Procedure for Evaluating Changes in Carcass Constituents Accompanied by Effects on Growth. (32388)

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One of the most typical experimental protocols utilizes a uniform group of growing animals which are divided in half; one-half is exposed to an experimental variable while the other half is not. Following this "treatment" measurements are made of tissue constituents and the differences between control and experimentally treated animals are compared. These findings are usually expressed as quantity or activity, per g of liver, heart, body weight, etc. One of the distressing aspects of this approach is that "treatment" may affect the growth rate as well as the fat and water contents making expressions of this kind often of questionable significance.

This problem has been dealt with in the laboratory as follows(1,2,3): It is known that organ weights (W) for most organs may be expressed as a function of body weight (B) in the relationship: Log W = a + b log B (4). This is also true of organ weight *vs* femur length. The latter can be used to determine the weight of a particular organ at the time when body growth ceased. The femur length is a function of the maximum growth achieved by the animal. When this idea was extended, it was found to provide a valid basis for evaluating the results of certain experiments. The total quantity of a given constituent in an organ or in the organism may be compared with the quantity which it should have contained on the basis of the size of the animal or indirectly from femur length.

An example of the application of this procedure along with new refinements will be demonstrated. Mice were starved and then refed and changes in the contents of the carcass of nitrogen, calcium, potassium and formed creatinine were studied.

Methods. Male albino mice weighing 22 to 33 g obtained from a commercial supplier, were individually housed in plastic containers (7 × 5 × 2½″) on a layer of sawdust. Control animals were selected at random. All were given water and Purina Laboratory Chow *ad lib* for one week. Some experimental animals were then given water only for 1, 2, 3 or 4 days and killed by cervical fracture. Other experimental animals were refed after they had lost 30% of their starting weight. Upon refeeding, the latter were given Purina Laboratory Chow and, in the drinking water, 2% dextrose solution (W/V) containing 0.05% (V/V) Berocca-C (Roche Laboratories) vitamin complex for 2 days. The control animals also received this solution for 2 days. The various experimental and control groups were designated as follows:

Group	Treatment	Day when killed
AI	Control	0
AII	"	9
AIII	"	20
B	Fasting	1
C	"	2
D	"	3
E	"	4
F	Lost 30% body wt; refed 3 days	7
G	" " 5 "	9
H	" " 10 "	14
I	" " 16 "	20

Immediately after death, the legs were stretched and taped flat and X-rays taken of the animals for a measurement of femur length. The instrument used was a General Electric Model D, Type 3 X-ray instrument. Source to target distance was 84 cm. A current of 25 ma was applied for 3 seconds. Femur lengths were determined as an average of 4 measurements from the X-ray image using a Lufkin vernier caliper.

The pelts were then removed, cleaned in a uniform manner, and retained for a separate study. The carcasses, including the heads and tails, were dissolved in 3 volumes (W/V) of 90% formic acid by autoclaving at 15 lb pressure (250°F) for one hour.

Total carcass nitrogen was determined by

TABLE II. Differences Between Observed and Calculated Values Following Starvation and Refeeding.

Group	No.	Mean body wt, g: Starting	Starvation	Refed	Δ L, cm	Δ N, mg	Δ K, meq	Δ Cr, mg	Δ Ca, meq
B	8	27.8	23.9		−.013 ± .013*	− 43.9 ± 13.7	−.132 ± .029	−4.45 ± 1.02	− .634 ± .304
C	8	29.2	22.9		−.022 .011	− 70.0 ± 12.0	−.130 ± .030	−5.43 ± .84	−1.620 ± .245
D	8	27.1	19.3		−.021 .011	−106.2 ± 16.8	−.282 ± .051	−5.49 ± 1.33	−1.873 ± .447
E	8	28.4	19.4		−.025 .015	−130.7 ± 12.9	−.425 .028	−7.07 .96	−2.485 .513
F	8	28.0	19.6	28.3	−.014 .006	− 35.8 ± 14.2	−.080 ± .046 (7)	+3.27 ± 1.47 (7)†	− .708 ± .551(7)
G	8	29.2	20.2	30.6	−.013 ± .012	+ 6.5 ± 16.4	+.022 ± .041	+5.56 ± 1.24	+ .010 ± .251
H	8	27.8	19.2	30.3	−.006 ± .013	+ 7.3 ± 23.2	+.025 ± .055	+3.82 ± 1.43	+ .032 ± .506
I	12	27.6	19.3	33.9	+.056 ± .008	+ 12.3 ± 10.3	+.076 ± .029(10)	− .85 ± 1.17(10)	—

Δ = Differences between observed and calculated values based on equations 1-5, Table I.
* Standard error of mean.
† Numbers in parentheses represent numbers of animals in each group.

an automated Kjehldahl procedure as previously described(5).

To one ml of formic acid-digestate was added .95 volume of the quantity of 2 N (potassium- and calcium-free) NaOH required to neutralize 1 cc to phenolphthalein in the cold. This was followed by 1 ml of 10% sodium tungstate and centrifugation. To 1 ml of supernatant was added 3 ml of water and alkaline picric acid solution, according to the standard procedure for determination of creatinine(6). Creatine could be measured quantitatively following autoclaving with formic acid as described and employing the standard conversion factor of 1.16. It was assumed that nearly all of the formed creatinine was derived from creatine phosphate. The quantity of total-body formed creatinine was calculated.

The supernatant was also used to measure total potassium by flame photometry and total calcium by the procedure of Kingsley (7).

Results. Ln-ln regressions are shown in Table I. They were derived from data ob-

TABLE I. Regressions Correlating Femur Length with Total Body Nitrogen, Potassium, Formed Creatinine, Calcium, and Body Weight.

		P
1)	$\ln L = -.23876 + .19106 \ln W$, $r = .731$,	$< .01$
2)	$\ln N = 5.39565 + 2.43665 \ln L$, $r = .813$,	"
3)	$\ln K = -.58930 + 2.22700 \ln L$, $r = .696$,	"
4)	$\ln Cr = 2.79585 + 2.53086 \ln L$, $r = .765$,	"
5)	$\ln Ca = 1.91637 + 2.64876 \ln L$, $r = .789$,	"

W = weight (g), L = femur length (cm), N = nitrogen (mg), K = potassium (meq), Cr = formed creatinine (mg), and Ca = calcium (meq). r = correlation coefficient.

tained from 27 normal mice ranging in weight from 22.3 g to 37.0 g. In each case the correlation coefficients were high and the regressions were statistically significant. From the observed femur length the expected values for nitrogen (N), potassium (K), calcium (Ca), and creatinine (Cr) could be calculated for individual animals. The differences between the observed values and expected values were calculated.

Starvation. Predicted femur length based on prestarvation weight (equation 1) was compared with actual values. In animals of groups B, C, D, and E, respectively, mean

femur lengths were less than predicted ($-\Delta L$, Table II). However, the differences between observed values and calculated values were not statistically significant. If these differences represent a decrease in femur length, a value of $-.025$ cm in an animal with the shortest femur length used in this experiment (1.348 cm) would result in a decrement of less than 2% in length and would underestimate calculated N content by less than 5% using equation 2. Such changes would not seriously affect calculations of N loss based on the assumption that no change in femur length had occurred.

Difference between observed and prestarvation values for N, K, Cr, and Ca for groups B, C, D, and E were calculated using equations 2-5 and are shown in Table II. In group E, $-\Delta N$ was 23.3% of the calculated prestarvation value. The mean value of $-\Delta K/-\Delta N$ was $2.85 \pm .12$ meq K/g N. K/N was 2.3 for the control animals and did not appear to change in groups B, C, and D but was 2.0 in group E. The mean value of $-\Delta Cr/-\Delta N$ was $.131 \pm .023$ for $-\Delta N$ less than 80 mg and $.058 \pm .004$ for losses exceeding 80 mg of N. The mean value of $-\Delta Ca/-\Delta N$ was $2.37 \pm .24$ meq Ca/g N.

Refeeding. During refeeding the mice exhibited formed creatinine values which greatly exceeded that predicted from femur length, even when the animals still exhibited a net loss of N. However, the predicted value was observed when growth was reinitiated. Growth, as manifested by increment in femur length above the prestarvation value, did not begin until after 10 days of refeeding. By the fifth day of refeeding N, K, and Ca had reached the prestarvation values.

Discussion. The findings justify the hypothesis that changes in the quantity of body constituents in the presence of fluctuations in protein metabolism and growth may be evaluated on the basis of femur length. Evaluation of such data on the basis of weight may result in error due to fluctuation in fat and water contents. This present scheme permits the use of animals which are not identical in weight and size and determines deviation in the quantity of a given constituent from that which is expected on the basis of size.

With regard to the actual experiment, there were several points of interest. During starvation $-\Delta K/-\Delta N$ was approximately 2.8 meq/g while K/N was 2.3 in the control and refed animals. This is related to the fact that connective tissue rich tissues are low in K as pointed out by Forbes and Hurst(8). $-\Delta Cr/-\Delta N$ fell rapidly for small losses in N and Cr was increased above the expected value while N deficit still existed during refeeding. This is consistent with the role of creatine phosphate as an immediately available energy storage substance. It decreases rapidly with depletion of energy stores and accrues in advance of protein synthesis upon refeeding.

Summary. The principle is espoused that even though effects on growth occur, changes in the quantity of body constituents which are accompanied by alterations in body nitrogen, fat and water contents may be evaluated on the basis of femur length. Linear ln-ln regressions may be derived with femur length as the independent variable. As an example of the application of this principle, mice were starved and refed, the carcass was dissolved in concentrated formic acid and total body nitrogen, potassium, calcium and formed creatinine were measured. Differences between observed values and values expected from femur length were determined. Decrement in potassium (meq), calcium (meq), and creatinine (mg) per g of nitrogen lost were respectively: 2.9, 2.4 and .13 (for losses of less 80 mg of nitrogen and .058 for losses greater than 80 mg of nitrogen). During refeeding formed creatinine exceeded predicted values until continuation of growth occurred. Nitrogen, potassium, and calcium reached prestarvation values before growth continued.

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