOHN W. C. BIRD

*Abstract.* The gastrocnemius muscle from white mice (Swiss Webster, female; NLW, male and female; varying in age from 6 to 13 weeks and in body mass from 8 to 36 gm) were analyzed by means of *Huxley's Equation for Heterauxic Growth* where double logarithmic plots were performed of muscle size as a function of body mass. These mice had been grown in normal gravity. Relative wet mass, relative dry mass, and percent dry mass did not display significant changes with body mass. Percent noncollagen nitrogen [NCN] in the dry muscle, however, did show an effect which was not significantly different from that anticipated from *Galileo's Principle of Similitude*:  $[\text{NCN}] \propto (\text{Body Mass})^{1/3}$ .

In formulating the *Principle of Similitude* in 1638, Galileo (as cited by Wunder *et al.*, 1968, pp. 20-32) proposed that the adversity imposed by an increase in body weight demands compensation in supporting structures of stouter shape and/or of stronger materials. These modifications, Galileo observed, are necessary to prevent what would otherwise be a disproportionately large force of weight for every unit of cross-sectional area of supporting structure. He argued that this demand applies not only to man-made structures but to biological structures as well. An appreciation for the application of this principle to inanimate structures has been exhibited by engineers and builders for many centuries (Bozajian, 1965, as cited by Wunder *et al.*, 1968, p. 24). Its relevance to animate structures has been the subject more recently of a few biophysical discussions (Thompson, 1942, pp. 25-51, and Lietzke, 1956, as reviewed by Wunder *et al.*, 1968, p. 23, and Rashevsky, 1948, pp. 569-629, as cited by Wunder, 1965, p. 448). In analyzing differential growth, however, most life scientists have ignored gravitational influence, particularly with respect to the demands of similitude. Part of this neglect might be attributable to the fact that compliance to this demand can come from more subtle changes than direct mass measurements of organ size.

Specific adjustments during chronic centrifugation have been noted in developing mice or hamsters for two structures which would play a major role in supporting gravitational force. These were the femur bone (Wunder *et al.*, 1960, as discussed by Wunder *et al.*, 1968, pp. 25-26) and the gastrocnemius muscle (Wunder, 1961, and Briney and Wunder, 1962 [as cited by Bird *et al.*,

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1963]; Bird *et al.*, 1963; Wunder and Bird, 1967). This has recently been confirmed for the adductor muscle of chickens by Burton *et al.* (1967, as cited by Wunder *et al.*, 1968, pp. 27,94).

If an extensor muscle such as the gastrocnemius, which functions as a major antigravity structure, is able to compensate for the demands of similitude, it would contribute to an animals' survival capability. The present study investigates the possibility that this muscle does possess the capacity for developmental adjustment as the normal force from gravity (body weight in a one-G environment) increases relative to cross-sectional area for support.

#### METHODS AND MATERIALS

Two strains of mice, NLW and Swiss Webster, were employed in this study. The ages, strains, numbers, and sexes of the individual mice are indicated in Fig. 5. Pregnant mothers of the NLW mice were shipped to us from a supplier. The young were then reared in the laboratory until the age of 5 weeks when they were placed about the periphery of the rotating centrifuge employed for exposure of the mice described in the accompanying paper (Wunder and Bird, 1969b). The NLW strain was employed in order to continue use of the same strain employed for earlier studies concerned with body growth and survival. Both sexes were employed with this strain.

The Swiss Webster strain proved to be more feasible because a greater number of large litters of the same sex could be obtained simultaneously from a supplier. The mice arrived at our laboratory when they were four weeks old and were also placed about the periphery of the centrifuge when approximately five weeks of age.

All animals had constant access to identical diets of Purina Laboratory Rat Chow and uncooked potato. The potato served as the only source of water. Temperature was maintained at 22°C. Approximately 4 mice were grouped together in each 13 x 16 x 10 cm compartment of the cages. Sawdust was placed on the cage floors. At terminations, various body measurements were performed, including wet mass, dry mass, and noncollagen nitrogen content (NCN) of the left gastrocnemius muscle. Dry mass was based on measurements made after the organs had been dried for 72 hours at 105°C. Lilienthal *et al.* (1950), as cited by Bird *et al.*, 1963) have proposed (NCN) as an index of the sarcoplasmic and contractile proteins. In an earlier report, Bird *et al.*, (1963) noted that NCN served as a better index of muscular compensation for gravitational adversity than did wet or dry weight. NCN determinations were performed by a modification (Bird *et al.*, 1963) of the method of Lilienthal and co-workers. These involved the NaOH extraction of NCN, micro-Kjeldahl digestion, and colorimetric measurement

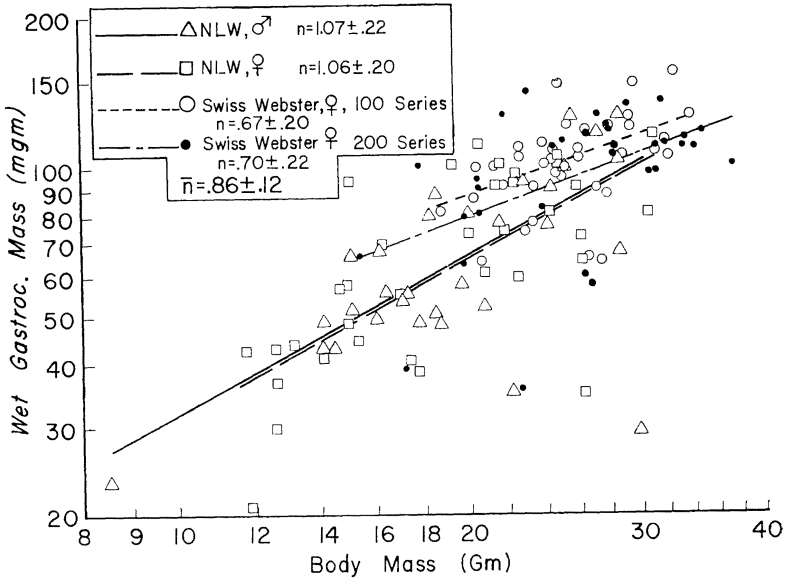


Figure 1. Wet muscle mass as a function of body mass.

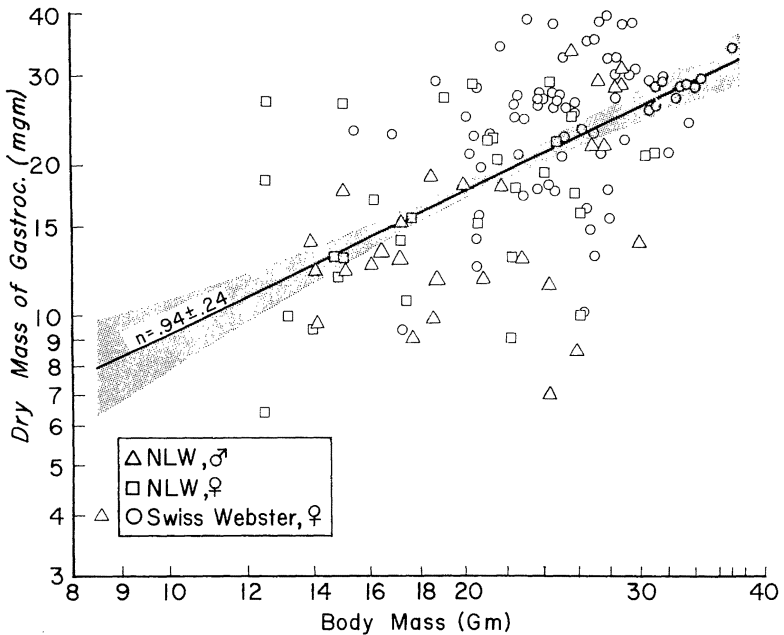


Figure 2. Dry mass as a function of body mass.

of nitrogen.

The exponents  $n$  or  $n'$  for various muscle measurements were evaluated from double logarithmic plots of muscle size or concentration in the muscle respectively versus body mass (Figs. 1-5). These constitute *Heterauxic Growth Curves* (also known as allometric curves) as described by Huxley (1932, as cited by Wunder, 1965, p. 442):

$$(\text{muscle size}) \propto (\text{body mass})^n, \text{ or} \quad [1]$$

$$(\text{concentration}) \propto (\text{body mass})^{n'}. \quad [2]$$

If concentration and relative muscle size do not change with animal size  $n'$  should equal zero and  $n$  should equal unity.

Values for the exponents ( $n$  or  $n'$ ) are computed from the least-squares slopes (or regression coefficients) of the data plotted on double logarithmic scales in Figs. 1-5 in accordance with the linear relationship between log size or log concentration and log body mass anticipated from equations [1] and [2]. Predicted standard errors of the ordinate are shown.

In Fig. 1 for size equal wet muscle mass, curves from the differing strains or sexes were significantly different from that obtained by combining all data. For that reason, the value of  $n$  obtained by averaging those slopes from each set of data is employed in the analysis of experimental results. For Figs. 2, 3, 4, and 5 slopes based on all control data were employed because they do not differ from those for individual sexes or strains.

The slopes obtained from a simple straight-line regression could not be demonstrated to differ significantly from unity in Figs. 1, 2, and 4 or from zero in Fig. 3. For that reason, the time and expense involved in a multiple regression analysis to consider the effect of age did not seem warranted. For the curves in Figs. 1-5, the exponent  $n$  or  $n'$  is evaluated from simple regression.

The regression coefficient for the log (concentration of NCN) for upon log (body mass) did significantly differ from zero ( $n' = 0.43 \pm 0.08$ ) in the first row of Table 1. With the data in Fig. 5, several multiple regression analyses were performed according to the method of least-squares estimation for multiple regression using the Gaussian Elimination Method of matrix inversion (Graybill, 1961). The line displayed is based upon:

$$\log (\% \text{ NCN}) = -0.584 \pm 0.028 + (0.367 \pm 0.085) \log (B \div 10\text{gm}) + (0.0036 \pm 0.0087) \text{wk}^{-1}W,$$

where  $W$  is the age in weeks beyond the age of 5 when experiments began. Other regressions for this data are listed in Table 1.

Student's small sample "t" test was used as the criterion for significance, with  $P \leq 5\%$  accepted as revealing a significant difference. Standard errors for derived quantities, were computed in accordance with the methods described by Worthing and Geffner

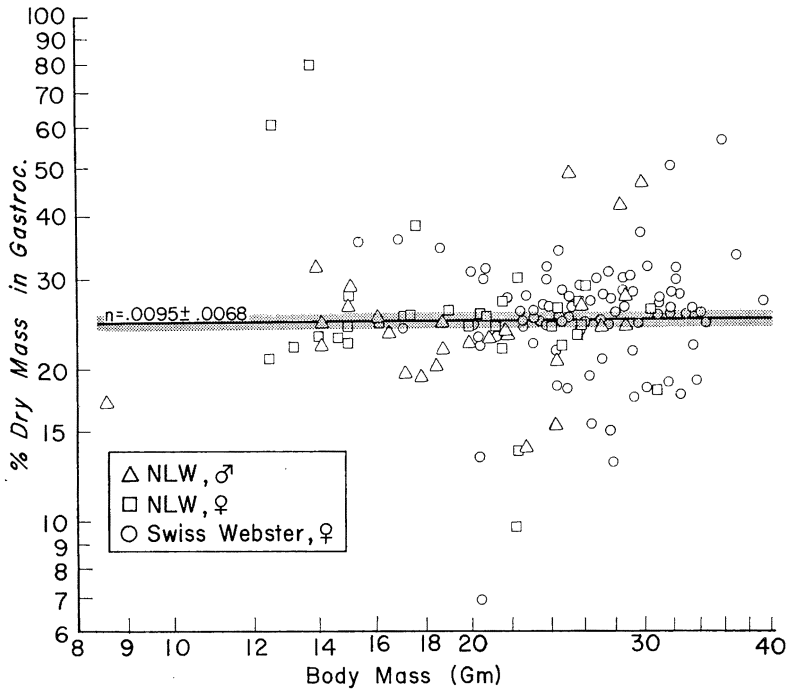


Figure 3. Percent dry mass as a function of body mass.

(1943, p. 213). A review of the experimental and mathematical procedures applicable to studies of this nature has been previously published by this laboratory (Wunder, 1965, p. 375).

#### RESULTS AND DISCUSSION

In our normal terrestrial environment (i.e.,  $g = 1.0G$ ) compliance with similitude is represented neither by the total muscle mass (Fig. 1), by dry mass (Fig. 2), nor by total concentration of dry material (Fig. 3). As wet mass or dry mass represent the more common measurements for organ growth, it is not surprising that much of the conceivable compliance with similitude by growing animals has gone unnoticed. In Fig. 5 where noncollagen nitrogen (NCN) concentrations in dry muscle are displayed, the value for  $n^2$  of  $0.38 \pm 0.17$  is not significantly different from the theoretical  $1/3$ . More refined measurements may eventually show that other changes are involved and that agreement with our theory is merely fortuitous. Present data, however, can be explained by a very simple model. This model assumes that with respect to gastrocnemius muscles of these mice growing at  $1.0 G$ , all demands of similitude are met by changes in NCN content as a function of body mass.

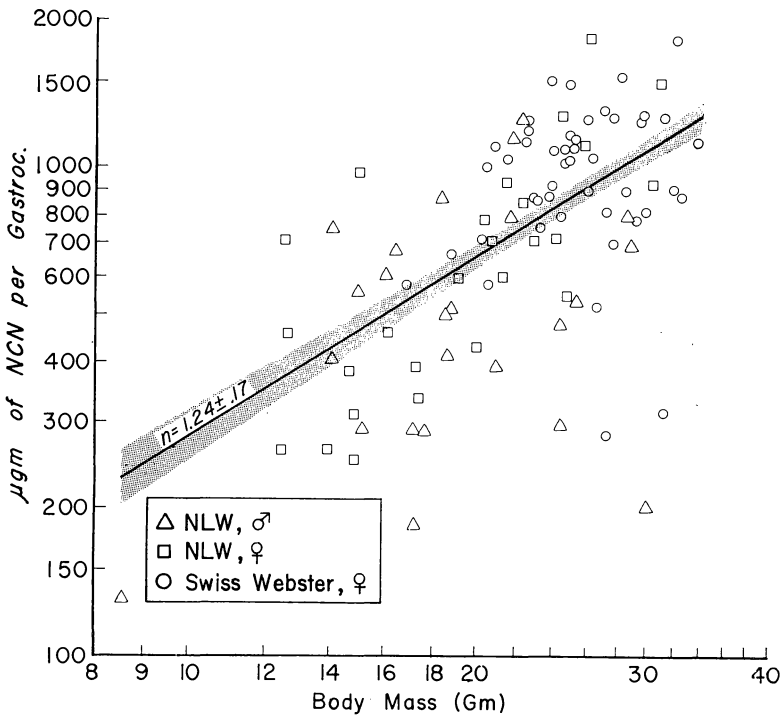


Figure 4. Mass of noncollagen nitrogen (NCN) per muscle as a function of body mass.

The validity of this model will become more obvious later when one realizes that this  $n'$  ( $0.38 \pm 0.17$ ) is independent of the animal's age, and that when the effect of mass upon weight is magnified by centrifugation the NCN concentration is further augmented.

For the most simple biological considerations the demands for compensation to applied forces should increase in proportion to the product of field intensity  $g$  and body mass  $B$  to the one-third power ( $gB^{1/3}$ ), or in proportion to  $g \times$  height (Wunder, 1965, p. 375). Thus, for complete compensation, concentration  $[C]$  in terms of percent dry mass or percent NCN of the gastrocnemius muscle should be described by  $n$  equal  $4/3$  and  $n'$  equal unity.

For one-G conditions, values for  $n$  and  $n'$  in terms of the data displayed in Figs. 1-5. Values for slopes of wet mass ( $n = 0.86 \pm 0.12$  in Fig. 1) and percent dry mass ( $n' = 0.0095 \pm 0.0068$ , Fig. 3) are significantly smaller than those anticipated in a model which assumes complete compliance with similitude. The data are indecisive for dry mass ( $n = 0.94 \pm 0.24$ , Fig. 2). Although the slope for total NCN is not significantly different from unity, ( $n =$

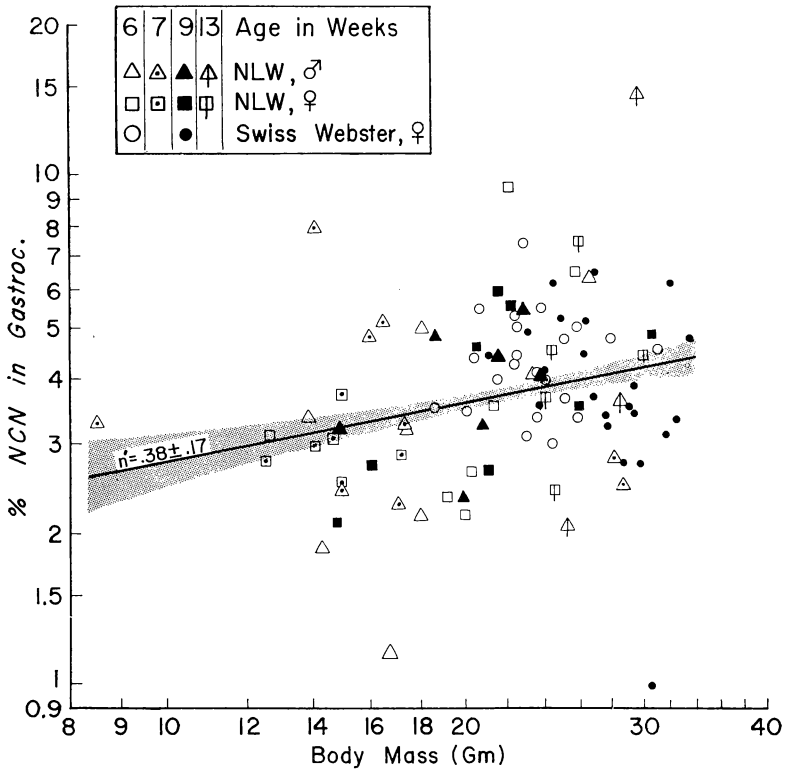


Figure 5. Percent NCN in dry muscle as a function of body mass. The  $n'$  of  $0.38 \pm 0.17$  is the multiple regression coefficient as listed in the first row of Table I. An  $n'$  of  $0.43 \pm 0.08$  as listed in the simple regression listed in the second row of Table I would correspond to comparable slopes in Figs. 1-4.

$1.24 \pm 0.17$ , Fig. 4) it is compatible with the predicted slope of  $4/3$ .

With development of NCN per unit of muscle dry mass,  $n'$  agrees with the theoretical value of  $1/3$ . This is true when  $n'$  is equated either to the simple regression coefficient,  $0.43 \pm 0.08$ , listed in the second row of Table 1 or to the multiple regression coefficient,  $0.38 \pm 0.17$ , listed in the first row of that Table and displayed in Fig. 5. The second value is empirically corrected by subtracting from it that contribution which might be attributed to the increasing age which accompanies increasing body mass. Both values are significant but not measurably different from each other. In the third column of Table 1 are listed multiple regression coefficients which show that those effects of age which are independent of mass could not be measured. The higher muscle NCN content associated with increasing body size could be either a direct



TABLE 1

Regression Coefficients of log (%NCN) in Gastrocnemius Muscles Upon:  
 log (gm of body mass)<sup>n'</sup> Age in Weeks (beyond the age of five weeks) $\beta_1$ ,  
 Sex (M = 1 and F = 2) $\beta_2$ , and Strain (NLW = 1 and SW = 2) $\beta_3$ ;

$$\log (\% \text{NCN}) \text{ at } 1.0 \text{ G} = \beta_0 + n' \log 10 \frac{\text{B (gm)}}{\text{gm}} + \beta_1 (\text{age}) (\text{wk}^{-1}) + \beta_2 (\text{Sex}) + \beta_3 (\text{Strain})$$

$\beta_0$	$n'$	$\beta_1$	$\beta_2$	$\beta_3$
-0.58 ± 0.03	0.38 ± 0.17	0.004 ± 0.009		
-0.58 ± 0.04	0.43 ± 0.08			
-0.47 ± 0.01		0.014 ± 0.008		
-0.59 ± 0.09	0.28 ± 0.22	0.01 ± 0.01	0.02 ± 0.03	0.04 ± 0.05

adjustment by the organism to greater weight Bg, a reflection of genetic factors, or in part a reflection of other factors, such as low-fat content.

Extension of the line in Fig. 5 has been found applicable to the rat even for conditions that had been interpreted by others to indicate that low protein diets caused a decrease muscular [NCN] (data of Mendes and Waterlow, 1958, as analyzed by Wunder and Bird, 1969a).

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