STUDIES OF TWO INBRED STRAINS OF MUS MUSCULUS; WITH PARTICULAR REFERENCE TO THE EFFECT OF THE EXTREME DILUTION GENE (c^e) ON LONGEVITY, AS ASSOCIATED WITH RATE OF GROWTH, METABOLIC RATE AND OTHER FACTORS

By

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INTRODUCTION

In recent years the process of aging, with its attendant variations and problems, has received considerable study. The approaches to the problem are diverse: biochemists have endeavored to detect possible influences of chemical changes in the tissues on aging, whereas geneticists have been primarily concerned with the influence of hereditary potentialities on longevity (Cowdry, 1942).

Two inbred strains of mice were specially developed for this investigation of the inheritance of longevity. One of them was called the black and tan strain, the other was the extreme dilute strain. The two strains differed only in the presence or absence of the "c^e" gene (as far as could be determined). An attempt was made to determine whether any definite difference in length of life could be established as a strain characteristic, and also to determine whether any association might exist between longevity and rate of growth, metabolic rate, and other differential characteristics.

At the outset, it should be pointed out that results of such a study would probably not be applicable to humans, or to the length of life of other animals, except in the sense that it gives one some basis for interpreting data from other forms. Contrary to general opinion, as Asdell (1946) has pointed out, the chronologic method of comparison does not fit physiologic conditions and is thus subject to gross error. Asdell summarizes his discussion by stating that, "No general system of age equivalences could be set up covering the whole life span. It could be done for equivalent physiological periods (such as gestation, length of uterine development, etc.) but as soon as these change to a

marked degree, a new set of equivalents would have to be established. Man fits less well into any of the systems than any other species because of the many peculiarities of his physiological behavior."

A major part of this investigation has been concerned in the comparison of the rate of growth and body size of the black and tan and the extreme dilute mice. Many other workers have studied the inheritance of size in mice. A correlation has been found to occur between inheritance of coat color and body size. It was concluded that some color genes increased (Feldman, 1935), some decreased, (Castle, 1941), and others had no observable effect on body size (Castle, 1938).

The brown gene (b) was the first of the coat color group to which was attributed an influence on body size. Green (1931) made numerous investigations on the inheritance of body size and its association with the brown gene. He concluded, however, that the chromosome bearing the brown gene also contained genes influencing body size, rather than that the brown gene itself exerted an effect on size. Feldman (1935) later concluded that the brown gene had a direct physiological accelerating action on growth. His conclusions were supported by Castle (1941).

Several coat color genes that have a dilution effect have also been believed to influence body size. They are Maltese dilution (d), leaden (ln), pink eye (p), and pallid (pa). Dilution has an accelerating influence, whereas the other three have retarding influences on growth. According to Castle and others (Castle, Gates, and Reed, 1936) the influence of blue dilution in mice is of the same nature as brown but less in amount. Cumulatively, brown and dilution have a greater accelerating effect on growth than either acting alone. The order of increasing size is as follows: blacks are smallest, then come blues, next browns, and last dilute browns. Pink-eyed dilution (p) seems to

reduce body size slightly (Castle, Gates, Reed and Law, 1936) and leaden (Castle, 1941) decreases it appreciably. Pallid (Castle, 1941) has a stronger retarding effect than pink-eye or leaden. Of particular interest is the interaction between brown and leaden, and brown and pallid. Brown leaden animals are smaller than black leaden individuals. In combination with pallid, brown also has a further diminishing effect on size. Thus the stimulating action of brown is reversed when it is associated with leaden or pallid. In addition, the combination of leaden and pallid is more retarding than either by itself.

A number of physiological and morphological characteristics have been attributed to the action of the lethal yellow gene (A^Y). Several of the various effects could well be due to a more fundamental endocrine disorder which may be conditioned by "A^Y". The effects pointing to this endocrine disorder are as follows: marked obesity, especially in females; low basal metabolic rate; shortened reproductive period; and shortened life expectancy for females. Danforth (1927) made a study of hereditary adiposity in mice using yellow animals. As a result of his findings, he assumed that the "A^Y" gene itself was responsible for adiposity (because no crossovers occurred). Since that time numerous workers have recognized the fact that yellow mice are more obese than non-yellows. Castle (1942) found that lethal yellow increases general body size in mice more than pallid (pa) decreases it, as regards both weight and body length. Dickerson (1946) made a further analysis of the underlying causes of obesity in yellow mice. He concluded that the yellow gene produced obesity by increasing appetite and reducing energy requirements. Dickie and Woolley (1946) weighed 161 mice (yellow and non-yellow) at monthly intervals. Peak weights were recorded between

seven and eighteen months of age and after eighteen months the yellows decreased in weight so that by twenty-four months they were approximately the same as non-yellow littermates. Further, some thin yellow mice were observed. They appeared perfectly normal. This raised the question of whether "A^y" itself or a closely linked gene was responsible for the obesity.

The albino series has received comparatively little attention as far as its influence on body size is concerned. Castle (1938) concluded that albinism (c) had no effect on body size. Law (1938) used a group of extreme dilute (c^e) animals in his experiments, but he was primarily interested in the short ear gene and drew no conclusions re the "c^e" gene. This summarizes the major work that has been done on coat color gene body size determination. There remain a few remarks in regard to body size and growth in general.

Stefan Kopec (1938) carried out an experiment in which 24,000 daily weights were taken of sixty-three female and forty-nine male black-and-white spotted, white, and lilac mice. He is one of the few workers who took daily weights of his animals. He found that males showed non-cyclic weight increase with irregular fluctuations, while the females showed marked cycles of weight change. These cycles appeared at the time when sexual maturity was attained; decreased in intensity with age, and ended with cessation of sexual activity. An average cycle lasted approximately thirteen days. Weight changes in males and females of different strains were similar. Apparently he did not detect the effect of (p) in his lilac mice.

Howard (1941) studied nine inbred strains of mice, three of which had a high incidence of mammary carcinoma, and six with low tumour in-

She weighed the mice weekly from birth to seventy days and

noted that differences existed between strains in rate or growth. In this case correlations were made with cancer susceptibility and not with coat color. A correlation was indicated between mammary cancer susceptibility and normal growth rate of young animals in inbred lines of mice. This was based on the age at which males and females diverged in weight. The three strains having a high incidence of spontaneous mammary gland carcinoma in breeding females, first showed a significant difference in weight between the sexes at eight, eight, and seven weeks of age. In the six (low incidence of mammary carcinoma) other strains, significant sex differences in weight appeared at five or six weeks of age. This has no direct bearing on the problem at hand other than to throw an interesting sidelight and show another factor at work in the complex process of growth.

Several workers have experimented with selective breeding for size, using mice. MacArthur (1944) has raised two races from one foundation stock. Successive generations of selections for body size produced a small race and an exceptionally large race. In 1944 the difference between these two races exceeded the mean weight of the small race. Less grams of body weight were lost by the minus selections, than were gained by equal effort at plus selection; and less grams were gained or lost by females than by males. These data agree with the view that size genes or modifiers multiply each other's effects and do not simply add to or subtract from the body weight some definite and constant number of grams. Goodale (1941) also carried on selective breeding experiments although his endeavors were directed to increasing size and he made no attempts to produce a "small" race. Generation after generation he continued to get an increase in body weight

through his selection.

One of the strains used in this investigation carries the factor for extreme dilution (c^e). This gene was first reported by Detlefsen (1921) and is an example of a mutant caught in the wild (others have been "Aw" and "pa"). Quoting from Detlefsen's paper: "On August 31, 1920, Mr. J. E. Knight of Weldon, Illinois, who exterminates rodents from corn cribs, poultry houses and the like, brought to my lab a young male mutant mouse which he had captured in a corn crib, located on a farm seven miles from the nearest town." On first examination, the animal looked like an ordinary black-eyed white mouse with dirty hair. Subsequently it became darker, and Detlefsen knew he had a new mutation. He made several test crosses and the mutant segregated out in the F_2 generation in all cases. He concluded that he had discovered one of three allels of the albino group. He designated the mutant gene as "c^d". Since that time it has often been called "Himalayan dilution" (c^H), since it was believed to be analogous with the gene producing the Himalayan rabbit. The extreme dilute mouse and the Himalayan rabbit, however, bear little resemblance to each other. Himalayan rabbits have pink eyes, white fur and intensely pigmented ears, nose, feet and tail. Extreme dilute mice have a uniformly faintly colored coat and dark eyes. Besides, a genuine "Himalayan mouse" has recently been caught wild on the island of Spiekeroog in the North Sea, which really resembles the Himalayan rabbit. The symbol, "c^e", is now usually used to designate extreme dilution and is the one recommended by the Committee on Mouse Genetics Nomenclature (1940). Extreme dilution is a member of the first linkage group (I) and is linked to pink-eye, shaker-1, and oligodactylie (Jour. of Heredity, vol. 36, no. 9, p. 271).

Extreme dilution is a member of the albino series of genes. Except for "C," which is completely dominant, the other allelomorphs of the series produce a "blending" type of inheritance, both factors acting together in the individual to produce a given fur color. "C" allows the full expression of the other color genes present. There follows a gradation of pigment-loss which finds its lower limit in the albino mouse, homozygous for the most recessive allel (c), whose coat and eyes have lost all pigmentation. The series from full color (C) to albino (c) shows a gradual reduction of first the yellow, and then the black pigment. The intermediate allelomorphs identified at the present are: intense chinchilla (c^{i}), chinchilla (c^{ch}), and extreme dilution (c^{e}).

The investigation this paper is concerned with was initiated in 1940 with the development of special strains for the study of longevity. The strains were developed by the usual methods without selection for any particular characteristic other than general health and vigor as determined by the appearance and behavior of the mice. Particular attention was given to the extreme dilute mice, as they had apparently not been previously investigated for size and related factors. Weight variations have been used as an index of growth. The term "adult body weight" seems a rather poor one as it is difficult to define accurately. Therefore, the mice have been weighed at frequent intervals, and rate of growth rather than absolute body size has been deemed of importance. Other characters investigated for variability between strains have been the following: sex ratio at twenty-one days of age; metabolic rate from one month to twelve months of age; litter size in general and according to parity; and longevity.

MATERIALS AND METHODS

Development of Strains. The original stock of mice from which were subsequently developed the present inbred strains, was a heterogeneous group of mice purchased at random from fanciers in Milwaukee. This stock of animals exhibited a wide variation in coat colors, many different color genes being represented. In addition, the mice showed exceptional vigor, rapid growth rate and high fertility and fecundity. After crossing all strains indiscriminately, one pair of black and tan mice heterozygous for "a^t" and "c^e" (Cc^e a^ta) was used as "the original pair" from which the inbred lines were started. In the eighth generation of brother and sister matings, one vigorous pair was used to initiate the stock. From this pair (#1), each member of which was heterozygous for three coat color factors (Cc^e a^ta Bb), three pairs of offspring were used to start the strains of black and tan, extreme dilute on black and tan, and brown and tan. Each of these strains was then inbred by brother and sister matings. Genotypes of the three inbred strains were as follows: CC a^ta^t BB; c^ec^e a^ta^t BB; and CC a^ta^t bb. The "a" factor had been bred out by selection. Other than that, no particular criterion except general vigor and state of health was used in the selection of the pair to continue the next generation. While all offspring in one generation were grouped for study, only one pair became the parents of the subsequent generation. In this way strains were obtained that had the same general origin with a known history as regards their previous characteristics. Also the undesirable features of albino animals were avoided as well as the action of numerous special genes, such as cancer susceptibility or resistance, found in the available inbred stocks so often used for genetic research. The difference

in phenotypic appearance between the members of the three strains was of value in preventing any confusion in identification that might have taken place by using three different strains having the same phenotypic appearance.

The data presented in this paper were obtained from stocks of mice that were being used at the same time in a study of the histological changes accompanying the aging process. Since a very large number of animals were required in this work, all crosses between the stocks were eliminated with the exception of a sufficient number made to ensure the fact that the stocks continued to be genetically identical with the exception of the "c^{e}" gene. It is recognized of course, that such rigid selection could readily produce numerous recombinations of minor or modifying genes and in this manner produce sublines with widely different genotypes. However, as there is no practical way of ascertaining this fact it had to be ignored in this study. On the other hand, similar breeding even within a strain as a whole will produce the same result. It may be mentioned that widely different sublines of the strains used showed close similarity to each other in the characters that were under investigation.

Records. Careful records were kept of all the mice, a triplicate system being used. In this manner the data were automatically grouped in three different ways. A card was made out for each mouse pair. On this card was given the number of the pair, the strain, and the date of mating. The main purpose of the card was to record litter data. Each litter born was entered on the card with the following information: date of birth, number in litter, number of males, number of females, number weaned, and disposition. Thus, at a glance, the cards gave one

the number of litters had by each female, in addition to other valuable and pertinent information. Each member of a mouse pair had a weight sheet. The sheet was so arranged that weights taken over a two-year period could be recorded on a single sheet. Date of birth was also given for each mouse on its weight sheet. Finally, the matings were all recorded in two bound books. Each mating was given a page number and on this page all "vital statistics" and any unusual observations were recorded. The book page number was incorporated into the number of the mating. Thus, pair number 111-A21 meant that the animals' parents were #11 and a full record of their history could be found in Book A on page 21.

Care of Animals. It was found that for the purposes of determining growth rate it was best to keep the mice in pairs. External conditions were more easily kept constant in that manner. There was also afforded more opportunity for observation of individuals than if the animals had been kept in large groups in cages. There was especially better feeding control by separation into pairs. Allee (1941) had found that when mice were kept together in cages in large numbers, certain groups dominated at different times and thus all did not have equal opportunity to secure food. Another advantage for raising the mice in pairs was that the frequent weighings were more quickly made in this way; and, naturally, information on individual litter birth dates and sizes was much more easily ascertained. The animals were consequently housed in enamel pans eight inches in diameter and three inches deep (Hagedoorn, 1939). Hardware cloth screen quarter-inch mesh was cut into square tops which were clamped onto the top of the pan. A paper tag bearing the complete number of the pair was fastened on to the wire top

by means of a paper clip. These pans seemed to provide adequate room for one pair of mice plus one litter. Sawdust was placed in the pans and changed once a week. The animals were ensured a constant supply of water by having water bottles which were inserted through the wire top. All the mice received a diet of Friskies.

The mice were examined daily to check for litters born, and to be sure that food and water were present in adequate amounts. Litters were kept with their parents until twenty-one days of age; at times they were not weaned until they were thirty days old. At weaning time, the males were put in one pan and the females in another. At two months of age the desired brother and sister matings were made and any excess mice that were kept were ear-marked and kept together in a pan (sexes separate). Unmated animals were kept in the same manner as the pairs.

Weighing. Many of the animals were weighed daily in order to observe more closely any day-to-day changes that might occur. Irregular fluctuations were found to occur, the slightest environmental change being apt to cause a marked fluctuation in weight. After the strains had been inbred for some time and the variability of the environment had been reduced to a minimum, weights were taken at ten-day intervals. Litters were weighed at birth (i.e. at least within the first six hours after birth), the entire litter being weighed as a whole and the average taken of each litter. All weighings were made on a spring-type balance and were made to the nearest half gram.

Litter Size and Sex Ratio at Weaning. Litter size was determined as carefully as possible although it may not always have been accurate if still births occurred or if the mothers exhibited cannibalistic tendencies. Normally, the females devour the placentae and fetal membranes

and often the still-born young. Some female mice eat some or all of their living young, but it is not a frequent occurrence. However, if a female does exhibit cannibalism she generally does so with all her litters.

The sex ratio was determined at weaning. This gave more of a survival rate of males compared with females than an ordinary sex ratio. It was assumed that the primary sex ratio was 1:1. MacDowell and Lord (1925, 1926) found that in litters wherein there was no prenatal mortality, the sex ratio was equality (416 males, 415 females).

Determination of Oxygen Consumption. General metabolic rate, expressed in terms of oxygen consumption, was determined on males of the black and tan and extreme dilute strains. The method used for the measurement of oxygen consumption was a modification of the one used by Williams, Phelps, and Burch (1941). It is a very simple method, requiring a minimum of time and equipment. The apparatus was constructed with two units, so that two animals could be run at the same time. Figure 1 shows a diagram of the apparatus. Instead of measuring the oxygen directly, a definite volume of water is measured which is used to displace the same volume of oxygen. The time required for the mouse to consume the displaced oxygen is measured. Essentially, the apparatus consists of two metering jars, two animal chambers, a frame to hold them, and necessary connecting glass and rubber tubing. The equipment is placed in an aquarium which serves as a water bath. Oxygen is supplied from a cylinder. In this apparatus the usual U- tube manometer was dispensed with, the inlet tube of the metering jar serving the double function of a passage for the metered water and of manometer.

The animal chambers were fitted with pieces of copper screening to provide a platform for the animals. Soda lime was placed under the

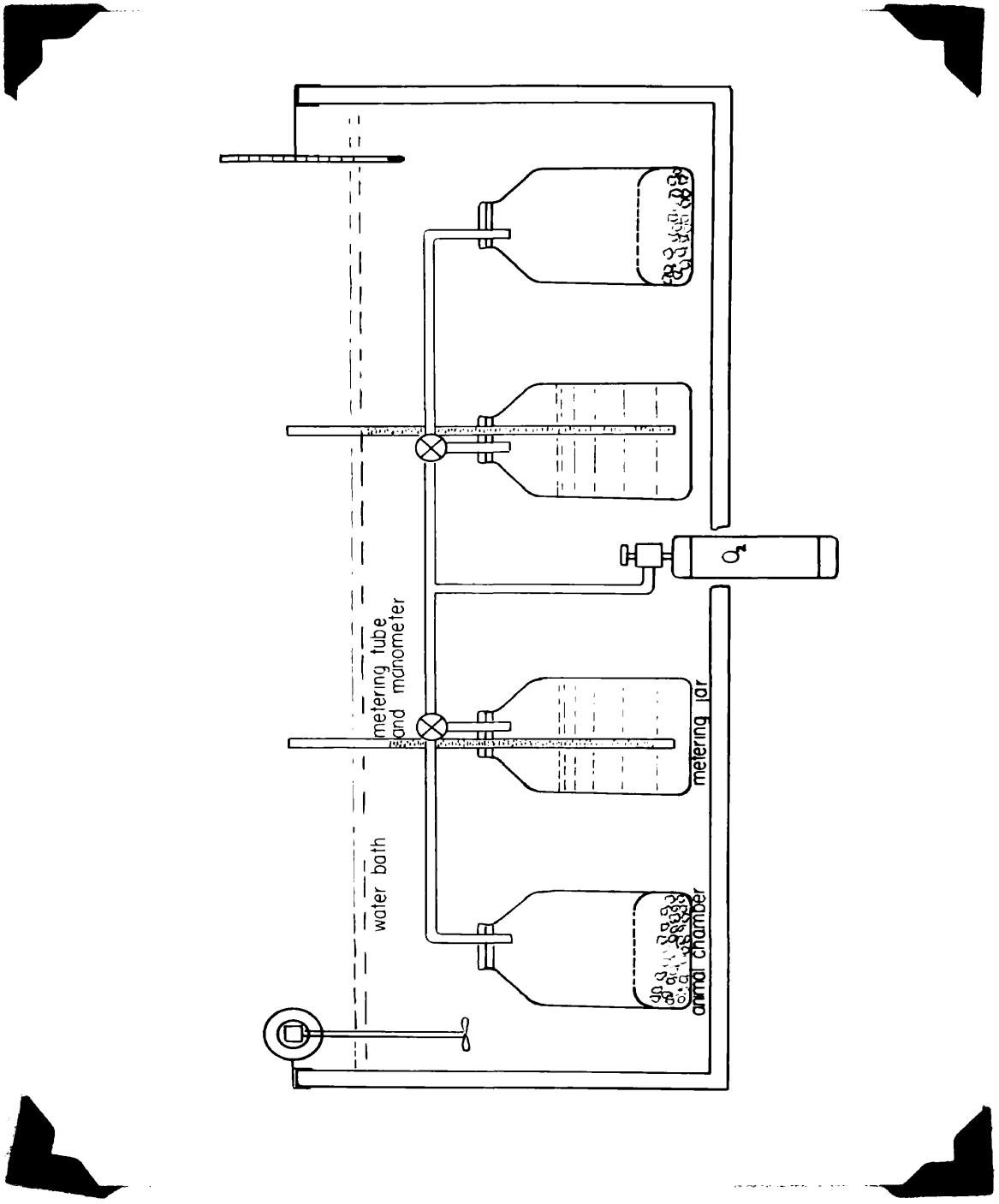

Figure 1. Diagram of apparatus for oxygen consumption determination.

screening for the absorption of carbon dioxide. The soda lime was changed each day and agitated vigorously after every run. The entire apparatus was tested for leaks overnight, after each day's runs.

At the beginning of a day's experiments, the metering jars were filled with water. Oxygen was then admitted into the jars and part of the water was thus displaced. After weighing, the animals were placed in the animal chambers. The experiment proper was commenced after half an hour's time had elapsed, the mice receiving their oxygen from the cylinder during this time. Starting time coincided with the time when the water level in the glass tube was even with the water level in the jar. Fifty cubic centimeters of water were then let into the tube with a burette. The two levels again coincided when the 50 cc. of oxygen displaced by the water had been consumed by the animal. The barometric pressure and the temperature of the water bath were recorded for each run.

Reduction of data to standard conditions of pressure and temperature of 760 mm. of Hg and degrees C + 273, and to 100 grams of body weight was accomplished through application of the gas laws in the following relation:

$$M = 50 \times \frac{60 \times 100 \times P \times 273}{t \times W \times 760 \times T} = \frac{107{,}763 \times P}{t \times W \times T}$$

where

M = metabolic rate (cc./hr./100 gm.)
t = time for consumption of 50 cc. oxygen (minutes)
P = barometric pressure (mm. of Hg)
W = weight of mouse (grams)
T = temperature of water bath (degrees C. + 273)

By way of explanation for the use of the above method, it should be noted that the investigation was not concerned with a determination of basal metabolism, but rather merely as an index of the comparative

differences between the two strains. Males alone were used because of the complications arising from the recurring oestrus cycle of the female.

The experimental animals were divided into the following age groups: one month, three months, six months, nine months, and twelve months. The mice were starved before determinations were made. The digestive processes of the mouse cease entirely in a few hours (Chevillard, 1935); the mouse thus has perhaps the shortest period of digestive activity of the warm-blooded animals. As a consequence, the animals used in these metabolism tests were most probably in the post-absorptive state, having been starved for a seven-hour period prior to the experiment.

Due to the fact that mouse metabolism varies with changes in environmental temperature (Herrington, 1940), the tests were all conducted at a constant temperature. Twenty degrees Centigrade (20° C.) was chosen because it was close to the average room temperature and thus most easily maintained in the research laboratory in which the work was done. A simple water bath with an electric stirrer was used for the temperature maintenance.

The animals were placed in the apparatus and were allowed a half-hour period of orientation before the experiment was commenced. This reduced movement down to normal. Several runs were made consecutively on each animal. If the first two runs varied greatly, a third determination was made.

RESULTS

Rate of Growth. Mean weights of male mice of the two strains studied are shown in Table 1. The figures represent the results of 3,272 weight determinations. The average standard deviation for both strains is 2.6. It may be seen from the table that the weight ranges

TABLE 1. Mean weights of black and tan and extreme dilute male mice.

Age (days)	Black and tan N	Black and tan Mean (grams)	Extreme dilute N	Extreme dilute Mean (grams)	Difference
Birth	67	1.2 ± .013	73	1.2 ± .009	
21	32	7.3 ± .330	26	7.7 ± .292	.4
30	83	13.0 ± .346	39	13.0 ± .353	
40	106	17.7 ± .267	41	16.9 ± .407	.8 ± .48
50	146	20.6 ± .200	80	20.3 ± .231	.3
60	154	23.1 ± .224	90	22.1 ± .218	1.0 ± .31
70	136	24.6 ± .176	103	23.9 ± .252	.7 ± .30
80	125	26.1 ± .216	88	24.8 ± .262	1.3 ± .33
90	100	27.2 ± .268	86	25.9 ± .270	1.3 ± .37
100	90	28.4 ± .308	87	26.5 ± .272	1.9 ± .40
110	70	28.8 ± .348	89	27.5 ± .285	1.3 ± .44
120	29	28.7 ± .464	91	28.1 ± .238	.6
130	30	29.3 ± .503	89	28.7 ± .224	.6
140	31	29.4 ± .467	73	29.4 ± .305	
150	28	29.1 ± .379	75	29.9 ± .327	.8 ± .49
160	27	30.0 ± .412	73	30.3 ± .318	.3
170	34	30.4 ± .377	66	30.6 ± .325	.2
180	41	30.9 ± .375	59	31.0 ± .403	.5
190	37	30.6 ± .412	55	31.3 ± .442	.7
200	36	31.2 ± .398	57	31.6 ± .391	.4
210	34	31.9 ± .377	56	32.1 ± .419	.6
220	31	32.1 ± .509	17	34.7 ± .788	2.6 ± .93
230	31	32.2 ± .526	16	34.6 ± .745	2.4 ± .91
240	30	32.5 ± .575	16	35.3 ± .820	2.8 ± 1.00
250	31	32.7 ± .634	16	35.8 ± .935	3.1 ± 1.12
260	33	33.5 ± .628	10	36.4 ± .847	2.9 ± 1.00
270	30	33.6 ± .727	12	30.6 ± 1.21	3.0 ± 1.41
280	24	34.4 ± .775	11	30.2 ± 1.09	1.8
290	19	34.4 ± 1.01	11	30.4 ± .906	2.0

from 1.2 grams at birth to 34.4 to 36.4 grams at 290 days. At 140 days the mean weight of both strains is the same (29.4 grams). The two strains similarly show almost identical weights from birth to forty days. From forty days to 130 days the black and tan mice are heavier than the other strain although the difference is not statistically significant in all cases. It is from sixty days to 110 days that the black and tan animals are significantly heavier than the extreme dilute ones, the difference being 1.9 grams in favor of the black and tans at 100 days. From 150 days to 290 days (160-day weight is an exception) it is the extreme dilute strain which is the heavier. From 220 days to 270 days the extreme dilute males significantly outweigh the black and tan animals. The difference in weight is as great as 3.1 grams at 250 days. The growth rates of both inbred lines are presented graphically in figure 2. The curves are similar in shape, each one having one rather sharp break. The black and tan male mice have a break in their curve at 150 days, whereas the one in the extreme dilute curve occurs at 210 days (two months later). In each instance there is an increase in weight, the increase being sustained and augmented as the animals grow older.

Rate of Oxygen Consumption. One hundred ninety-six oxygen consumption determinations were made on male black and tan and extreme dilute mice. Tables 2 and 3 show the results in some detail, whereas table 4 contains the reduced data. Figure 3 represents the results graphically. The metabolic rate, expressed as number of cubic centimeters of oxygen consumed per hour per hundred-gram weight, varied from 215 to 877. The data were grouped according to age and strain. Referring to table 4, one sees that the black and tan male mice have a greater average

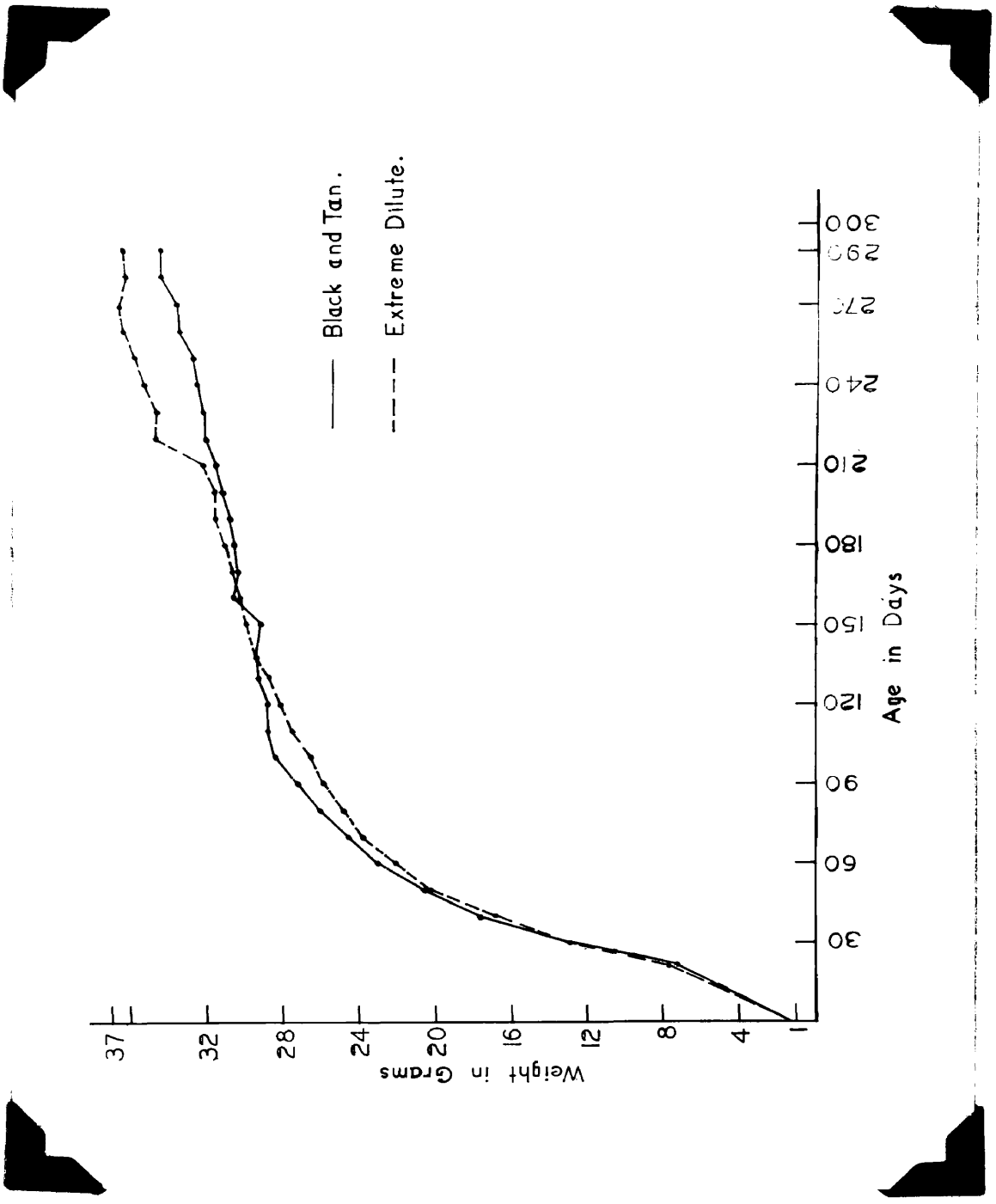

Figure 2. Growth rates of black and tan and extreme dilute male mice.

TABLE 2. 98 oxygen consumption determinations on extreme dilute male mice (after a 7 hour starvation period).

Age (mos.)	Weight (gms.)	cc.O_2/hr./100 gm. 1st run	cc.O_2/hr./100 gm. 2nd run	Age (mos.)	Weight (gms.)	cc.O_2/hr./100 gm. 1st run	cc.O_2/hr./100 gm. 2nd run
1	18	519	539	6	34	359	315
1	16	537	467	6	30	358	388
1	18	527	517	6	31	388	421
1	18	482	497	6	28	401	383
1	16.5	518	506	6	33	392	365
1	11	825	793	9	30	429	310
1	12	828	774	9	33	370	329
1	16	669	801	9	33.5	302	358
1	14	574	607	9	31.5	384	421
3	27	386	397	9	33	326	304
3	29	329	445	9	30.5	366	316
3	30	345	376	9	29	352	352
3	30	495	402	9	38.5	315	315
3	25	393	334	9	35	325	293
3	25	391	402	9	42	330	300
3	33	377	375	12	30	330	295
3	29	382	342	12	35	327	299
3	28	384	417	12	34.5	324	298
3	25	320	340	12	39	310	370
6	26	427	371	12	36	350	318
6	36	365	314	12	30	499	492
6	36	373	357	12	34	335	283
6	34	379	302	12	31	334	301
6	31	374	337	12	33	255	357
				12	31	371	350

TABLE 3. 98 oxygen consumption determinations on black and tan male mice (after a 7 hour starvation period).

Age (mos.)	Weight (gms.)	cc.O_2/hr./100 gm. 1st run	cc.O_2/hr./100 gm. 2nd run	Age (mos.)	Weight (gms.)	cc./O_2/hr./100 gm. 1st run	cc./O_2/hr./100 gm. 2nd run
1	11	610	603	6	30	356	382
1	12	670	675	6	28.5	428	384
1	8	744	417	6	28.5	382	437
1	9	805	804	6	28	408	323
1	9	780	765	6	27	336	375
1	9	820	851	6	29	429	317
1	11	755	755	9	25	330	444
1	11	702	739	9	28	430	446
1	9.5	855	875	9	31	310	425
1	10	877	821	9	32	377	390
3	27	422	465	9	29	380	404
3	26.5	444	550	9	32	248	212
3	26.5	396	427	9	27	380	377
3	27	542	441	9	31	373	407
3	27	598	614	9	28	413	413
3	28	536	512	12	34	343	403
3	31	340	384	12	32	384	310
3	24	342	417	12	34	434	309
3	29	344	352	12	29	308	330
3	24	385	321	12	29	412	406
6	26	341	412	12	26	335	315
6	27	307	356	12	30	204	215
6	30	339	419	12	34	326	326
6	28	345	306	12	29	341	281
				12	30	386	384

Figure 3. Oxygen consumption rates of male black and tan and extreme dilute mice.

TABLE 4. Oxygen consumption rate of male mice.

Age (months)	Black and tan N	Black and tan Average O_2 consumption cc./hr./100 gm.	Extreme dilute N	Extreme dilute Average O_2 consumption cc./hr./100 gm.	Difference
1	20	750 - 24	18	610 - 30	140 - 38
3	20	442 - 19	20	385 - 10	57 - 21
6	20	372 - 9	20	372 - 6	-
9	18	379 - 15	20	345 - 6	36 - 16.1
12	20	347 - 12	20	343 - 14	4

metabolic rate than the extreme dilute animals at the ages of one month, three months, nine months, and twelve months, although the difference at twelve months is not great enough to be of significance. At six months of age the two strains have the same metabolic rate. No lowering of the rate was found to occur in the extreme dilute males between nine and twelve months. No appreciable variation occurs in the black and tan mice between six and nine months of age. During this first year of life, the black and tan individuals show a greater range of metabolic variation than the extreme dilute. Black and tan mice have 66% greater "range of variation" than the extreme dilute ones from one to twelve months. In general, the black and tan strain is slightly more variable than the extreme dilute strain, according to the average standard deviations. Tables 2 and 3 show extent of individual variation. Some animals were quite variable in successive determinations, while there were five mice wherein two runs gave identical results in each case. Extreme dilute mice showed less individual variation in general than did the black and tans. The average is 40 cc. for the former and 48 cc.

for the latter. The six-month-old extreme dilute mice seem to be the most stable, having a 32 cc. average fluctuation for individuals. In the black and tans the twelve-month group had the lowest fluctuation (37cc.), while six-month-old mice showed an average fluctuation of 58 cc. These figures represent amount of individual variation in metabolic rate. The weights of the experimental mice are given in tables 2 and 3. No appreciable effect on the metabolic rate seems to be exerted by the weight of the animals. Table 5 shows the differences in oxygen consumption rates at various age intervals.

TABLE 5. Differences in oxygen consumption rates at various intervals.

Strain	From				
	1-3 mos.	3-6 mos.	6-9 mos.	9-12 mos.	1-12 mos.
Black and tan	-308	-70	+7	-32	-403
Extreme dilute	-225	-13	-29	0	-267

Litter Size. Litter size according to parity is shown in figure 4. The second litter is the largest one for the black and tan females. Extreme dilute females produce their largest litter with the third pregnancy. Average number of young in each litter are presented in table 6. Regardless of parity, black and tan mice have an average litter size of 6.8 animals, and extreme dilute mice have a 6.6 litter size on the average.

Sex Ratio at Weaning. Table 7 shows the sex ratio at weaning of the strains of mice studied. The males exceed in the black and tan, whereas in the extreme dilute line an excess of females survived to weaning age.

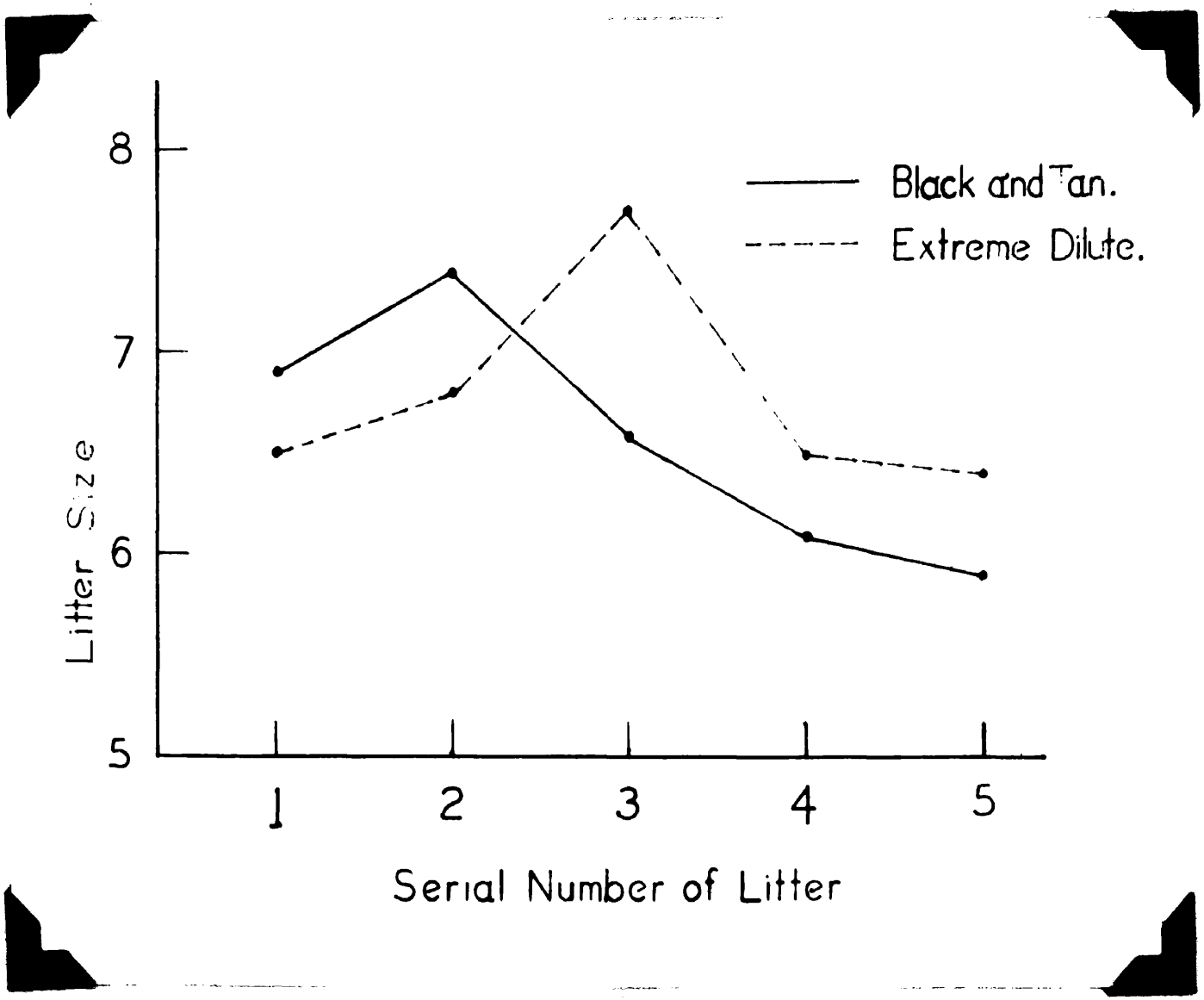

Figure 4. Average litter size according to parity.

TABLE 6. Litter size. (Data from 269 litters.)

Serial number of litter	Average number of young	
	Black and tan	Extreme dilute
1	6.9	6.5
2	7.4	6.8
3	6.6	7.7
4	6.1	6.5
5	5.9	6.4

TABLE 7. Sex ratio at weaning.

Strain	Males	Females	Total	% Males	% Females
Black and tan	326	250	576	56.6	43.4
Extreme dilute	113	167	280	40.4	59.6

Longevity. Longevity records of 348 male mice have been obtained. The percentage still living at various age levels is presented in figure 5. Table 8 shows the percentages and the actual numbers. The largest drop in percentage living occurs between eight and twelve months in the black and tan individuals. At sixteen months only 27.4% of the original 203 males is present, while 67.14% of the 140 extreme dilute males are still living. It is from twenty to twenty-four months that the sharpest percentage drop occurs in the extreme dilute individuals.

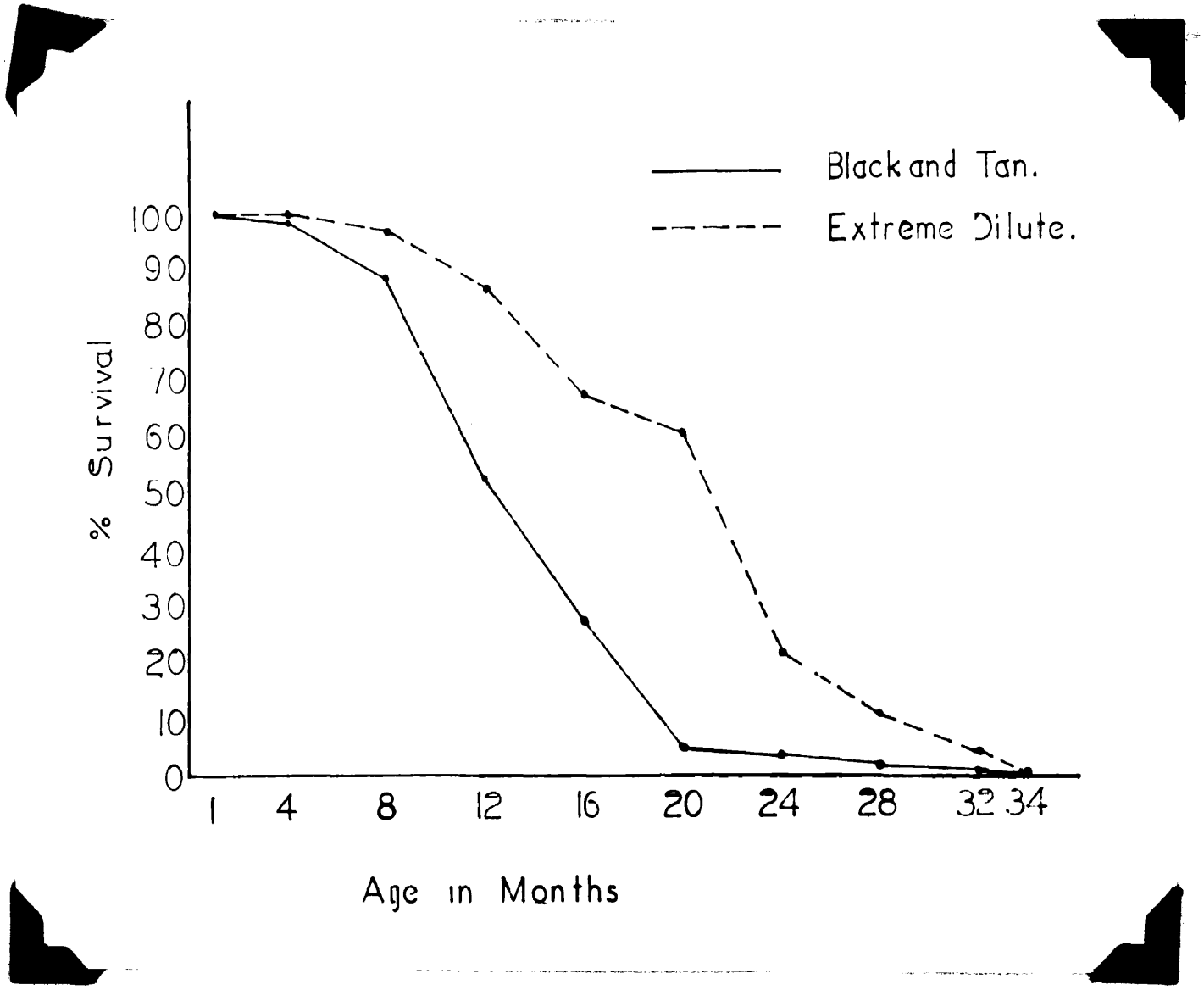

Figure 5. Survival rates of male black and tan and extreme dilute mice.

TABLE 8. Survival rate of male mice.

Age (months)	Black and tan		Extreme dilute	
	Number living	%	Number living	%
1	208	100	140	100
4	205	98.5	139	99.2
8	184	88.4	135	96.4
12	109	52.4	121	86.4
16	57	27.4	94	67.1
20	10	4.81	85	60.71
24	8	3.85	30	21.42
28	3	1.44	15	10.71
32	1	0.48	6	4.28
34	0	0.00	1	0.71

DISCUSSION

General Observations. Of the three strains initiated at Marquette University, only two have been maintained up to the present time. It was in the brown and tan line that the unfortuitous combination of genes probably occurred that is so often associated with inbreeding. The resulting loss of vigor and fertility necessitated the giving up of this strain and continuing on with the black and tan line and the extreme dilute line. These two strains have shown no loss of vigor as a result of close inbreeding, but appear as healthy in the present twenty-fifth generation as they did in the earlier generations. The environmental conditions under which they have been raised have remained as nearly constant as possible. Both at Marquette University and at the University of Maryland, the same type of room was provided for them. As a matter of fact, in each case, it was a basement room with a window and a ventilator. The same food (Friskies) has been used without any supplementary feeding. Thus it can be assumed that the change in geographical location did not actually change the immediate environment of the mice. Slonaker (1912) found that rats reared in very different geographical areas have essentially the same span of life.

The black and tan strain seemed to be more active in general than the extreme dilute strain. This was noted during the course of the many daily observations but was particularly brought to light when the series of orientation experiments to the oxygen-consumption determination apparatus was run. The extreme dilute mice were more quickly resigned to the confinement in the small glass chamber, whereas the black and tan mice were extremely active for at least fifteen minutes. They did not calm down to "normal" activity rate until approximately half an

hour had elapsed. Possibly as a consequence of the greater activity of the black and tan animals they likewise consumed more food than the extreme dilute mice. This was noted in a general way, since actual amount of food consumption was not measured. However, it became quite evident that while the extreme dilute mice would have some food in their pans two and three days after a supply had been placed there, the black and tan mice exhausted an equivalent amount in a shorter time (one or two days).

Some laboratories make a practice of isolating pregnant females. It was found more advisable to maintain the mouse pairs together throughout pregnancy and the subsequent birth and rearing of the litter to weaning age. From the observations, it appeared that the male was not a detriment in the rearing of the litter.

Rate of Growth and Body Size. The average body size of the black and tan and the extreme dilute mice has been found to be 30.5 and 31.5 grams respectively, at 180 days of age. In both strains, however, the weight curve moves upward well after the 180th day, so that at 210 days of age the average weight of the black and tan is 31.5 grams and for the extreme dilute, 32.1 grams. On the basis of these results, considering the 180-day weight as the adult body weight as has been done by all previous workers, one would conclude that the gene for extreme dilution does not have any effect upon average body size.

In regard to the use of an arbitrary 180-day weight as the adult weight of a mouse it might be well to point out that according to Asdell's interpretation of chronologic aging, the actual adult weight would vary with each strain of animals used. This would mean that the adult body weight of any strain would vary with the final weight obtained.

However, as there is no general agreement as to how much of the total weight should be considered adult weight it has been impossible to present any better interpretation of adult weight.

As the animals grow older, it has been noted that there is a definite increase in the weight of the extreme dilute mice with age. Unfortunately, the data are too scanty to make any definite generalizations except to point out that the animals become appreciably heavier with age. In the numerous dissections of older mice it was observed that this increase in weight, as might be expected, was accompanied by an increase in the amount of fatty tissue present.

The rate of growth varies between the two strains studied. The extreme dilute animals have a slower growth rate but a final higher weight than the black and tan individuals. At the age of 140 days, the extreme dilutes overtake the black and tans and proceed to grow larger than these. A rather abrupt upward surge in the growth rate occurs in each strain. In keeping with the idea that the extreme dilute mice are "slower", their break occurs at a later age (210 - 220 days) than does the one in the black and tan line (150 - 160 days). The increase in size occasioned by this spurt of growth is a real one since it is maintained and gradually augmented. Thus it is not a case of irregular fluctuation or a temporary or abnormal increase. No variation has been noted in rate of growth as a result of inbreeding.

Since the only difference between the two strains of animals is the presence or absence of the "c^e" gene it seems likely that slower growth rate may be associated with this gene. No doubt there are other genes affecting growth rate and body size also, although in this case the "c^e" gene seems to have the major influence and to be felt in the genic balance. The results indicate that the multiple factor hypothesis

as originally formulated by Nilsson-Ehle (1909) and Emerson and East (1913) is perhaps not quite correct. They assumed that in cases of blending inheritance of quantitative characteristics, the difference between the parents was the result of action by different genes located in different chromosomes; that these were devoid of dominance and had a cumulative action. Recombination among such genes was assumed to account for the increased variability of succeeding generations. If the above were essentially true, one would get more variation in each succeeding generation of the mouse strains. This was not found to be so. Rather, it was found that the results agree more nearly with Castle's newer interpretation of the multiple factor hypothesis (1941). He states that instead of all genes affecting a quantitative character being devoid of dominance, dominance might be lacking in some cases, partial in others, and complete in still others. Instead of their joint action being cumulative, it might be sometimes cumulative, sometimes conflicting. Location of several size genes in the same chromosome would result in linkage and so give greater apparent influence to constituents of the group. Thus the "c^{e}" gene might quite conceivably play a major role in determining the slower growth rate and subsequent larger size of the extreme dilute mice. Other probable related effects of this gene will be discussed subsequently.

Litter Size. The females of the two strains of mice under consideration produce about the same size of litter. Litter size depends on number of eggs liberated at ovulation and the rate of prenatal mortality. (Gruneberg, 1943) The differences in litter sizes that do occur in these two strains are probably due to differential pre-natal mortality rather than to variations in fecundity, since pre-natal

mortality is probably associated with body size. MacArthur (1942) found that within a species, litter sizes are largest in the larger-bodied races of laboratory animals. It is felt that this may be a direct consequence of the larger body size - there is less prenatal mortality, every fetus having a better chance for survival in the larger uterus of the larger mouse. Thus a gene affecting larger general body size would most probably cause the larger litter size indirectly. It will be noted that the first two litters of the extreme dilute mice are smaller than the first two of the black and tan. However, these litters were born to females in the age where the black and tan group surpassed the extreme dilute group in body size. In the third, fourth, and fifth litters the reverse condition occurs, larger litters being born to the now larger extreme dilute females.

Both in rats (Asdell, 1941) and mice (Gruneberg, 1943) the first litter or so is usually smaller than subsequent ones. Asdell states that this is an indication that reproduction is a stimulus to the ability of the female to reproduce and lactate. He found that virgin female rats showed irregularities and cessation of oestrus cycles earlier than did the breeding groups. This was further evidence for a stimulating effect due to reproduction. In the black and tan line, the maximum litter size was attained at the second litter, while in the extreme dilute line it was the third litter which exhibited the maximum size. Again, this is another example of the black and tan strain being faster in reaction and the extreme dilute strain being slower but with ultimately larger results - having a larger average litter when they have their maximum litter size. It may be concluded that in general, litter size does not differ appreciably in the two strains. However, the important

point to remember is that it takes the extreme dilute females longer to reach their best productivity, and having reached it they subsequently tend to surpass the black and tan females in later litters. Gates (1925) determined that litter size influences weight of the individual mice both on the first day and on the twenty-first day after birth, through the available food supply, but that there was no differential mortality favoring either small or large litters. Since the average litter size of the two strains studied was similar, the above facts do not introduce an error into the weight or the longevity data.

Sex Ratio at Weaning. Most sex ratio determinations have been made at birth and there is general agreement that under normal conditions the primary ratio is 1:1 as would be expected. Prenatal mortality could possibly alter this ratio. However, prenatal mortality appears to affect both sexes equally (Gruneberg, 1943), being non-selective. Significant deviations have been found to occur from the 1:1 ratio, however. Bittner (1936) found that diet affected it and Asdell (1941) found that in rats the late-bred females produce fewer males than do the females bred as young animals. Since all the mice in this investigation received the same diet and were bred at the same age (two months), a 1:1 primary sex ratio has been assumed, and the sex ratio determined at twenty-one days. In the extreme dilute strain, the females appeared to have a better chance of survival, comprising 59.6% of the total number of mice at twenty-one days of age. In the black and tan strain the reverse condition was found to be true although the male percentage (56.6) did not exceed the female percentage as much as the female exceeded the male in the first mentioned strain. The reason for this differential mortality appears obscure, the point to emphasize being the

fact that this is another factor wherein the two strains exhibit a difference.

Rate of Oxygen Consumption. Williams, Phelps, and Burch (1941) determined oxygen consumption of guinea pigs. The apparatus used for the determinations under discussion here, was a modification of the one they described, the method used being essentially the same as theirs. Their guinea pigs were given a five to ten-minute rest period in the chamber before the run was started. However, this was found to be too short a period of orientation for the mice. Consequently, a series of tests was run in which oxygen consumption for consecutive intervals was determined, noting the time it took for the animal to consume each ten cc. of oxygen until 110 cc. had been displaced. Thus there were eleven readings for each animal. Several mice of each strain were used. The figures were converted to cc. oxygen/hr./100 gms. (formula used by Williams, et al, 1941) and curves were plotted. It was noted that in each instance the curves leveled off after approximately thirty minutes had elapsed. This denoted the cessation of the abnormally vigorous activity induced by being placed in the changed environment of the animal chamber. Thereafter, there was some activity, but there was no way of controlling this. As Herrington has said (1940), "Since no measurements under any conditions are ever strictly basal, there appear to be no serious objections and many advantages (particularly with small animals) in accepting activity as normalized by populations and long periods as a condition of the standard metabolism, both within and without the zone of thermal neutrality." Considering the results obtained above, it was decided to give the mice a thirty-minute rest period in the animal chambers before beginning the run, and to accept any activity occurring thereafter as normal.

Reduction of data to cc. oxygen/hr./100 gm. weight was followed by Williams for guinea pigs and this form of expression was adopted for the mouse data. Various other investigators have favored other modes of expression. Riddle (Benedict, 1938) referred heat production to the average adult weight characteristic of the species, in his work with doves. Others have used surface area as an expression of size. However, in most instances surface area has in turn been derived from weight and not been actually measured. Benedict (1938) has reviewed the different ways of expressing metabolic rate and he concluded that it can be maintained that the general body composition of adult animals of any one species is much the same irrespective of size; hence, one may use the body weights as measured in comparing adult animals of the same species without particular reference to the actual composition of the body.

Fuhrman, McLin, and Turner (1946) obtained metabolic rates for mature albino mice. They found a significant difference between morning and afternoon values. However, they subsequently determined that it was not the time of day that induced the difference but rather the degree of activity. Activity was correlated with time of day and thus gave the illusion of a variation in metabolic rate between the morning and the afternoon. Since all of the determinations on the black and tan and extreme dilute males were made at the same time of day (early afternoon) the degree of activity associated with differences in time did not influence the obtaining of comparative values for the two strains of animals.

Many interesting observations were made on the basis of the 196 oxygen consumption rate experiments that were conducted. One might

assume that the first determination for each animal would be higher than the second if it differed at all. However, this was not found to be true in all instances. Therefore, it is best to take an average value rather than the lowest value to be the most accurate, because there are nearly always variations between consecutive runs, and these are unpredictable and variable.

The extreme dilute male mice were found to have a lower metabolic rate than the black and tan males. The difference was especially great in the one-month-old group. It was less in the three-month-old group, but still significantly lower. At six months of age the two strains were strikingly similar, the values for average oxygen consumption (cc./hr./100 gm.) being identical for the two groups. However, at nine months the black and tans again exceed the extreme dilutes in metabolic rate. At twelve months the difference is not great but still the extreme dilutes have the lower value. The much lower metabolic rate of the extreme dilute mice at one month and three months possibly signifies an association with their slower growth rate, the faster-growing black and tan males having a higher metabolic rate accordingly. Further there is a rather long time interval wherein there is no fluctuation in metabolic rate, which occurs in both strains. Again, the extreme dilute mice are "slower", reaching this period later than the black and tan mice do. It is between nine and twelve months of age that the extreme dilute animals remain more or less constant in their rate of oxygen consumption, the corresponding period for the black and tan animals occurring from six to nine months. Another analogy may be drawn between growth rate and metabolic rate when one considers that at six months the two strains do not differ in metabolic

rate and are also most similar in body size. The twelve-month-old black and tan mice have a lower rate than the nine-month-old ones, suggesting that physiologically, a twelve-month-old black and tan mouse is older than a twelve-month-old extreme dilute mouse - since approaching senility may be determined by the point at which metabolic rate drops. As McCay (1942) has said, " . . . . senescence is much more than a matter of time." Since the two strains vary primarily in regard to presence or absence of the "c^e" gene, the variations encountered in metabolic rate might well be ascribed in part to the influence of the "c^e" gene. Schopbach, Keeler, and Greenberg (1943) have associated metabolism and heart rate with variations in coat color in rats. They found that the wild gray Norway rats operate at lower basal metabolic rates than the Wistar albinos. They concluded that the coat color genes were the most important factor conditioning the inheritance of these traits, although they added that other genes were most likely involved also. Boettiger (1941) has determined experimentally that thyroxin raises the basal metabolic rate. Thus it is possible that the "c^e" gene affects the thyroid gland and indirectly the metabolic rate, growth rate, and associated factors. Further experimentation is needed to determine the mode of action.

Longevity. Available data point to the fact that the extreme dilute males are longer lived than the black and tan males. Of 140 extreme dilute males, 60.7% were still living at twenty months, whereas at the same age only 4.8% of 208 black and tan males were still alive. It can readily be seen that the difference between the two strains is striking. The possible inheritance of aging has been investigated in rats. Sperling, Loosli, Barnes, and McCay (1946) used white rats in an

effort to determine the effect of diet and coffee upon the life span. They found that neither diet nor coffee had much effect on the life span, but that the heredity of the rats had a definite effect. In McCay's numerous experiments (1942), he found that the life span in rats was flexible and that retarded animals tended to outlive those that matured normally. The results obtained on the mice are similar to McCay's although arrived at in a different manner, since McCay produced the condition of slow growth rate artificially. From the set of results obtained, it would seem that the life span of male mice is in some manner associated with rate of growth and metabolic rate. Since the environment of both strains of mice was the same, the variations were most probably of an hereditary nature. Since the main known genetic difference between the strains was the presence or absence of the "c^{e}" gene, it might well have played a major part in determining longevity either directly or indirectly.

SUMMARY AND CONCLUSIONS

Data are presented on observations of two inbred strains of mice which have been investigated for a period of seven years to determine variations in length of life and any association that might exist between life span and metabolic rate, rate of growth, body size, and litter size. These two strains differed only (as far as could be determined) in the presence or absence of the "c^{e}" gene. Otherwise, both strains were specially developed black and tan mice produced by brother and sister matings. The mice were reared under identical conditions and received the same diet throughout the course of the investigations. Twenty-five generations of each strain have been studied. The observations reported are based on male mice from the fifteenth to the

twenty-fifth generation. From these studies the following conclusions have been drawn:

1. Evidence has been presented which indicates a genetic basis for length of life in mice.

2. Associated with longer life span there is a lower metabolic rate, a greater body size, and a slower growth rate.

3. There is an indication that the slower growing strain produces largest litters later in life than the more rapidly growing strain. The third litter is the largest in the extreme dilute females; the second one is the largest in the black and tan females.

4. The sex ratio at weaning has a greater percentage of females than males in the slower growing strain.

5. The gene for extreme dilution is associated with longer life span, lower metabolic rate, greater ultimate body size, and slower growth rate.

6. Extreme dilution does not have any effect upon body size at 180 days.

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