

A LABORATORY STUDY OF GROWTH RATE IN YOUNG
MICROTUS PENNSYLVANICUS

By

Theodore Fred Whitmoyer

AN ABSTRACT

Submitted to the College of Science and Arts
Michigan State University of Agriculture
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fulfillment of the
requirements for
the degree of

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Approved

Don W. Hayne

AN ABSTRACT

To analyse growth of young voles, Microtus pennsylvanicus, according to recognized statistical procedures, adult animals were bred in captivity under laboratory conditions. Females were isolated when pregnancy was observed so that the young could be weighed and measured. A total of 320 young animals representing 59 litters were marked at birth and individual records were maintained from which instantaneous growth rates were computed. Statistical analyses of these rates were made. Some of the young and mothers were subjected to various experimental treatments by exchanging half of the offspring between two litters and imposing two different disturbance levels on each (disturbed every two days or every seven days) by handling in measuring and weighing.

Since instantaneous rates of growth were calculated for each individual animal, variability could be used as a statistical tool. Analysis of the data revealed the following information:

- (1) No statistical differences were shown to exist between instantaneous growth rates of males and females during the first four weeks of age.
- (2) Large amounts of variation prevent reliable estimates of age by weight criteria.
- (3) No distinct growth periods were found during at least the first three weeks of growth when analyzed from

day-to-day individual instantaneous growth rates.

(4) Although animals within any given litter were consistent in their rate of growth, the litter's average rate fluctuated during the first three weeks, and a difference was readily identified between litters for short periods.

(5) Growth rates of various litters differ so that a significant difference is shown to exist from week to week, but for the over all period no differences between litters were shown.

(6) Influencing factors other than heredity, degree of disturbance, parent history, size of litter, and month of birth affect growth rates of young Microtus. These factors may represent climatic factors of the immediate environment, but were not identified in this study.

Sex recognition after the first week by means of external linear measurements was statistically validated. Other external measurements revealed no differences between sexes for length of tail or hind foot to three weeks of age at which time 51 per cent of the average adult tail length and 89 per cent of the average adult hind foot length was attained. Ear pinnae of all animals unfolded by the fifth day and all animals had at least one eye open by the eleventh day.

The study poses several interesting problems with regard to growth rate and heredity, environment, and speciation.

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I. INTRODUCTION

A. Problem and Objectives

Interesting accounts of growth and development of several microtine species have heretofore been published, but with little use of statistical devices to express the variability observed. In the present study, animals were bred in the laboratory to obtain information concerning at least the minimum amount of variability which might be found in such characteristics as change in weight, external linear measurements, secondary sexual characters, and also that variability of growth resulting from the disturbance encountered in obtaining the data. It is expected that greater variability may occur in natural populations.

B. Review of Literature

In literature dealing with the reproduction of small rodents there is little use of statistical tools in studying variability of growth rate within a taxonomic unit. Gates (1925), Svihla (1932, 1934, and 1936), and McCabe (1950), all working with Peromyscus, point out interspecific and intersubspecific differences in growth rates, weight, and litter size, but say little about variation within species or subspecies discussed. Among others, Parkes (1926) and Retzlöff (1939) have studied growth of the white mouse

relative to litter size, and Rabasa (1952) reported variation in growth rate in older white rats in relation to number per cage and temperature. Morrison, et al. (1954) has tabulated a sketchy comparison between several species of Peromyscus and Microtus, as well as a species of Clethrionomys and Dicrostonyx.

Studies of several species of Microtus can be characterized according to content of (a) population dynamics -- Leslie and Ransom (1940), (b) laboratory techniques and studies -- Baker (1932), Poiley (1949), Harrington (1955), Greenwald (1956), Ecke and Kinney (1956), and Ransom (1934), and (c) field or natural history studies -- Bailey (1924), Bodenheimer (1949), Cowan and Arsenault (1954), Hatt (1930), Hamilton (1937 and 1941), Hatfield (1935), Howell (1924), Goin (1943), Barbehenn (1955), and Jameson (1947). Of these papers, anatomical variation is dealt with mainly by Howell (1924) and Goin (1943). In literature of the genus Microtus, complete daily growth data is found only in Hamilton's (1937 and 1941) work on M. pennsylvanicus, that of Cowan and Arsenault (1954) on M. oregoni, and that of Hatfield (1935) on M. californicus, although no thorough statistical examination of variability is made in any of these. Barbehenn (1955) has shown that "differential growth and size of individuals is not readily attributed to soils, weather, or forage composition" in the field, but no information is given showing variability in daily growth rates, nor do Howell (1924) and Goin (1943) include these valuable data.

It remains that only Cowan and Arsenault (1954) have made use of instantaneous rates which may so easily be used for comparing relative growth in different populations. Brody's monumental work (1945) reviews the theory behind instantaneous rates of growth (Chapter 16), and an interesting discussion of the more elementary mathematical concepts involved may be found in Granville (1946, pp. 65, and 168-171). A simplification of Brody's formula (page 508) $k = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$ for use with electric calculator and slide rule may be expressed by the mathematical equivalent:

$$k = \ln \left(\frac{W_2}{W_1} \right) \div t$$

where k = daily instantaneous rate of growth.

$\frac{W_2}{W_1}$ = ratio of the surviving proportion of weights.

t = time interval in days between observations.

For invaluable aid in the use of other statistical concepts, reference is made to Ostle (1954) and Snedecor (1946). Burt (1954) is used here as the authority for taxonomic identification of the species of Microtus found in Michigan; the same book contains a valuable discussion of linear measuring techniques (page 34).

In more recent papers, Greenwald (1956) has studied reproduction of Microtus californicus, and Ecke and Kinney (1956) have sought to age Microtus pennsylvanicus from seventeen to sixty days of age by using age-molt correlation.

Harrington (1955) has criticized the use of external linear measurements in aging young Microtus, and suggests that bone clearing and staining techniques are valid for determining the age, the method being reasonably accurate to the day for the first two weeks, to the week for the first two months, and to the month for the third and fourth months. This latter method of course, is, as yet, of no use in field investigation.

II. MATERIALS AND METHODS

A. Breeding Stock

Wild voles, males and pregnant females, were brought to the laboratory from a field study at East Lansing, Michigan, during September and October, 1955. Most of these animals previously had been captured and marked by toe clipping. Offspring of the earlier litters from the wild animals were also used for breeding.

Matings were made between available females and several males chosen for signs of sexual activity, using up to eight females with one male. This schedule produced numerous litters, but does not allow ready analysis of genetical factors of growth.

B. Laboratory Methods

Food and water were continuously available to the captive animals. Water was administered by the usual annealed-end glass tube and inverted bottle, the distance of the tube end from the cage litter being varied as the young animals grew. Fresh food was administered daily and included corn and rolled oats with fresh carrots and quantities of lettuce trimmings. This lettuce was consumed in quantity by the mice, and was used not only for food and water, but also as nesting material. While long grasses

seemed to be preferred for nesting material during the spring, the greater number of litters were raised during the winter in the damp nests of decaying lettuce where rearing success was high.

Two types of cages, larger and smaller, were used. Active breeding occurred when five to eight females were placed with one adult male in a rather large area. An old aquarium measuring 43 x 20 x 23 inches and several porcelainized metal tanks measuring 29.5 x 22 x 15 inches were used for such breeding and for holding excess animals. Animals to be isolated for nursing, for various experiments, and for duration of pregnancy were placed in 12 x 12 x 12 inch galvanized metal cages, equipped with removable wire tops. These cages were randomly placed in four tiers of four cages each on movable metal racks. The cage floor was blanketed with two inches of coarse sawdust and fine wood chips through which, to a limited extent, the captive Microtus were able to burrow.

All animals were maintained in a basement laboratory except for one breeding enclosure which was located in an office for closer observation. The basement laboratory was open to outside ventilation throughout each day. No temperature records were kept; the ordinary temperature was about 70° F. with extremes of perhaps 50° F. to 75° F. The animals were illuminated for at least 16 hours daily, following findings of Ransom and Baker (1932).

C. Identification and Measurements

Individuals of each litter were identified shortly after birth by branding one or more quarters with a hot bent dissecting needle. Numbers given at this time ranged from one to eight (see Fig. 1), and served only to identify the animal within the litter. It was assumed that no impairment to growth resulted from this treatment. As the fur lengthened, it was removed with scissors locally, down to the branding scar. Whenever identification was questionable, that animal was not included in subsequent data. After the second week, a number of animals, mostly females, were more permanently marked by toe clipping (see Fig. 2).

Notes were made relative to certain qualitative items such as unfolding of ear pinnae, opening of eyes, and other characteristics not discussed here.

Linear measurements used were those of tail length, hind foot length, and the distance between the anterior border of the anus and the posterior trunk of the urinary papilla. All measurements were made on live animals without anesthetic, using a wooden metric graduated rule and following Burt (1954). Animals were weighed on a triple beam balance to the nearest one-tenth gram.

While many records were made of the time of measuring, the exact hour of parturition was seldom known, and thus twenty-four hour accuracy in age determination was the basis of computation. With some departures, most measurements were

Figure 1

Diagram to show method of marking
infants of a litter by means of a hot
bent dissecting needle.

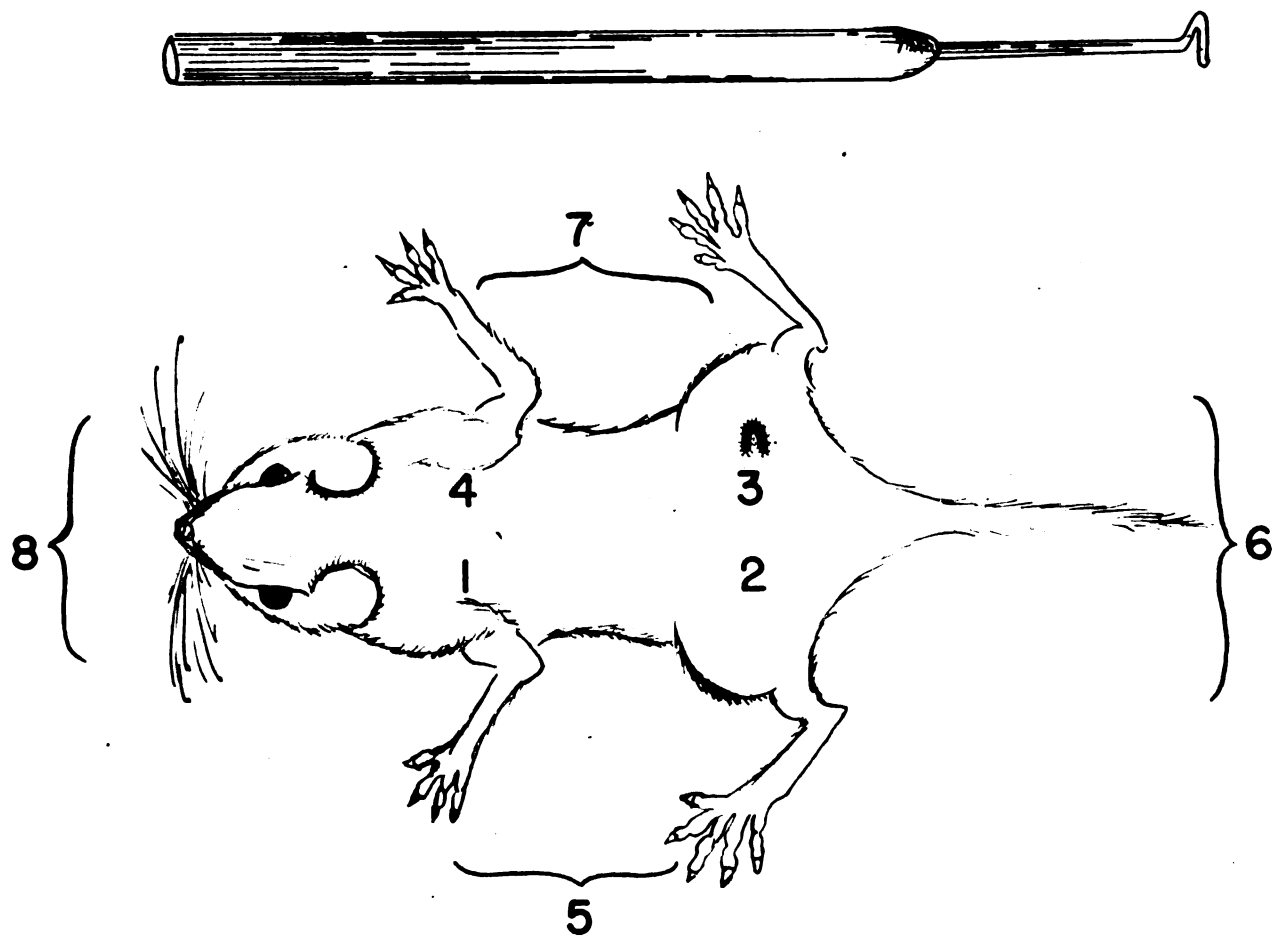


Figure 1

Figure 2

Method used for numbering older
individuals by means of toe clipping.
(Feet slightly enlarged for detail.)

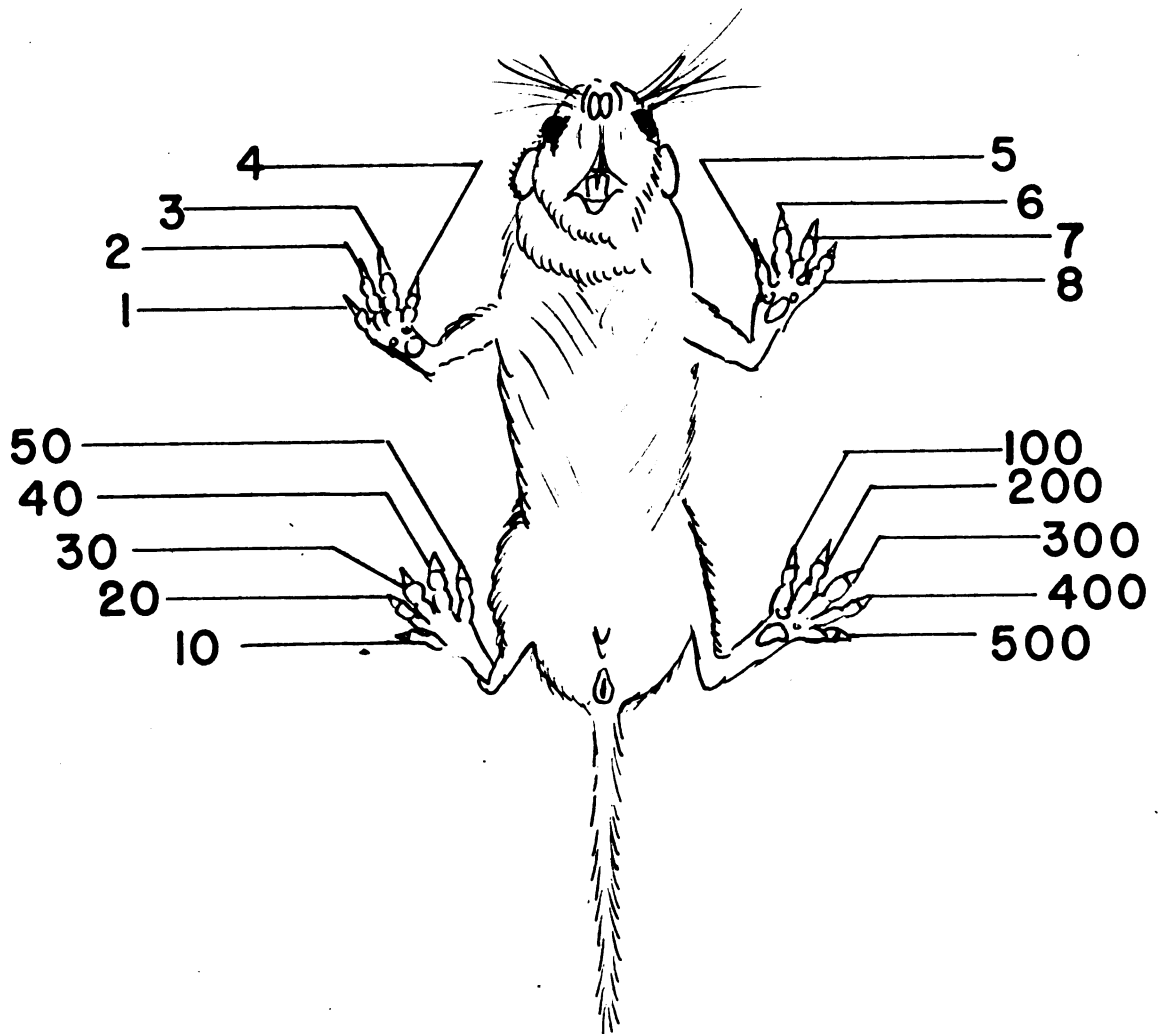


Figure 2

made between 7 P.M. and 1:30 A.M.

The litter to be measured was first isolated from the mother and placed in a small pan lined with a folded cloth. The parent was then measured and weighed in a ventilated aluminum cup with a spring top and replaced in the cage. Each juvenile was measured and weighed on the open platform of the balance, which was warmed by a 100-watt lamp placed about 10 inches away. After the young were two weeks old it was necessary to confine them in the aluminum cup for weighing.

Rate of growth is here discussed in terms of instantaneous rates, stated on a daily basis, as discussed by Brody (1945) and used by Cowan and Arsenault (1955). From the original weight data, instantaneous rates of growth were calculated by simultaneous use of an electric calculator and a log-log slide rule. The ratio of the change of weight was performed on the calculator to three decimal places. The natural logarithm of this quotient was then obtained from the scales of the slide rule and divided by the number of days in the period to put it on a daily basis. The facility of the method as compared to the use of tables recommends its use to biologists, many of whom may not be familiar with the advantages of the log-log slide rule.

The midpoint classes were arbitrarily chosen to allow the grouping of a number of individual records based upon slightly different times of measurement. In general, each midpoint class contains only those observations which

assure a strong central tendency about the midpoint. Arbitrary rules were set up to govern the grouping of data, for example, with a growth rate having a midpoint calculated at the sixteenth day of age, it would be grouped in midpoint class ten.

D. Experimental Treatments

To study the possible effects of (a) dividing of a litter between two parents and the inclusion of another animal's young in a litter, (b) the frequent disturbance incidental to measuring the young animals, and (c) the animals nursed by a foster mother compared to those nursed by their own mother, each litter or part of litter raised in captivity was enrolled in one of the following experimental categories:

- (1) Split, disturbed, own mother
- (2) Split, disturbed, foster mother
- (3) Split, not disturbed, own mother
- (4) Split, not disturbed, foster mother
- (5) Not split, disturbed, own mother
- (6) Not split, disturbed, foster mother
- (7) Not split, not disturbed, own mother
- (8) Not split, not disturbed, foster mother.

(a) The Split category designates litters which included a number of animals, usually half of the total litter number, interchanged with another litter. To all appearances, such transfers were readily accepted by the females and were

carried out with no more than normal fatality of young. Whenever possible, such transfers were accomplished during the first three days following birth, and between litters born within two days of each other. The Not Split category designates those litters containing only offspring of one parent. When a litter was split, animals were chosen for transfer to a foster parent or retention with the true parent according to coin-tossing or a table-of-random-numbers procedure.

(b) Those litters of the Disturbed classification were "disturbed" by handling at least every other day during the first three weeks after birth, and those in the Not Disturbed category were handled only at seven-day intervals. No doubt all litters were also disturbed to some degree incidental to the daily feeding schedule.

(c) Own Mother versus Foster Mother refers to the parent nursing the animals. "Own" animals were raised by the true parent, and "Foster" animals were raised by a foster parent.

III. ANALYSIS OF RESULTS

A. Weight vs Age Growth Curves

Daily mean weights for both sexes combined are shown in Figure 3, plotted on a linear scale along with one and two standard deviations on either side of the mean. Assuming a normal distribution, the outside curves (two standard deviations) include 99 per cent of animals, while the inside curves (one standard deviation) include about 66 per cent. In attempting to age a young animal of known weight, one would read across horizontally from the weight on the ordinate scale, only to find a wide range of corresponding ages. This variability, as observed in the laboratory, suggests a poor field relationship in judging age from weight, condemning use of weight as more than a general criterion in aging. Although Cowan and Arsenault (1954), indicated a close correlation between weight and age up to thirty-two days after birth for M. oregoni, the present study indicates that the variability reduces the usefulness of this fact for M. pennsylvanicus.

Daily weights of males and females have been combined above because statistical examination failed to reveal substantial differences. The Fisher "t" test, comparing the daily mean weights between males and females, shows no statistically significant difference (based upon the 5 per cent

Figure 3

Mean weights of males and females combined (circles) with one and two standard deviations on each side of the mean, plotted on a linear scale.

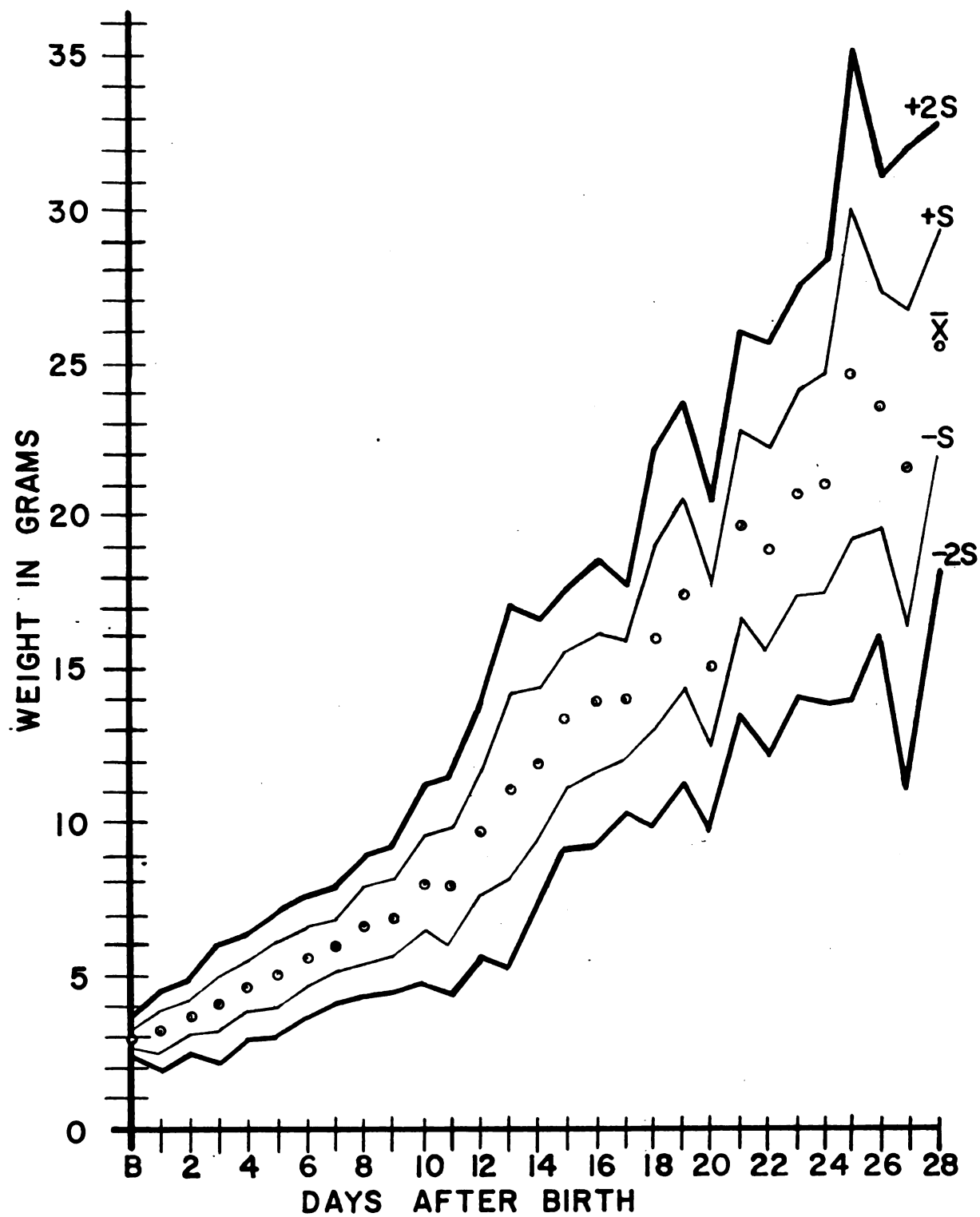


Figure 3

level of significance) up to the twenty-eighth day, excluding the twenty-sixth day, when for reasons not clear, the difference is very highly significant (based on the 1/10 of 1 per cent level of significance). In general, the lumping of data from both sexes for an overall daily mean for the first three weeks of age is validated by this test.

B. Instantaneous Rate of Growth

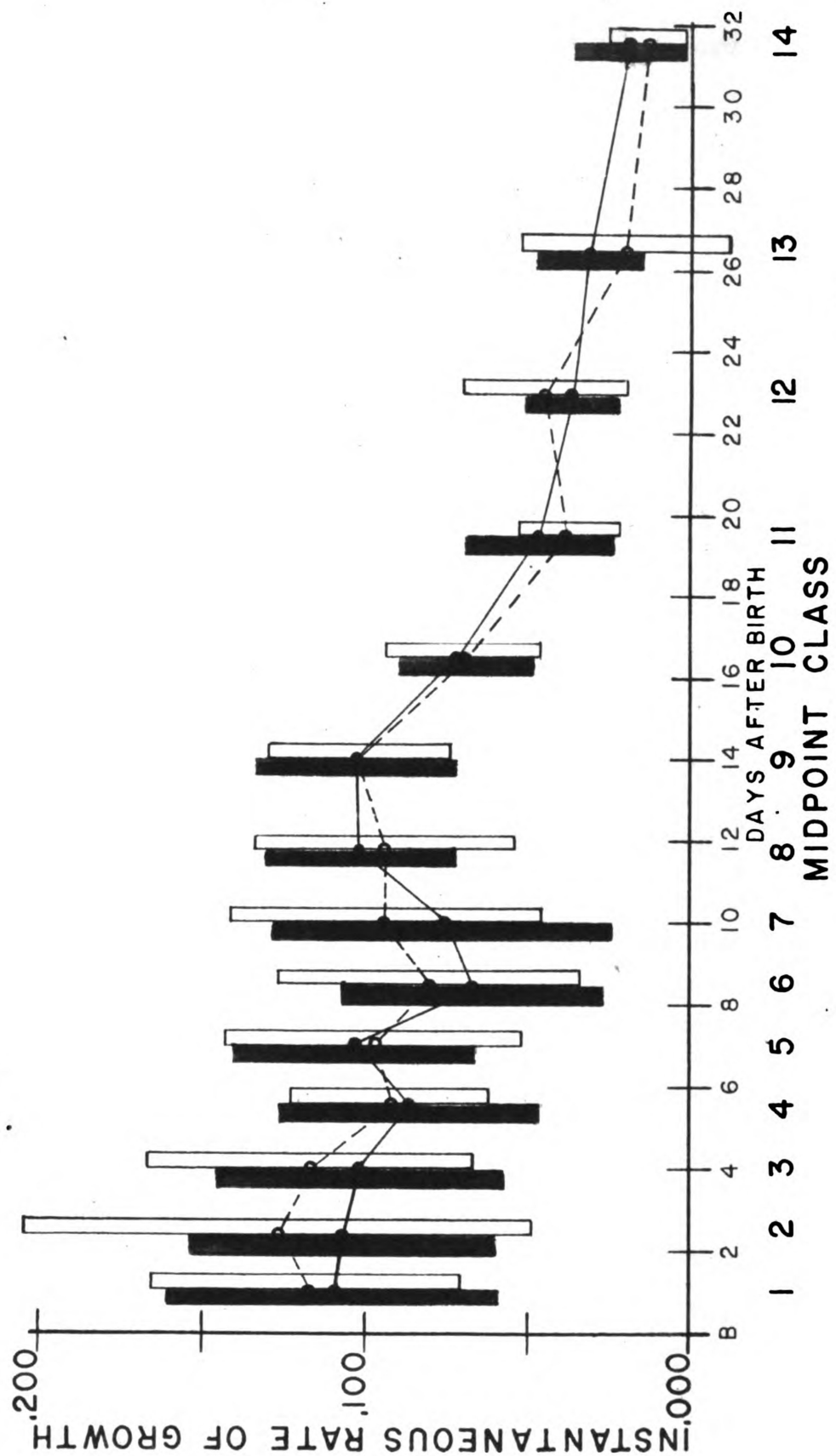
The values for individual instantaneous rates of change in weight are shown separately for each sex in Figure 4. One standard deviation is plotted on either side of each mean as an indication of the variability. For none of the time periods shown is there a significant difference between the means of the two sexes. As previously explained, the midpoint classes were set up to accommodate for the fact that not all the calculated growth rates were related to exactly the same periods of time. A cursory examination of this figure suggests that the mean of individual instantaneous rates of growth varies about a value of 0.100 for the first fourteen days, followed by a transition on the sixteenth day, after which a gradual reduction results in a value of about 0.015 by the thirty-second day. There is little apparent difference in the variability exhibited by either sex, but there is a suggestion of reduced variability with increase in age.

A closer analysis shows that there is statistically a highly significant difference (at the one per cent level

Figure 4

Mean instantaneous rate of growth of 117 males (solid line) and 90 females (broken line) plotted according to mid-point classes, with one standard deviation on either side as an expression of the variability. (Solid bars represent male standard deviations, open bars female standard deviations.)

Figure 4



of significance) between the overall mean of the first nine midpoint classes (first fourteen days) and the overall mean of the tenth to twelfth midpoint classes (sixteenth to twenty-third day), both using combined data of males and females. Analysis of variance tests also show highly significant differences within each group of midpoint classes, though it must be recalled that the reliability of such a test is questionable because of non-uniform variances and non-independence of data. The transition period represented by midpoint class ten (sixteenth to seventeenth day) may be included in either group or omitted entirely without altering results of the analysis.

The mean instantaneous rate of growth may be calculated in two different ways for any period.

- (1) By averaging the rates of growth calculated separately for individual animals, as has been done in the present study; and
- (2) By calculating a rate from the change in mean weight of a number of animals, as one must do when only mean weights are available.

To determine whether values obtained by the two methods might be compared, in the present study, eleven mean values corresponding the first eleven midpoint classes (first nineteen days) were calculated both ways. The mean difference (and standard error) calculated as method (2) value minus method (1) value was $.008 \pm .008$; with ten degrees of freedom, furnishes no evidence against a direct comparison of data obtained by the two methods. Subsequent calculations are based upon the individual rates

of growth because it gives information about variability of growth. A "t" test also shows no significant difference between daily data and data computed from coding into mid-point classes, hence all calculations are according to the latter grouping.

Figure 5 shows the mean weight, both sexes combined, plotted on a semilogarithm scale for the first four weeks. The slope of the line, representing the instantaneous rate of growth (see Brody, 1945, p. 517-518), approximates a straight line for short periods, and thus may imply constant rates; two periods are distinguished here. The rates of growth based upon the trend of the average weight growth curves have been calculated for the first nineteen days at $k = 0.097$, and for the nineteenth to twenty-eighth day at $k = 0.043$. Cowan and Arsenault (1954) have shown graphically a daily growth constant (k) and have calculated this value from mean weights of forty-one young M. oregoni. The daily instantaneous rate of growth calculated in the same manner has been divided by them into four distinct periods as follows:

<u>Days after Birth</u>	<u>k</u>
1 to 9	.1617
10 to 19	.0630
20 to 29	.0328
30 to 38	.0190

Although the use of mean weights for the calculation of instantaneous rates of growth require less calculating,

Figure 5

Daily average rate of growth for males and females combined plotted on a semi-logarithmic scale. The solid red line indicates the slope of the line which roughly represents the instantaneous rate of growth (k). (animals represented).

Figure 5

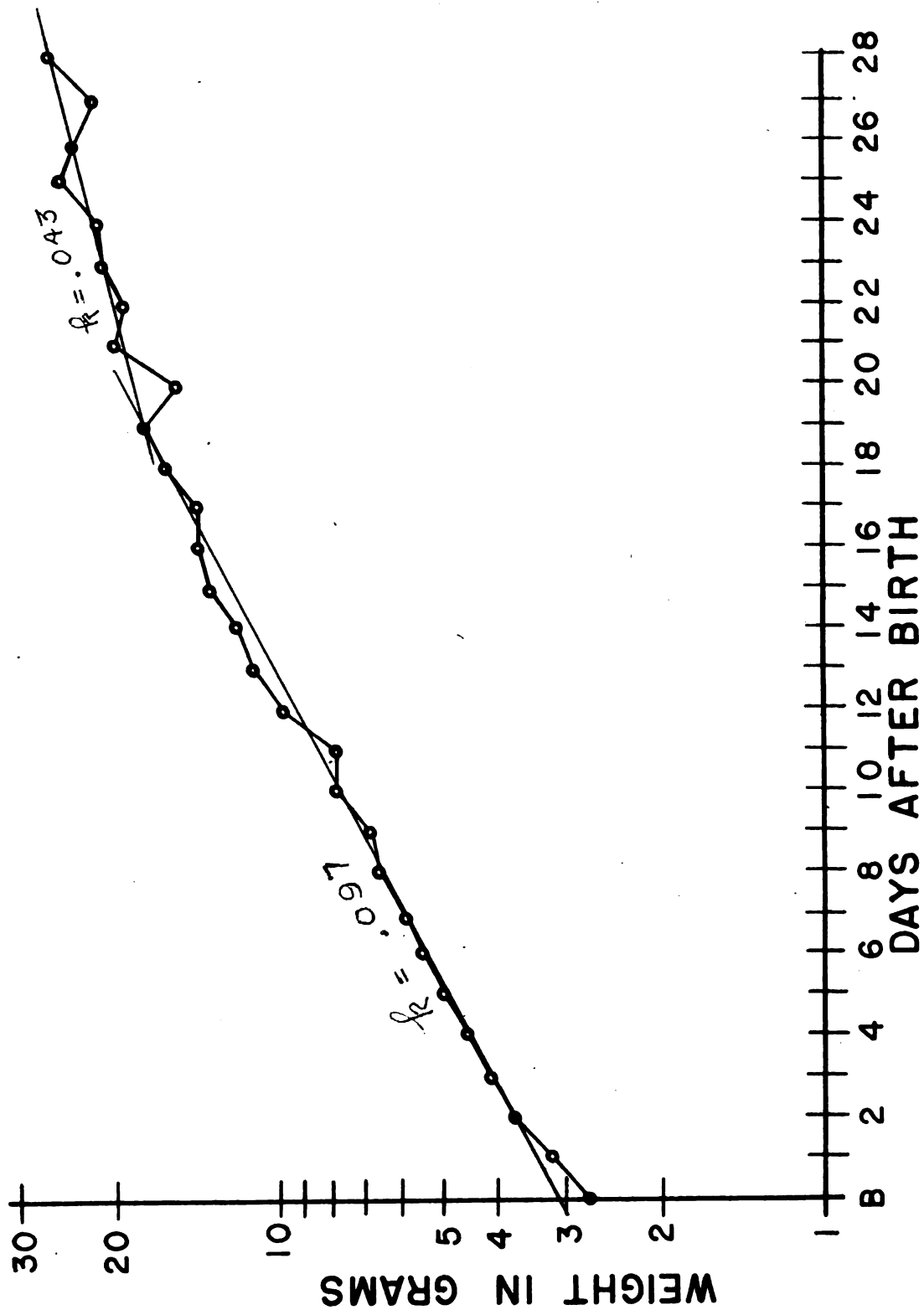
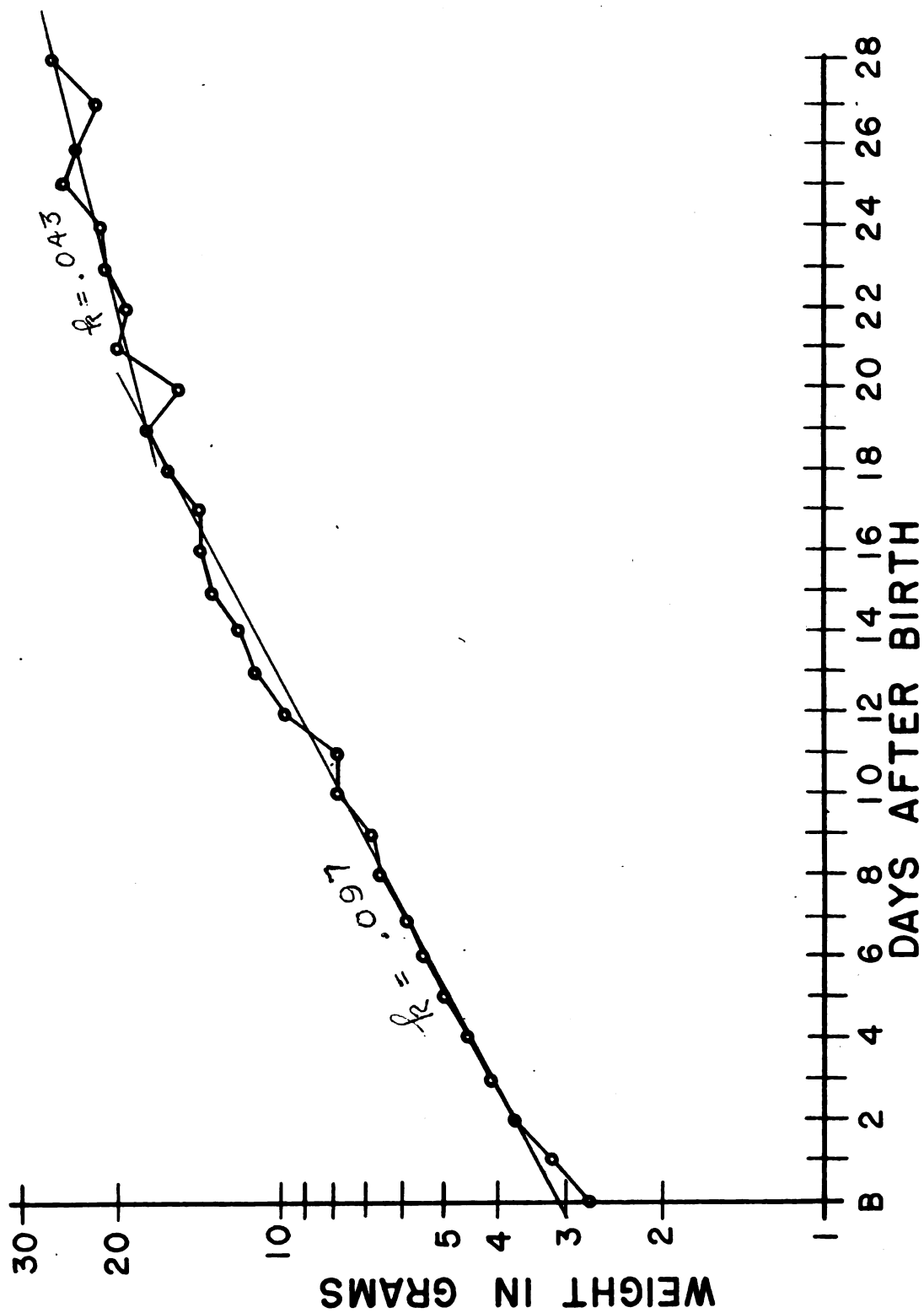


Figure 5

Daily average rate of growth for males and females combined plotted on a semi-logarithmic scale. The solid red line indicates the slope of the line which roughly represents the instantaneous rate of growth (k). (animals represented).

Figure 5



a serious limitation of this method is that there is no estimate of variability, thus preventing a statistical comparison of mean values.

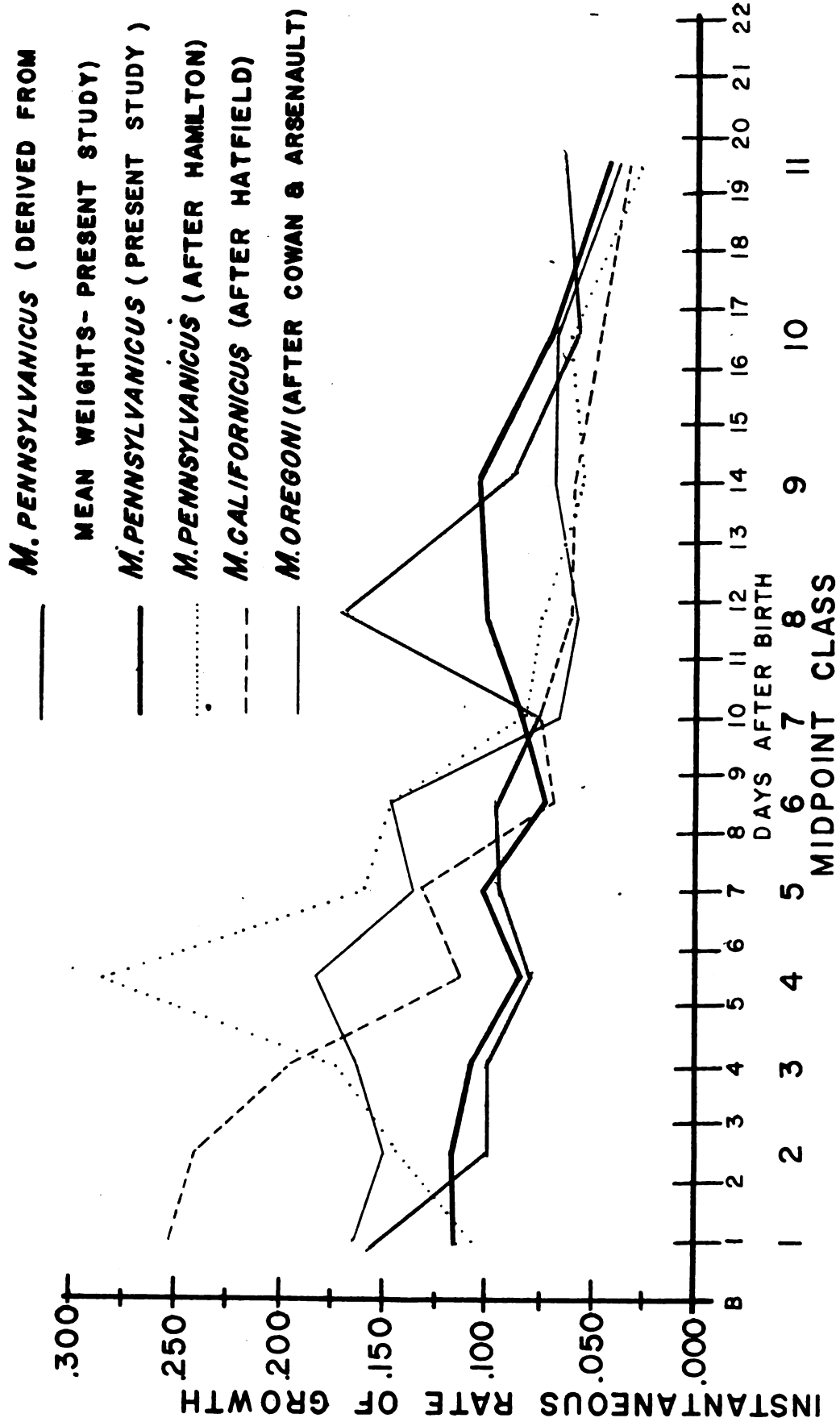
Cowan and Arsenault (1954) suggest that rates calculated from mean weight changes may represent the growth trend of a species or subspecies. Figure 6 shows a comparison of the rates of growth for three species of Microtus, the rates calculated for changes in mean weight during the first eleven midpoint class periods.

The heavy black line is the instantaneous rate curve for M. pennsylvanicus of this study, calculated from means of individual rates of growth, and the heavy red line indicates values derived from mean weight data. The two lines agree well except for one point, where chance exclusion of certain animals may have unduly altered the mean weights. The differences observed between Hamilton (1937 and 1941) and the present study, for animals of the same species, may indicate environmental or regional differences. For reasons not clear, the rates during the first ten days are higher for all other studies, while rates determined by the present study are higher than all others after the tenth day. A study of the growth of animals of several related species, or of animals from stocks collected at several points within the range of a single species, all raised in the same laboratory, would allow better understanding of this problem.

Figure 6

Mean instantaneous rates of growth
calculated for several species of
Microtus from various authors, and
plotted according to midpoint classes
of the present study.

Figure 6



C. Experimental Treatments and Rate of Growth

Statistical analyses of rate of growth differences were made in order to examine (1) whether litters differ in growth rate; (2) whether there are differences of growth rate between successive litters of the same female parent, and whether there are consistent differences among parents; (3) whether there are differences in growth rate according to size of nursing litter and month of birth; (4) whether there are differences in litter averages between litters conceived in the wild versus litters from females raised and bred in captivity; (5) whether the parent and litter effects are consistent over a three-week period; and (6) whether there are differences of growth rate caused by disturbance or change to a foster parent. No separate analysis of inheritance has been attempted. Except for (1) and (5) above, the analyses of variance use litter average instantaneous rates of growth for the first week, the averages being based only upon those young being reared by their own mother.

(1) When comparing the differences among litters with the variability of individuals within litters, highly significant differences are demonstrated (level of significance beyond 1 per cent), see Table 1. It is apparent from this test that there are far greater differences between litters than would expect to find by chance of sampling.

TABLE 1
ANALYSIS OF VARIANCE FOR AMONG AND WITHIN LITTERS

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Mean Square</u>
Among litters	40	2625
Within litters	110	124

(2) Average rates of growth for litters of the eleven females which gave birth to and reared at least two successive litters were examined in a further analyses of variance. The variation among litter averages is broken down into (a) differences among the eleven parents with regard to average growth rate of their offspring for the first week, (b) the comparison of growth of the one litter born in captivity versus the next consecutive litter (order of litter), and (c) the interaction of parents with order of litter, this last being the estimate of experimental error. As shown in Tables 2 and 3 there are no apparent differences at the five per cent level of significance. Thus it is evident from the above analysis that there are no evidences of difference among these parents with regard to rate of growth of their offspring during the first week and there is no evidence for any consistent difference between successive litters. Within the limits of the methods used, then, there is no evidence that a continued period of captivity of the mother influenced the rate of growth of her offspring. Table 2 indicates that there are several unusually high growth rates; these lack an explanation other than a possible hereditary effect.

TABLE 2
LITTER AVERAGE INSTANTANEOUS RATE OF GROWTH FROM ELEVEN
FEMALES EACH WITH TWO CONSECUTIVE LITTERS

<u>Female Parent Number</u>	<u>Litter "1" Average Growth Rate</u>	<u>Litter "2" Average Growth Rate</u>
11	.104	.055
14	.133	.101
15	.100	.114
16	.121	.128
19	.092	.127
20	.100	.116
21	.099	.079
24	.112	.069
29	.112	.092
32	.146	.145
33	.077	.116

TABLE 3
ANALYSIS OF VARIANCE BETWEEN TWO CONSECUTIVE LITTERS OF THE
SAME PARENT

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Mean Square</u>
Total	21	
Among Parents	10	676
Order of Litter	1	133
Error Term	10	453

(3) Because the growth rate of a litter might be somewhat dependent upon the "experience" of a mother, and because rate of growth of offspring might also be influenced as a wild animal becomes conditioned to a laboratory environment, the following analysis compares the first week average rate of growth of 4 litters conceived in the wild environment and born in captivity with the growth rate of second litters of the same females, bred in captivity. A comparison of the above with the first two litters of 5 females born and bred in captivity is also made. Information from one "wild" parent was not used because of heavy ectoparasite infestation and subsequent death of the young. The analysis showed no significant differences in rate of growth between the first and second litters or between wild and captive-raised parents (see Tables 4 and 5).

TABLE 4

LITTER AVERAGE INSTANTANEOUS RATE OF GROWTH FOR THE FIRST
WEEK COMPARING THE FIRST AND SECOND LITTERS GROWN IN CAPTIVITY
OF WILD-CAUGHT AND CAPTIVE-RAISED PARENTS

<u>Wild-caught Parent Number</u>	<u>Litter 1</u>	<u>Litter 2</u>
11	.104	.055
19	.092	.127
24	.112	.069
32	.146	.145
<u>Captive-raised Parent Number</u>		
15	.100	.114
20	.100	.116
21	.099	.079
29	.112	.092
33	.077	.116

TABLE 5

ANALYSIS OF VARIANCE OF DATA FROM TABLE

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Main Square</u>
Total	17	
Parents	8	.000709
Wild-caught vs captive-raised	1	.000147
Within category	7	.000789
Litters	1	.000046
Error (Parent $\times$ Litters)	8	.000509

(4) The effect of month of birth and size of nursing litter upon the growth rate was explored. The overall average litter size from fifty-nine litters born in captivity was 5.4 young per litter. Graphical analysis failed to reveal any distinct trend in average instantaneous rate of growth for the first week when plotted according to litter size, nor did there seem to be a trend in number nursing when compared month-to-month. It was evident, however, that few litters were born in the months of September, October, and November, presumably because of experimental procedure, nine litters being the total production for these months. (See Table 6) The five litters from November were a result of the adoption of an improved breeding environment which was then used during the last part of December and the ensuing months. For an analysis, data from September to November were grouped and compared with those from January, February, and March. The size of nursing litter was grouped into the categories "five and less" and "six and more". The analysis examined the litter average instantaneous rate of growth, for the first week, of only those animals which were the offspring of the female nursing them. An analysis of variance reveals no statistically significant differences between litter sizes or between periods of birth (see Tables 7 and 8).

TABLE 6
NUMBER OF YOUNG NURSING THAT SURVIVED THE FIRST WEEK ACCORDING
TO MONTH OF BIRTH

<u>Month of Birth</u>	<u>Number of Litters Grouped According to Litter Size Nursing</u>							
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>
September				1		2		
October							1	
November			2	2	1			
December	(No Animals Mated Prior to December 15th)							
January			3	3		4	1	
February		1		1	3			2
March		2	1	1	1	9		

Note: Litter size at birth has been augmented for experimental purposes in many cases.

TABLE 7
LITTER AVERAGE INSTANTANEOUS RATE OF GROWTH ACCORDING TO MONTH
OF BIRTH AND SIZE OF NURSING LITTER

<u>Size Group</u>	<u>September-November</u>	<u>January</u>	<u>February</u>	<u>March</u>
5 and less	.096	.121	.108	.084
6 and more	.107	.099	.097	.076

TABLE 8
ANALYSIS OF VARIANCE OF NURSING LITTER SIZE AND MONTH OF BIRTH

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Mean Square</u>
Litter size	1	.000112
Month of birth category	3	.000333
Error	3	.000094

(5) If the variability in growth rate during the first week after birth prevents us from obtaining clear information concerning the factors which influence growth, it may be that a better tool would be a longer period of growth. The following analyses will be concerned with the rate of growth during the first three weeks of age. These weekly periods have been selected arbitrarily and mean instantaneous rates have been calculated for the three weeks for 96 animals representing 30 litters.

The analysis of variance used here is a simple two-factor analysis, the factors being animals and weeks, and the error term the interaction of the two. The term "animals" is further broken down into meaningful comparisons, namely litters, and still further into parents and litters of the same parents (see Table 9).

(a) Between Litters. There are two possible tests of differences between litters:

(1) First, one may inquire as to whether these particular litters differ among themselves in growth over the three weeks. For this test we use as an error term the differences

between animals within the litters. In making this comparison, highly significant differences ($F = 12.1^{**}$ at 29, 66 degrees of freedom) are found. There is thus reliable evidence that these litters differed over the entire experiment.

(2) Second, we may investigate the litter average growth on the basis of how consistent the differences between litters remained when examined week-by-week. The error term here is the interaction weeks x litters. This test reveals no significant differences ($F = 0.805$ with 29, 58 degrees of freedom). This is a test of differences between litters in general, viewing the present litters as samples of some larger population of similar litters. In conclusion, then, we do not have evidence of consistent differences between litters.

(b) Between parents. There appear to be no significant differences among parents with regard to growth rate of their young, using as a basis of comparison the variation observed between litters of the same parent ($F = 0.865$ at 22, 7 degrees of freedom) for the total three week period.

(c) Between litters of same parent. There is, however, a highly significant difference between the growth of litters of the same parent when compared with growth of animals within litters ($F = 13.4^{**}$ at 7, 66 degrees of freedom) for the total three weeks of growth.

(d) Interaction: weeks x litters. Over the course of the experiment, high and significant interaction of weeks

with litters is observed when compared to the interaction: weeks x animals within litters. ($F = 11.8$, with 58,132 degrees of freedom). This means that the litters followed different growth trends from week to week. This high interaction or inconsistency was seen above, in the discussion of differences between litters.

(e) Weeks. In comparing overall growth rate during the first three weeks, it is obvious that there are significant differences when comparing either with the interaction of weeks x animals ($F = 83.3^{**}$) or with the interaction of weeks and litters ($F = 30.3^{**}$). These differences are explored elsewhere in this paper.

The apparent inconsistency of statements (a-1), (a-2), and (d) above, reflect the fact that the different litters fluctuated in rate of growth from time to time. Each member of a litter seems to follow consistently the litter fluctuation, and this agreement allows fairly precise comparisons of several litters at any particular age with the conclusion that there are significant differences between litters at that age (a-1). However, when viewed over the entire period, litters fluctuate considerably from week to week, some growing faster one week, some another. These differences may exhibit compensatory variations and are found to be significant when analysed by arbitrary time periods. These changes which occurred from week to week may represent hereditary or environmental influences, a possible environmental influence being day-to-day fluctuations in

the laboratory climate and schedule. Whatever the cause, it was not identified in this study.

It would seem further that growth rates might better be calculated so that the time interval, rather than being an arbitrary chronological unit, would be coincident with a particular physiological event in the life of the individual concerned. The determination of such events might greatly aid a comparative study of species growth differences, as well as setting landmarks useful in field studies.

TABLE 9
ANALYSIS OF VARIANCE FOR LITTERS, PARENTS, AND WEEKS

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Mean Square</u>
Animals	95	379.04
Between litters	29	1043.55
Between parents	22	1006.77
Between litters of same parent	7	1163.43
Between animals of same litters	66	86.61
Weeks	2	39,330.00
Weeks x animals	190	472.64
Weeks x litters	58	1297.64
Weeks x animals of same litter	132	110.14

(6) Disturbance incidental to measuring the young animals appeared to be one likely factor influencing rate of growth when this study was undertaken. To test whether such disturbance was truly a factor affecting rate of growth, and to measure the effect, if possible, certain experiments were set up. Several litters were subjected to a number of experimental treatments, as described previously (p. 11). In addition, a more precise experiment was devised to test the effect of disturbance independent of that of heredity, by means of a series of 2 x 2 Latin Square designs based upon the exchange of members of two litters, keeping equal the number nursing each of the females.

The overall comparison of the effect of various procedures (Table 10) has been made by an analysis of variance (Table 11). The experimental procedures 6 and 8 (see p. 11) were not represented by a sufficiently large sample, and are not included in Table 10. In comparing the variation within litters with that among litters, highly significant differences are indicated ($F = 7.04^{**}$), as has been encountered before in this work. The comparison of among procedures with litters within procedures indicates that there are fewer differences among the various procedures than one would expect to find by chance ($F = 0.31$).

The effect of disturbance was examined more directly by a series of six experiments, each of which was analyzed as a 2 x 2 Latin Square. Each experiment consisted of two females nursing equal-sized litters of young. Half of

TABLE 10
MEAN INSTANTANEOUS RATES OF GROWTH AMONG THE VARIOUS
EXPERIMENTAL PROCEDURES

<u>Experimental Procedure</u>	<u>No. of Litters</u>	<u>Total No. of Offspring</u>	<u>Mean Instantaneous Rate of Growth</u>
Split, Disturbed Own Mother	7	20	.100
Split, Disturbed Foster Mother	10	18	.092
Split, Not Disturbed Own Mother	6	14	.094
Split, Not Disturbed Foster Mother	8	16	.090
Not Split, Disturbed Own Mother	15	63	.101
Not Split, Not Disturbed Own Mother	11	49	.100

TABLE 11
ANALYSIS OF VARIANCE OF INDIVIDUAL INSTANTANEOUS GROWTH RATES
AMONG THE VARIOUS EXPERIMENTAL PROCEDURES

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Mean Square</u>
Total	179	
Among litters	56	.001718
Among procedures	5	.000558
Within procedures	51	.001831
Within Litters	123	.000244

the young born to each female were exchanged for half of the young of the other female. The one female and mixed litter were then subjected every second day to disturbance equivalent to the handling and measuring of the parent and the young, while the other female and litter were similarly disturbed only once every 7 days. Symbolically, if A and B represent the two females, and a and b respectively their offspring, then the 2 x 2 Latin Square is:

	Raised by female A <u>(Not Disturbed)</u>	Raised by female B <u>(Disturbed)</u>	<u>Total</u>
Raised by own mother	a	b	a + b
Raised by foster mother	b'	a'	a' + b'
Total	a + b'	a' + b	

where the comparison $a + a'$ versus $b + b'$ represents the effect of parentage. Repetition of the experimental design is necessary because in any single experiment the effect of disturbance is combined with that of any difference between females with regard to quality of care extended the young. This split litter procedure was designed to allow the raising of animals of similar hereditary backgrounds under different levels of disturbance, since hereditary differences of the young of different litters were assumed to be important. These experiments were not designed to furnish equally good information on the effect upon growth rate of hereditary factors, or of the "nursing potential" of the mother studied, although such studies would be valuable.

The original data of these experiments (Table 12) are first week average growth rates for half-litters. It has been assumed that any variation in growth rate would be found during the first week of age, and that a longer period may well hide such variation by compensatory effects previously mentioned. Examination of the data shows that the effect of disturbance over all experiments was extremely small, as was also the effect of whether an animal was raised by its own or by a foster mother. These facts are reflected in the analysis of variance (Table 13). There appears no significant differences between the average growth rate of parts of litters raised by their own or by a foster parent, nor is there evident any difference between the average growth rate of litters raised under disturbed conditions as compared with those raised under the less disturbed conditions. There is, however, evidence of differences among the various experiments or repetitions of the Latin Square (See Table 13).

TABLE 12

ORIGINAL DATA FROM REPEATED 2 x 2 LATIN SQUARE ANALYSIS OF THE EFFECTS OF DISTURBANCE; AVERAGE INSTANTANEOUS RATES OF GROWTH FOR THE FIRST WEEK OF HALF-LITTERS (Figure in parentheses is the number of animals in the half-litter.)

	<u>Disturbed</u>	<u>Not Disturbed</u>
Parent Number	568	562
Own Mother	.075 (3)	.074 (3)
Foster Mother	.085 (3)	.070 (3)
Parent Number	554	563
Own Mother	.109 (3)	.089 (2)
Foster Mother	.098 (4)	.091 (4)
Parent Number	541	550
Own Mother	.112 (2)	.145 (2)
Foster Mother	.097 (1)	.115 (1)
Parent Number	575	571
Own Mother	.082 (3)	.080 (3)
Foster Mother	.069 (3)	.088 (3)
Parent Number	137	502
Own Mother	.105 (3)	.092 (4)
Foster Mother	.101 (4)	.106 (3)
Parent Number	132	554
Own Mother	.077 (2)	.087 (3)
Foster Mother	.106 (3)	.084 (2)

TABLE 13
ANALYSIS OF VARIANCE COMBINED FROM DATA OF THE SIX 2 x 2
LATIN SQUARE EXPERIMENTS

<u>Source of Variation</u>	<u>Degrees of Freedom</u>	<u>Mean Square</u>
Total	23	
Disturbance	1	1
Experiment	5	920
Own <u>vs</u> Other	1	12
Parentage	6	101
Disturb x Experiment	5	145
Own x Experiment	5	202

D. Linear Measurements

Means and standard deviations were calculated for each sex for tail length, length of right hind foot, and the distance between the anterior border of the anus and the posterior base of the urinary papilla (See Fig. 7). For use in Figures 8, 9, and 10, the standard deviations were calculated by pooling the sums of squares and degrees of freedom ($n - 1$) for three consecutive days and associating the standard deviation thus obtained with the mean of the central day of the group, using the second, seventh, fourteenth, and twenty-first days respectively. This method increases the number of observations contributing to the standard deviation, which is presented here as a measure of variability, not as a basis for computing standard error. A great proportion of the variability observed in linear measurements may well be due to human error.

Only about fifty-one per cent of the average adult tail length of 51.5 millimeters (Burt, 1954, based on the midpoint of the range of 38 to 65 mm.) is reached at three weeks of age. The tail growth curve (Fig. 8) shows little difference between sexes, but variability tends to increase with age, perhaps because of increasing measuring error introduced as animals became more active.

Figure 9 suggests that the average adult hind foot length of 21.5 millimeters (Burt, 1954, based on midpoint of range given as 18 to 25 mm.) is nearly reached (89 per

Figure 7

Illustration to show measurement of the distance between anus and urinary papilla for the early determination of sex in young Microtus pennsylvanicus. (Measurement is about five millimeters on specimen shown.)

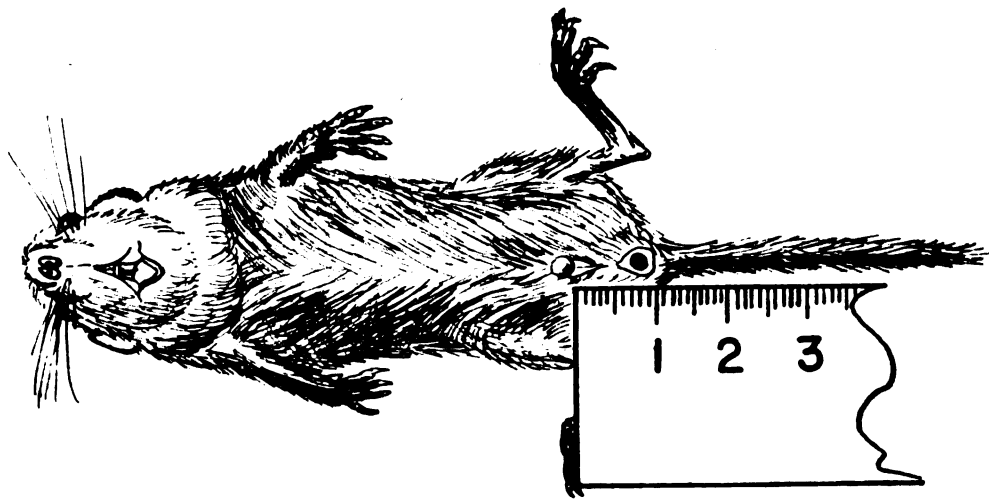


Figure 7

Figure 8

Change of mean tail length with increase of age for males (solid line) and females (broken line) with one standard deviation on each side of the means as an indication of variability (see text). Solid bar represents male and open bar represents the female standard deviations.

Figure 8

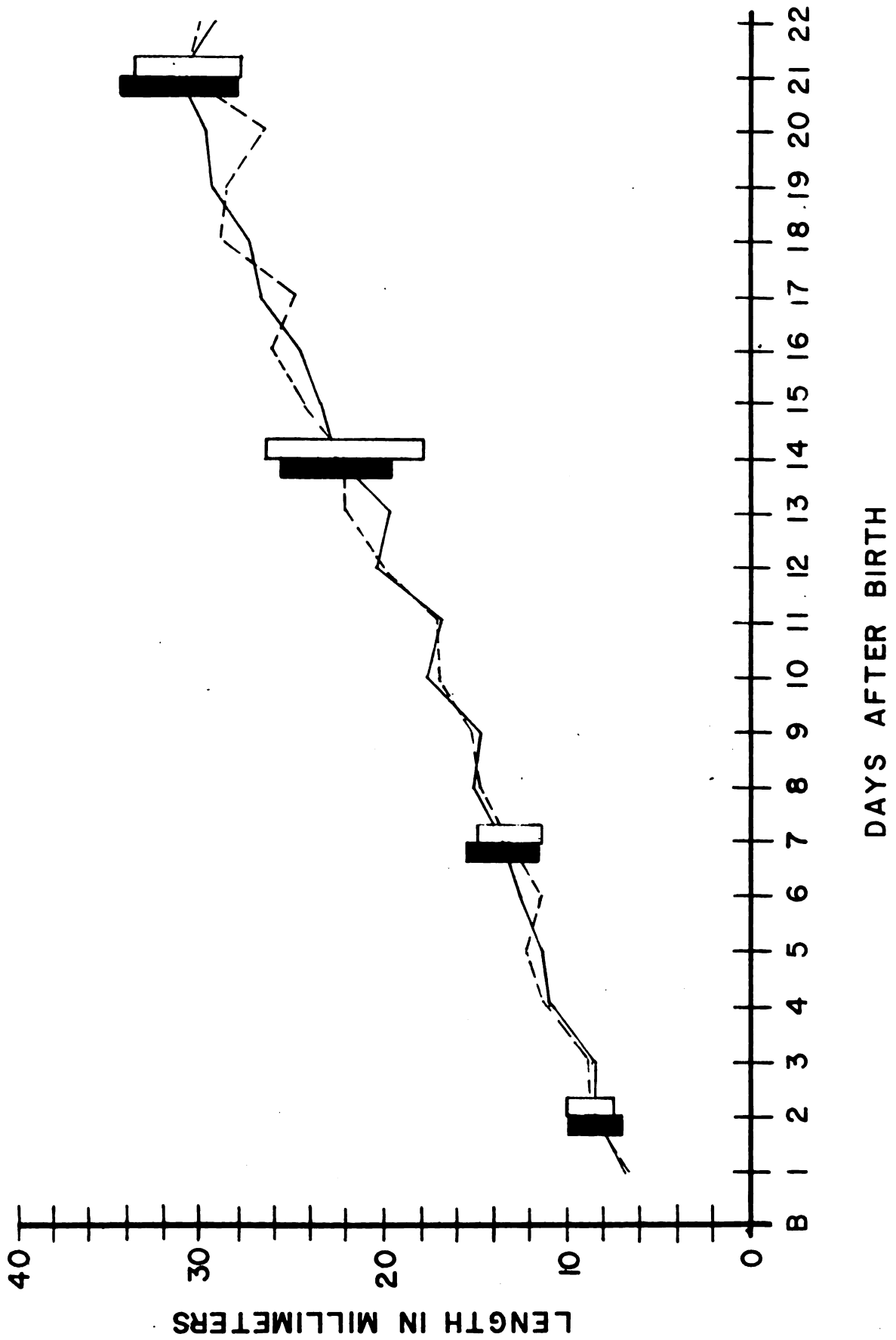
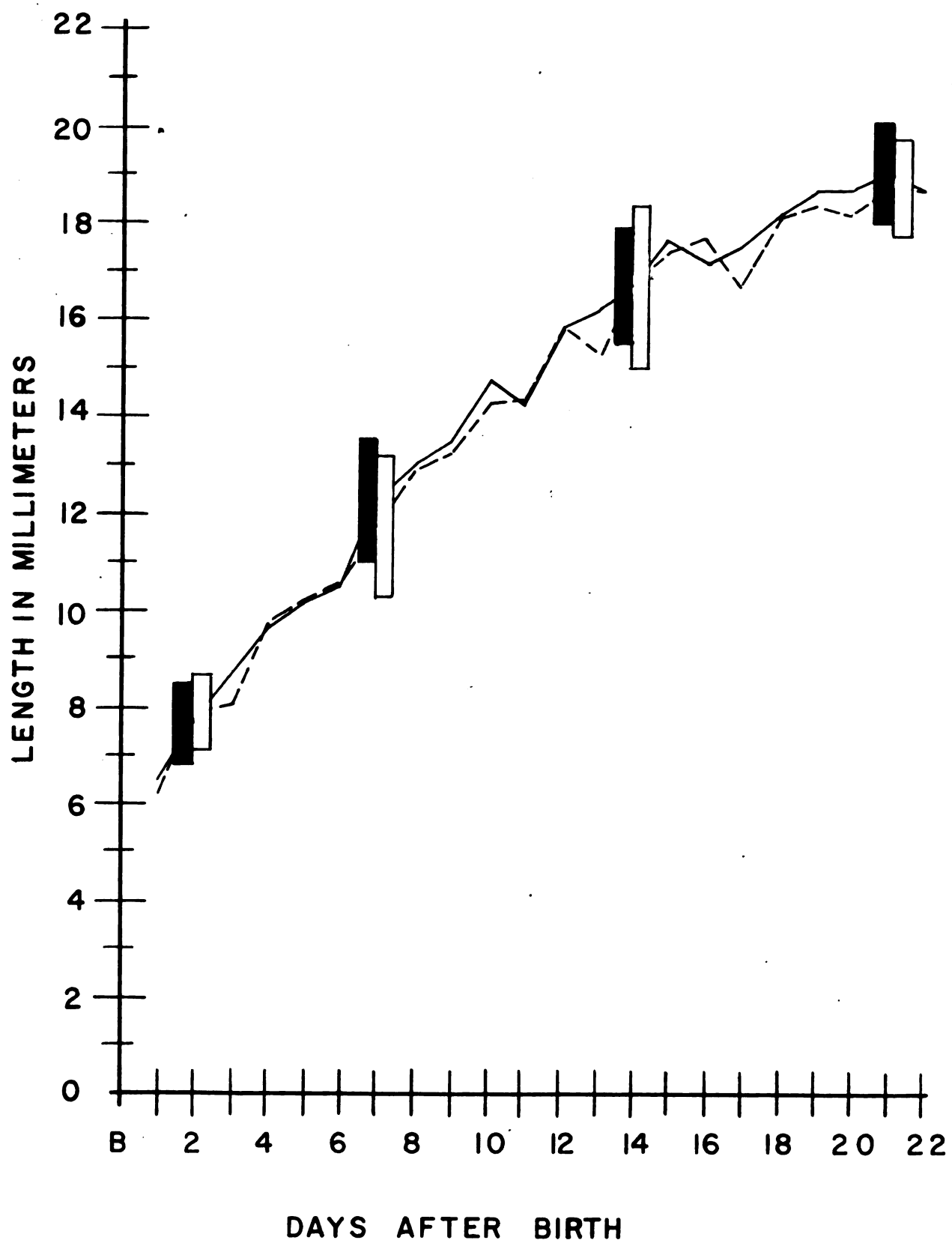


Figure 9

Change in mean length of hind foot with increase in age for males (solid line) and females (broken line) with one standard deviation on each side of the means as an indication of variability (see text). Solid bar represents male and open bar represents female standard deviations.

Figure 9



cent) by the end of the first three weeks. There appears to be no distinct trend in the variability as age increases, nor is there evident any significant difference between sexes concerning hind foot development.

As previously mentioned, the anus-urinary papilla measurement was made in an effort to determine externally the sex of young mice. No statistically significant differences between sexes were shown by use of the "t" test until the eighth day when a highly significant difference (at the 1 per cent level) was indicated. Except for the ninth day when the sample size was very small, each subsequent daily examination showed a very highly significant difference (1/10 of 1 per cent level of significance) between male and female measurements. Figure 10 graphically shows the trend of growth of this character after the first six days. It is therefore evident that a statistical basis exists for the use of this external measurement in sex determination of young Microtus after the first week. It should be noted that such determination would be more usable when animals of the same litter are compared with each other.

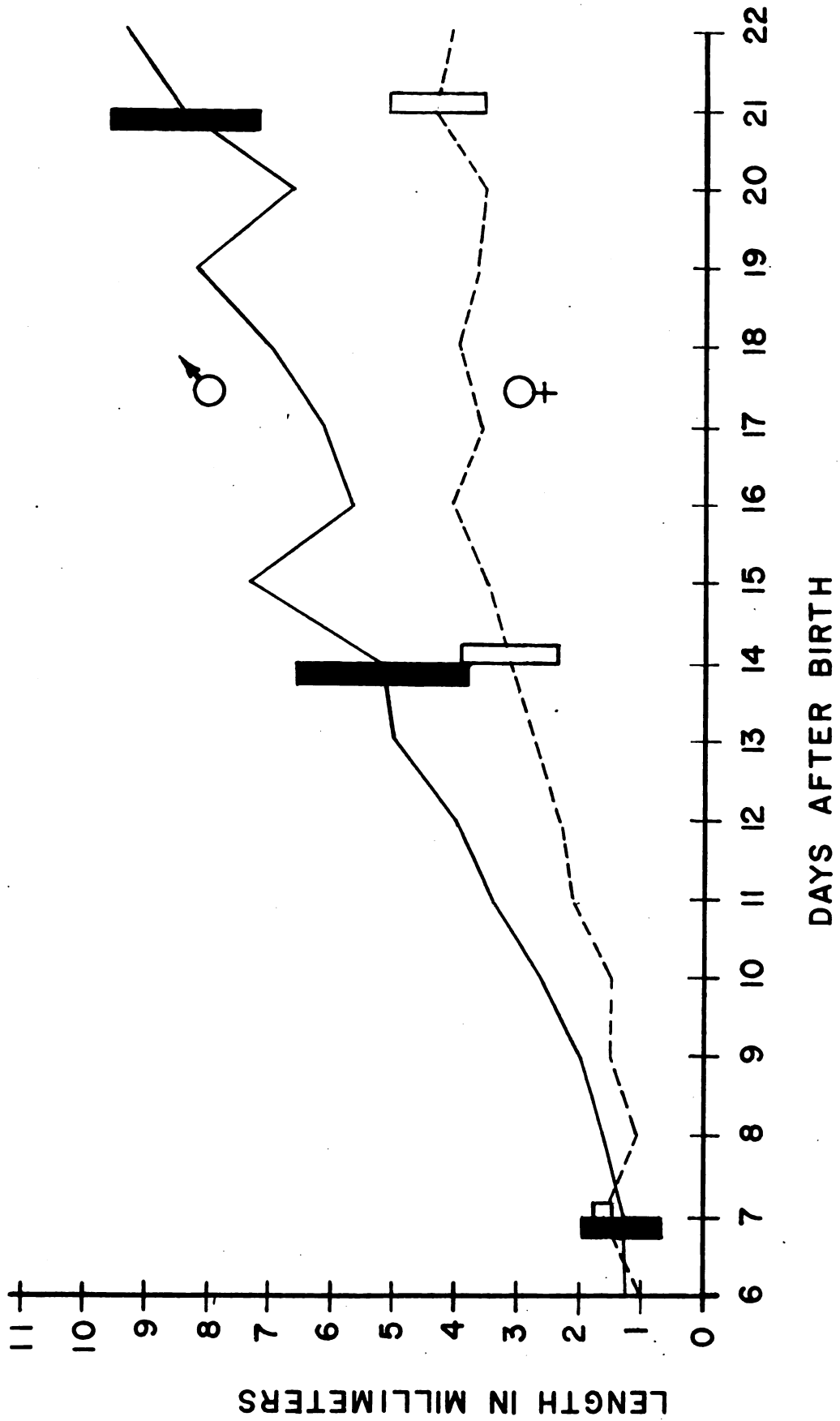
E. Qualitative Observations

External qualitative characteristics of the developing young Microtus were studied with the hope that they might be used to age young animals in the field. Such traits as the unfolding of the ear pinnae and the opening of the eyelids are easily observed. Although the subject is not

Figure 10

Curve representing the daily increase of the distance between the anus and base of the urinary papilla of males (solid line) and females (broken line). One standard deviation on each side of the mean represents the variability. Solid bars denote male standard deviations, open bars denote female standard deviations.

Figure 10



pursued here, regular black and white photographs may be readily used for recording proportional changes in body parts (see Appendix 1), although color and quality of the coat are probably best recorded by color photographic methods.

In no case were the observations of the animals spaced more than two days apart, therefore the day of ear pinnae unfolding and the day at which at least one eyelid is separated are subject to an extreme error of two days, with average error of less than one day. From Tables 14 and 15, it is observed that all animals had ear pinnae unfolded by the fifth day, and all had at least one eye in use by the eleventh day.

TABLE 14
PERCENTAGE OF EIGHTY-EIGHT ANIMALS WITH EAR PINNAE UNFOLDED
BY DAY INDICATED

<u>Days after Birth</u>	<u>Number</u>	<u>Percent Unfolding at Day Indicated</u>	<u>Cumulative Percent of Total</u>
1	18	20.5%	20.5%
2	30	34.1%	54.6%
3	29	33.0%	87.6%
4	8	9.0%	96.6%
5	3	3.4%	100.0%

TABLE 15
 PERCENTAGE OF 123 ANIMALS WITH AT LEAST ONE EYE OPEN BY
 DAY INDICATED

<u>Days after Birth</u>	<u>Number</u>	<u>Percent Opening at Day Indicated</u>	<u>Cumulative Percent of Total</u>
7	3	2.4%	2.4%
8	37	30.1%	32.5%
9	27	22.0%	54.5%
10	45	36.6%	91.1%
11	11	8.9%	100.0%

IV. DISCUSSION

It is evident from this study that only general conclusions may be made regarding the growth rate of the meadow vole, Microtus pennsylvanicus, as observed in the laboratory. Individual variability from environmental and hereditary factors is high and tends to obscure more specific and detailed information. It is thought that such variability as is observed in the laboratory may be less than that expected in the field, though this statement is only opinion until tested.

The day-to-day analysis of the instantaneous growth rate has shown little statistical basis for accepting the distinct growth periods described by Brody (1945) and Cowan and Arsenault (1954), nevertheless, it would seem that rates of growth calculated by physiological events in the life of the animals rather than arbitrary time periods may prove of interest. The use of individual rather than group instantaneous rates of growth allows the use of statistical tools, and supplies information concerning variability.

It is seen that although there is much evidence for differences between litters with regard to growth rate over short periods, there seems to be a compensatory action over a longer period which tends to obscure the short-term differences. Unexplained factors other than heredity, size of litter, month of birth, and degree of disturbance appear

to have influenced growth rate in this study.

Contrary to the findings of Cowan and Arsenault (1954), this study indicates that the aging of young Microtus pennsylvanicus by weight is subject to high error. It may be that weight combined with other information such as linear measurements and certain qualitative characters might prove a useful tool in the field.

A statistical basis has been demonstrated for the discrimination of sex in the young animals by measurement of the distance between the anus and the urinary papilla.

The study has undoubtedly raised more questions and problems than it has solved. Although the use of instantaneous rates of growth has shown its value in various computations, it may be asked: Is ^{there} a physiological time interval which would be of value in comparative growth studies? How much valuable information concerning speciation and animal distribution may be obtained from comparative growth studies? How do growth traits become inherited? Is there a threshold of response in regard to disturbance of a mother and the growth of her young? What environmental aspects affect growth rate, and how? The above are only a few of the questions which are presented.

V. SUMMARY AND CONCLUSIONS

(1) Animals were easily bred by including a number of females with one male in a large cage. The transfer of young Microtus to foster parents was successfully accomplished.

(2) No statistical differences were shown to exist between instantaneous growth rates of males and females during the first four weeks of age.

(3) Large amounts of variation prevent reliable estimates of age by weight criteria.

(4) No distinct growth periods were found during at least the first three weeks of growth when analyzed from day-to-day individual instantaneous growth rates.

(5) Although animals within any given litter were consistent in their rate of growth, the litter's average rate fluctuated during the first three weeks, and a difference was readily identified between litters for short periods.

(6) Growth rates of various litters differ so that a significant difference is shown to exist from week to week, but for the over all period no differences between litters were shown.

(7) Influencing factors other than heredity, degree of disturbance, parent history, size of litter, and month of birth affect growth rates of young Microtus. These factors may represent climatic factors of the immediate

environment, but were not identified in this study.

(8) A statistical basis has been shown for distinguishing sex after the first week of age by the linear distance between the base of the urogenital opening and the anterior border of the anus.

(9) About fifty-one per cent of the average adult tail length, and eighty-nine per cent of the average adult hind foot length is attained by the third week after birth in laboratory-raised animals. All of the animals were found to have their ear pinnae unfolded by the fifth day, and all of them had at least one eye open by the eleventh day.

(10) Several interesting problems have arisen from this study. Any attempt at a laboratory analysis of specific and subspecific differences in growth rate must give particular attention to the control of environmental conditions, either by control of laboratory conditions or by proper experimental design.

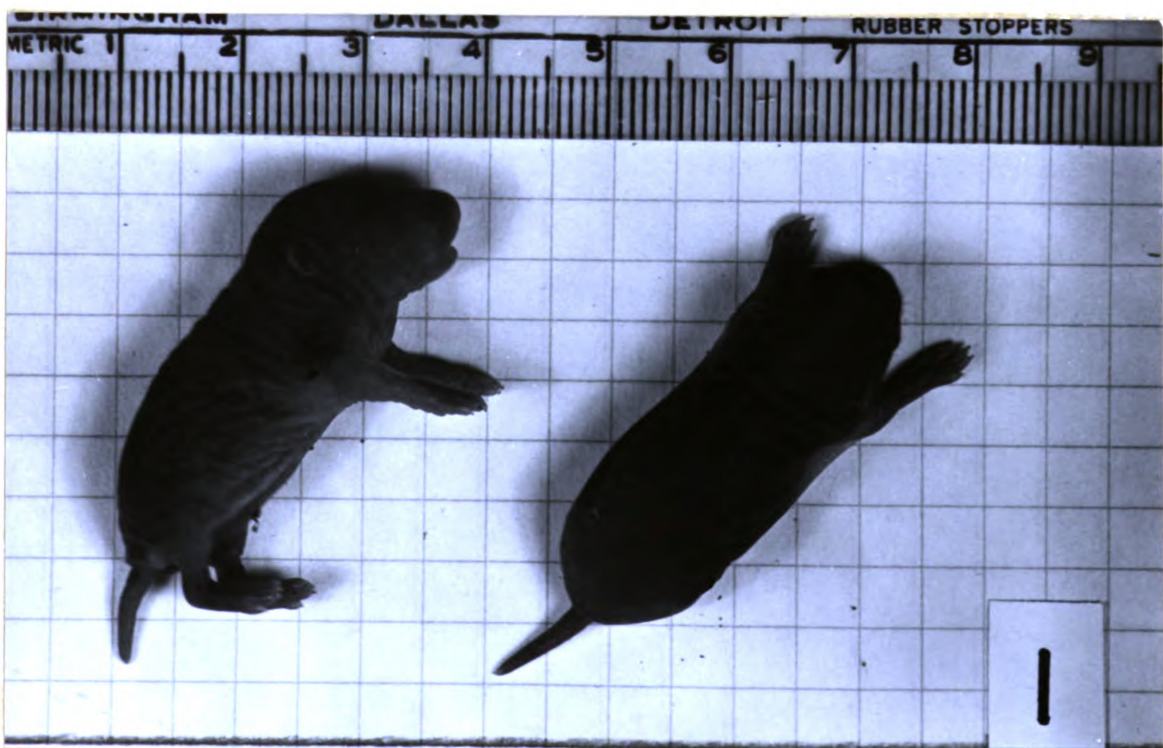
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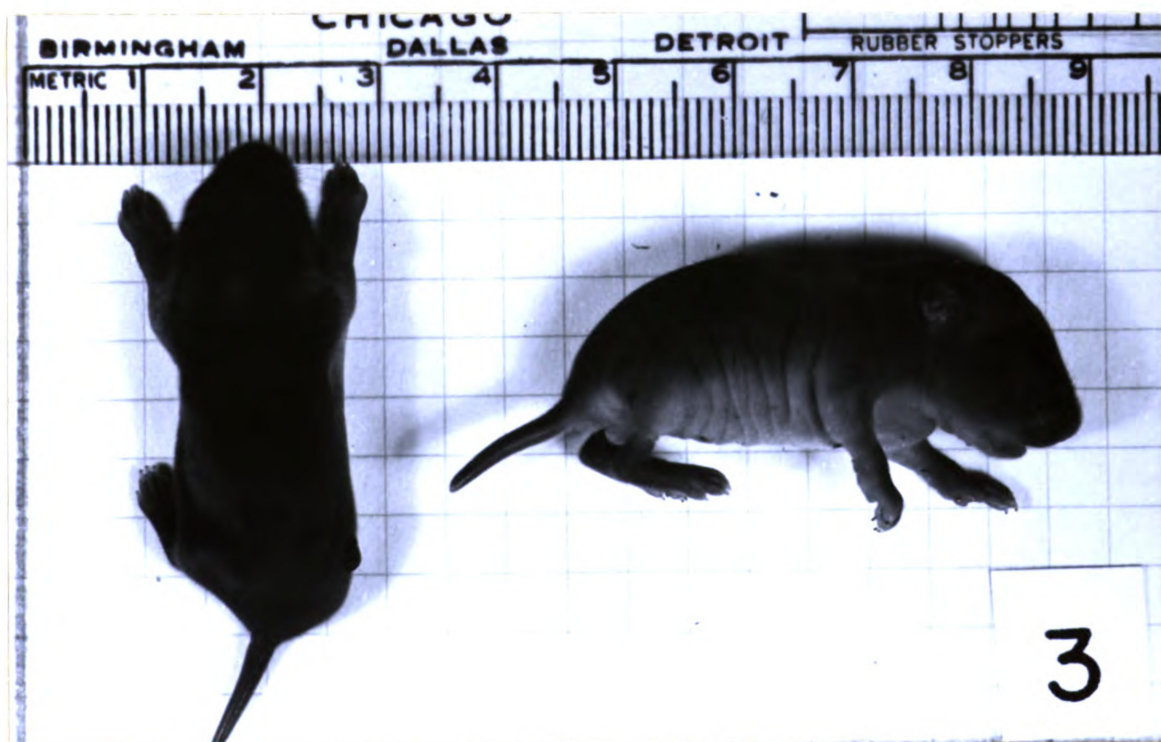
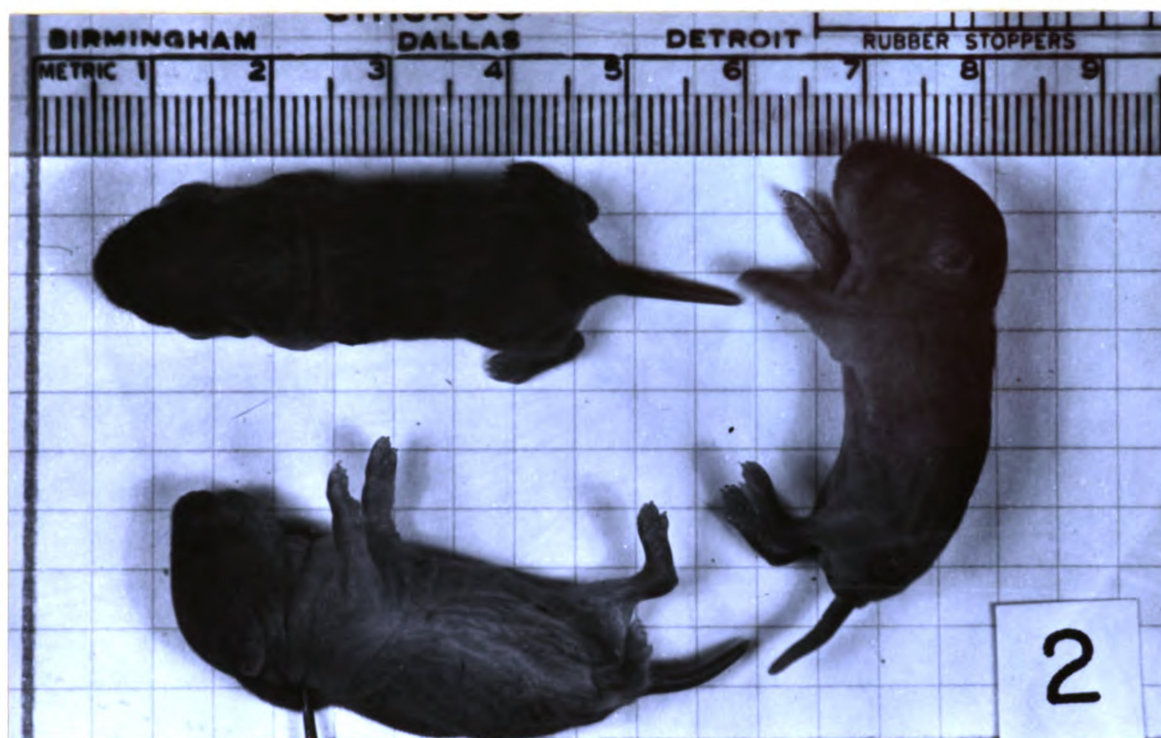
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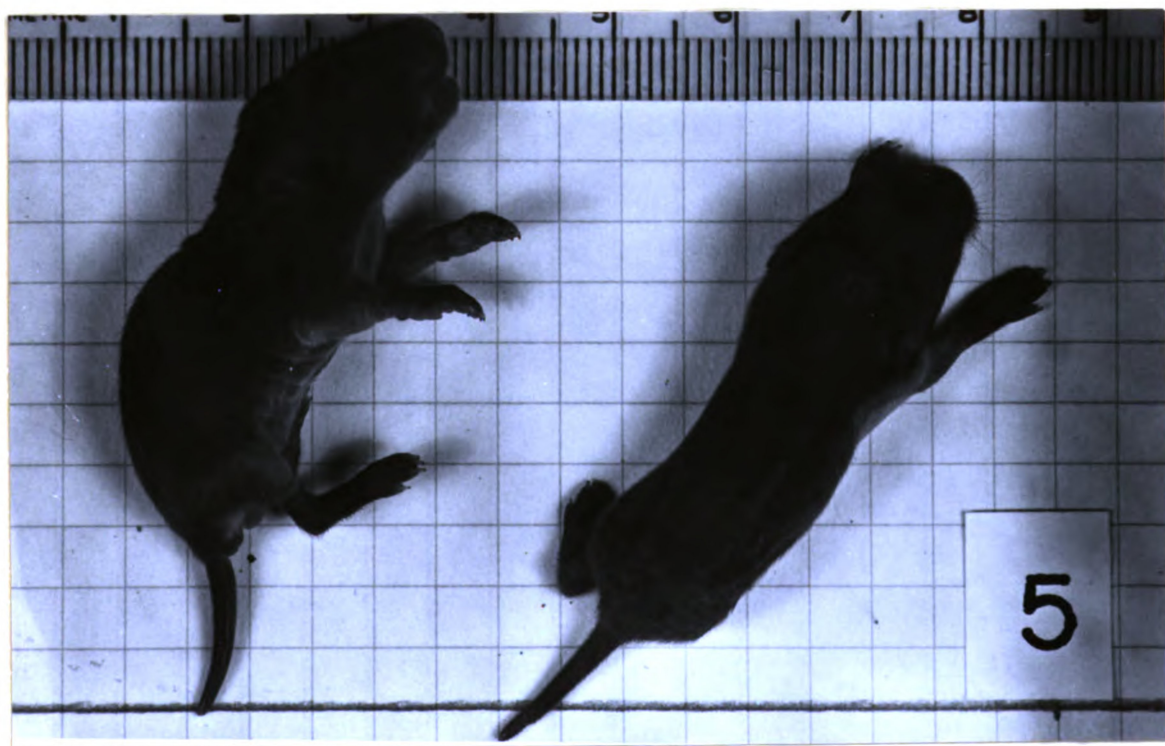
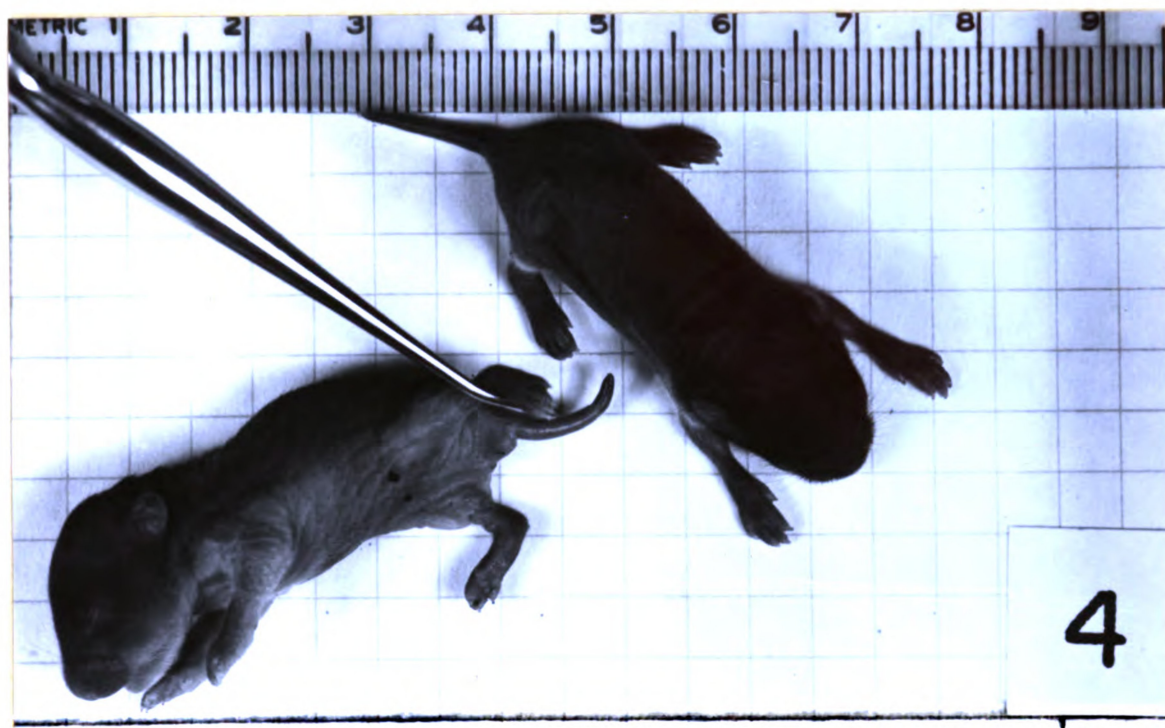
APPENDIX



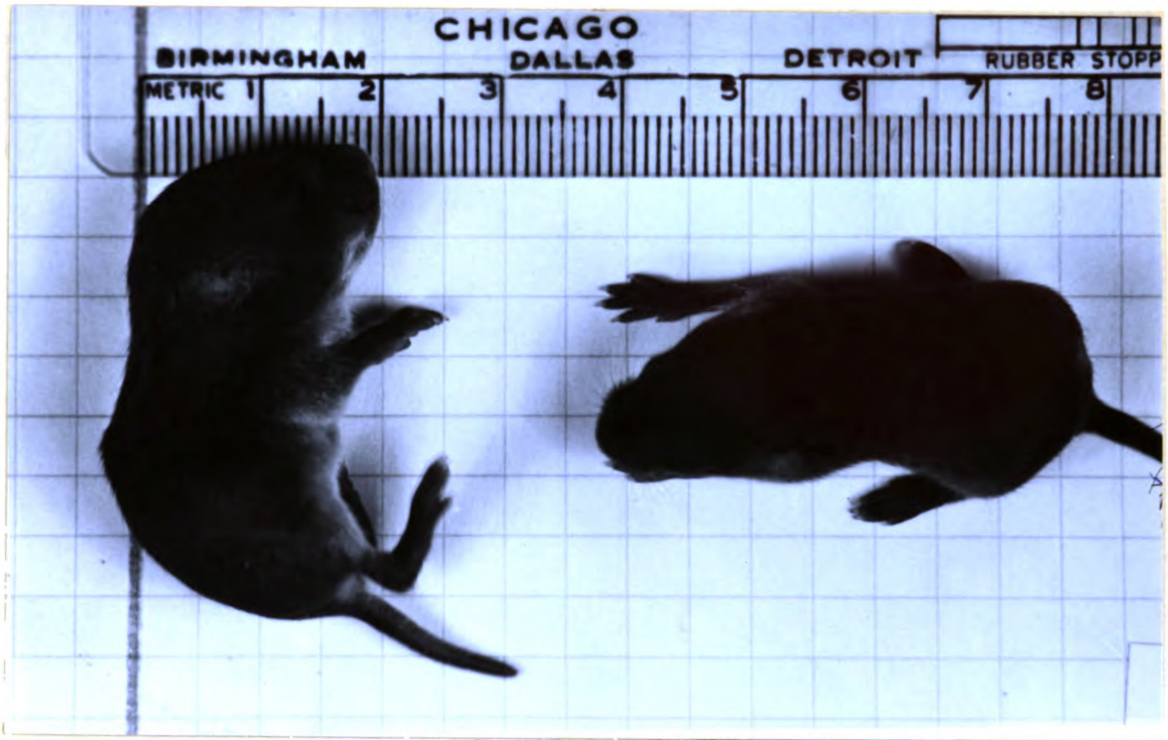
Young *Microtus* at birth (upper) and one day after birth (lower).



Microtus at two days (upper) and three days after birth (lower).



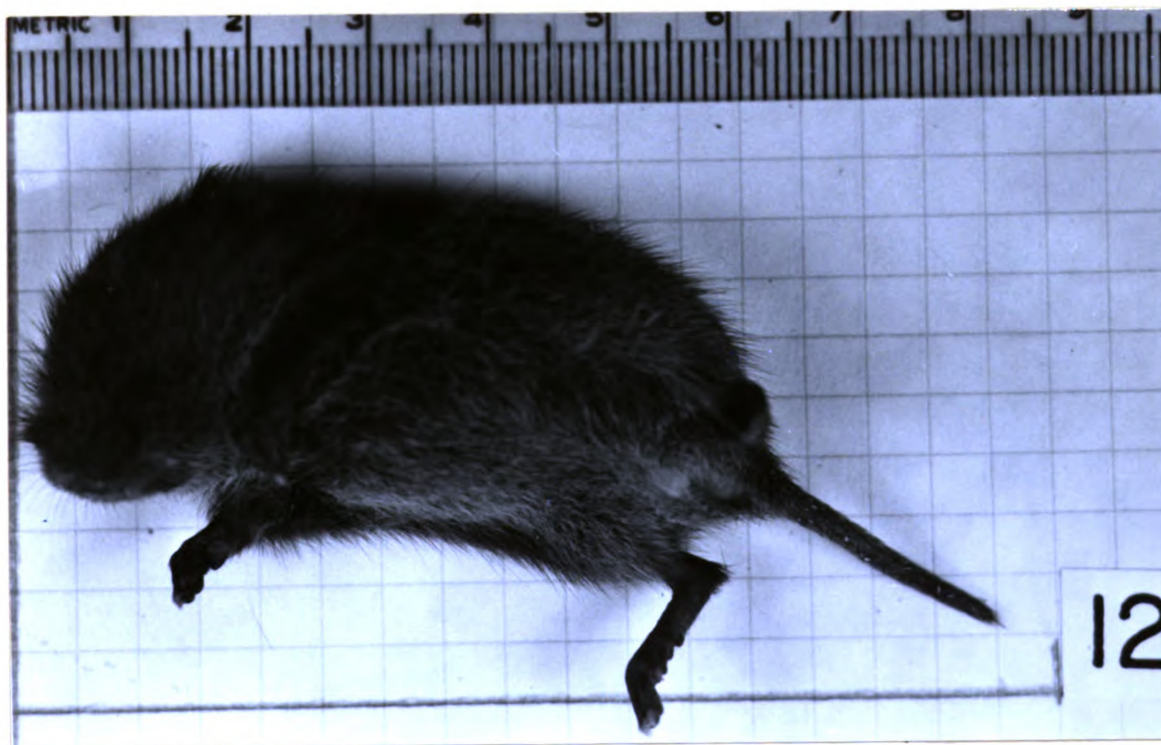
Microtus at four days (upper) and five days after birth (lower).



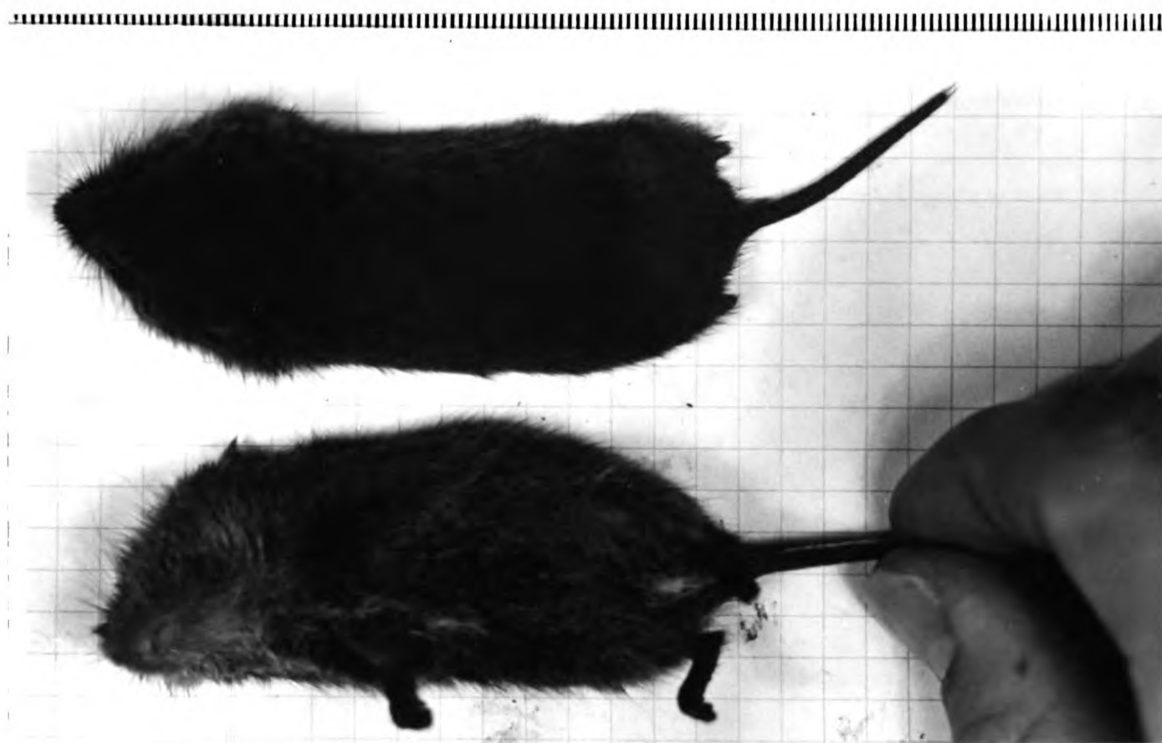
Microtus at six days (upper) and eight days after birth (lower).



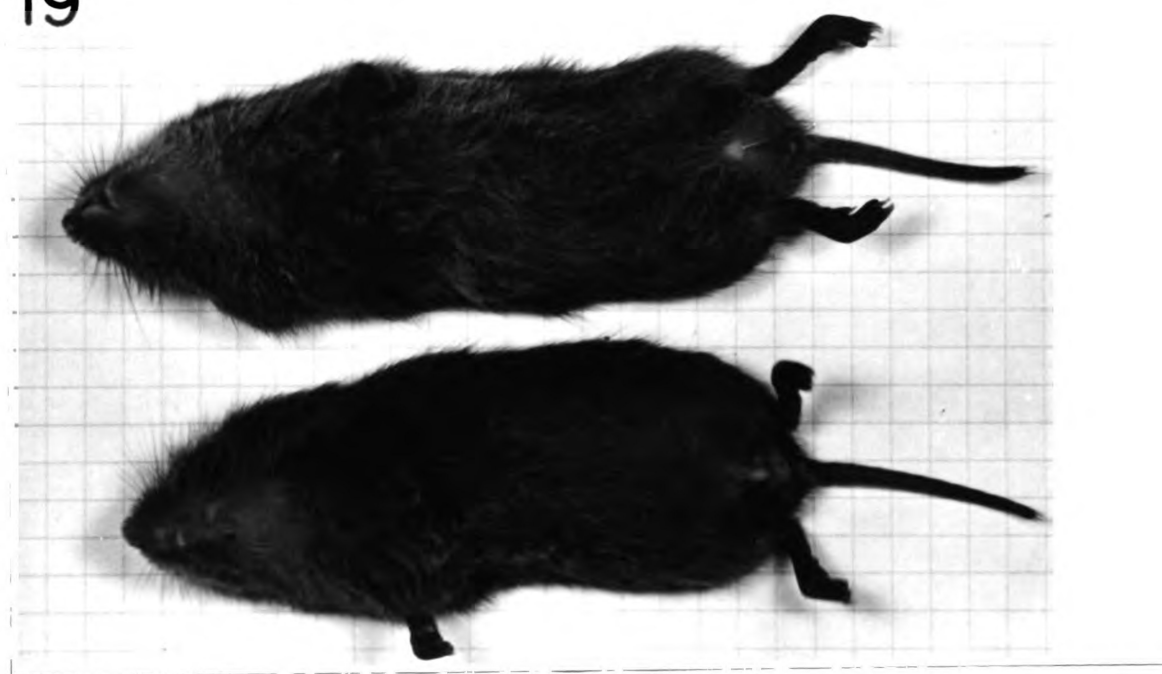
9



Microtus at nine days (upper) and twelve days after birth (lower).



19



Microtus at seventeen days (upper)
and nineteen days after birth (lower)



23

Microtus at twenty-three days after birth.



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