

## THE PRENATAL GROWTH OF THE MOUSE.

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The period before birth offers a unique opportunity for the study of mammalian growth. At no other period are the basic external influences, such as temperature, nutrition, and humidity, so accurately regulated; while the changes in speed, in relative magnitude, and in complexity are greater than at any other time. Yet the difficulties in the way of obtaining reliable data for this period are considerable. The rapidity of the changes, combined with the impossibility of determining the exact time of fertilization of a given ovum, necessitate large numbers; the dissection and handling of the smallest embryos is difficult and has seldom been attempted. Indeed the scarcity of data for this whole period explains a dangerous tendency to accept such data on their face value.

The data for man are subject to errors from pathological conditions of mother and embryo, as well as to gross inaccuracies in age. Averages for the rabbit are given by Friedenthal (1914), but these include weights of embryonic membranes and placentaë. The data for the rat are open to criticism which is presented later in detail. The most reliable figures are those for the guinea pig (Draper, 1920, and Ibsen, ms.). In spite of the extensive use of the mouse for studies on postnatal growth, no published data on its prenatal weight have been discovered by the authors. This paper presents such data from 115 litters, including 959 embryos, and also attempts to show a similarity in the progress of prenatal growth in certain vertebrates. Brody and Ragsdale (1922-23) and Brody (1925-27) have presented a method for finding the age equivalence for different animals based on the latter part of postnatal growth and upon conception age. Friedenthal (1914) had previously shown a similarity in the relative growth rates of different animals by plotting the logarithms of the weight against

the logarithms of the age. He emphasized the point that the similarity appeared only when the age was calculated from the time of conception. On the other hand, we are led to the conclusion that the age of embryos should be counted from the beginning of the embryo proper. This method reveals a closer harmony among curves of prenatal growth than has been previously demonstrated, and shows that the major differences in the curves of the mouse, the guinea pig, and the chick lie in the amount of tissue involved in the first organization of the embryo and in the length of the prenatal life.

*Embryo Age.*

In mammals, the separation of the egg from the main food supply is correlated with a precocious development of the trophoblastic elements of the morula. These form the yolk sac and the traeger, which effect the first connection with the maternal food supply. They are well developed before the visible organization of the embryo is started. This preliminary stage occupies an appreciable part of the gestation period. If natal and prenatal growth are to be compared, the embryonic membranes and placenta must be excluded; hence the growth curve of the embryo should start with the beginning of the embryo proper and not include the preliminary stage or the extraembryonic tissues. This proposition is shown to be justified by the data themselves.

Any definition of the beginning of the embryo proper must be largely arbitrary; as a practical criterion we propose the primitive streak stage. This establishes the main axis of the embryo and includes the differentiation of the anlage of the embryo proper. The time of its occurrence can probably be determined with as great accuracy as can the time of the fertilization of the ovum. The time between conception and the primitive streak stage is sufficiently long to make a considerable difference in the mathematical description of the embryo growth curves. And this difference is great enough to afford a statistical method that may make it possible to estimate the time of the primitive streak stage from the growth curve of the embryo. This method is shown to hold good for the mouse, the guinea pig, and the chick.

### *Material and Methods.*

*Ancestry of Embryos Weighed.*—The maternal grandmothers of the embryos came from the Bagg albino strain which has been inbred since 1913, and for the last twelve generations in our colony the inbreeding has been between brother and sister. This strain was chosen because of its good breeding and nursing qualities. It carries the genes for homozygous brown agouti self-color, dark eye, and intense pigment. The females used were over 3 months old and they had not nursed young for at least 3 weeks before this mating. The maternal grandfathers of the embryos came from the Storrs-Little strain which originated from the Little dilute brown strain, inbred since 1909, by one out cross with back crosses to the pure dilute brown strain for five successive generations. This strain has been inbred brother to sister for ten generations. The animals are pink-eyed dilute brown self-colored. The mothers of the embryos,  $F_1$  hybrids between the two lines just described, were nursed in litters cut down to six at birth. They were weaned when 4 weeks old and held in groups of six or less in mating boxes until over 3 months old before being mated. All embryos came from first litters. Their fathers, which were over 3 months old at the time of fertilization, came from a line (No. 89) of intense brown agouti self-colored animals which has been inbred in very large numbers in this colony, brother by sister, for nine generations.

The highly inbred lines were desired to give uniformity; but as the greatest vigor was also desired, hybrid mothers were used (Wright, 1922), with the hope of stabilizing litter size and reducing prenatal mortality. The fathers were taken from a third strain to reduce the segregation as far as possible. First litters were used because in these the prenatal mortality has been found to be the lowest. Breeding was held off until 3 months old to permit full body development, since hybrid embryos are frequently too large for an incompletely grown mother.

*Timing Copulations.*—The males were kept isolated in small boxes in front of the large boxes of females. For copulation the males were put into the boxes with the females for an hour each day, then all the females were examined for vaginal plugs with the aid of a pure silver probe. As soon as a plug was discovered the female was isolated in a small box where she was left without being touched again until the time assigned for taking her embryos.

*Diet and Care.*—Bread, fresh milk, canary seed, hemp, pin-head oatmeal, supplemented with cabbage twice a week, constituted the diet. The boxes were provided with shavings and cotton batting, and with the exception of those containing pregnant females, were cleaned every week. The boxes were kept in an equably heated laboratory.

*Dissection of Embryos.*—The embryos were taken during the months of November to April. The pregnant females were killed mechanically at the end of some multiple of 24 hours from the end of the hour the male was last with the female. This was accurate within a few minutes. The 19 day embryos are not included. Since most litters are born before the end of the 19th day, those not yet born are selected on the basis of prolonged gestation, and it is possible that a correlation

exists between the extended gestation and the condition of the embryos. Until such a correlation is disproved, curves of prenatal growth should exclude data beyond the minimum period of gestation.

Immediately on killing the mother the uterus was exposed and cooled; this suddenly reduced the vital processes of the embryos to a minimum and delayed actual death for a considerable time. Even 3 hours after the death of the mother, the hearts of the early embryos would pulsate if warmed. All degenerate or recently dead embryos were excluded. The heart beat was the best sign of life; in later embryos, the movements of the skeletal muscles. The general body color was markedly different in the rare cases of very recent death.

Embryos 13 to 18 days were removed under a Leitz binocular microscope magnifying 7.5 diameters. When removed from uterus and membranes, the umbilicus was clamped with forceps to reduce bleeding and severed close to the abdomen. A small glass spoon was used for lifting the embryo; after the excess fluid was blotted off, it was placed in a glass ring between cover-glasses, moved to the balance shelf on a convenient carrier, and lifted to the balance pan with forceps.

The membranes of embryos of 10 to 12 days were removed in Locke's solution under the Leitz binocular, lifted out of the solution to the cover-glass with the glass spoon, there freed from excess fluid with pointed rolls of paper toweling, the ring put in place and covered with the second cover-glass.

The whole decidual capsule for 8 to 10 day embryos was opened in Locke's solution by a single equatorial cut with fine scissors. This permitted the embryo to slip out from the decidua capsularis. It was then pipetted to a second container with a black paraffin bottom and fresh Locke's solution. With specially ground, hook-pointed needles and brilliant illumination under a Greenough binocular magnifying 30 diameters, the amnion, allantois, and remainder of the yolk sac were removed from the embryonic area. Cutting against the paraffin held the embryo in place until the dissection was completed. In many 8 day embryos the separation of the membranes had to be entirely by needle cutting, but in the 9 day embryos the membranes could be gathered between the blades of a fine pair of iridectomy scissors and nicely removed with two cuts. This dissection was exceedingly slow, 3 hours usually being required for a litter. When prepared, the embryos were pipetted directly to the cover-glass and the drop of fluid removed with the paper pointers while under observation with the high power binocular; then the ring and the second cover-glass added.

While two of the authors shared in removing the embryos older than 12 days, the younger ones were all dissected by the same person, thus ensuring a high degree of uniformity in the details of technique.

*Glassware.*—The glassware included numerous sets of five different sizes. Each piece of glass was etched with an identifying mark. Two sets of glass were used alternately for successive embryos. After an embryo was weighed, the glass was washed in water, rinsed in alcohol, and dried with a lintless silk cloth. The

weight of each set of glass was determined, after being washed in this manner, every day it was used.

*Balances.*—For 10 to 18 day embryos a Sartorius balance was used, which, in the hands of the observer who made all the readings, gave an accuracy within 1/10th mg. Eimer and Amend analytical weights, with whole gm. gold plated, were used with this balance. Embryos of 8, 9, and a few of 10 days were weighed on a Troemner Assay balance (new No. 30) with Troemner triple checked Precision button weights. The total load prescribed for this balance is 1.0 gm., but this was increased to over 1.5 gm. by substituting cover-glasses for the balance pans. The greatest load used was 0.3 gm. In actual practice this balance proved to be accurate within 1/100th mg. Both balances were provided with hinged side doors which were used exclusively in the process of weighing. Both pointer scales were read with a magnifying glass. The balances were kept permanently in position on a special rigid shelf bolted to a masonry wall that is subject to no sensible vibrations. When not in use each balance was kept inclosed in a case consisting of hinged top and sides fastened to the wall and operating free of the shelf without jarring the balance. All weighing was done within 6 months while the steam heat was on and the windows mostly closed (November 3, 1926, to April 17, 1927).

*Weighing.*—Embryos 9 to 18 days were weighed individually. While one was being weighed the next one was being dissected, and the work so timed that as soon as the second cover-glass was in place the embryo was immediately transferred to the balance pan. Embryos awaiting dissection were left in their intact amnions in the uterus. Embryos from two 8 day litters were weighed individually, but the other 8 day embryos were weighed in groups.

Zero was determined once a day on the Sartorius balance from five sets of five pointer readings. On the Troemner, five pointer readings were recorded before and after each embryo weighing and the average between these two deviations was used to correct the embryo weight for zero. All glassware was weighed by the overload method. For weights of all embryos under 18 days the last place was determined by one set of five pointer readings from which the deviation was calculated, corrected for zero, and divided by the sensibility constant. All pointer readings are on file.

*Histological Method.*—The embryos used for sectioning were fixed in either Bouin's fluid or Allen's  $B_{15}$ , and run carefully up to paraffin. Some were sectioned transversely and others longitudinally.

## RESULTS.

The average weights and the frequencies for each day appear in Table I. The distribution of individual weights for each day is shown in Fig. 1. In order to include all the data in one legible chart, the logarithmic scale is used for the weights. And in order to present graphically the number of individuals of each weight on each day, a

uniform scale is used for the abscissa, which thus gives both age and number of individuals. This chart shows the curve of the calculated means, the observed means, the modes, the ranges, and the classified distributions of individual weights for each day.

The distributions show: (1) that for each day individuals are found equal in weight to the mode of the day before. The regularity of this overlap may be considered evidence of as much as 24 hours variation in the interval between copulation and the fertilization of the ova. (2) Modes and means are generally close together. (3) The highest

TABLE I.  
*Weights of Mouse Embryos (See Text for Ancestry of Material).*

Conception age $t$	Embryo age $t - 7.2$	Frequencies		Average weight	
		Litters	Embryos	Observed $\Sigma W/n$	Calculated $.0000000456(t - 7.2)^{1.448}$
8	.8	11	93	.00008	.00009
9	1.8	8	76	.00147	.00173
10	2.8	10	88	.00860	.00869
11	3.8	10	101	.0329	.0265
12	4.8	11	77	.0762	.0622
13	5.8	11	100	.1298	.1240
14	6.8	11	74	.2288	.2213
15	7.8	10	75	.3651	.3656
16	8.8	12	95	.5926	.5677
17	9.8	11	89	.8467	.8408
18	10.8	10	91	1.190	1.198
Totals.....		115	959		

individual weights on each day form a curve nearly parallel to that of the means. (4) The means do not approximate a straight line.

Brody (1926-27)<sup>1</sup> shows that a straight line may be drawn through Stotsenberg's (1915) averages for the rat when these are plotted on semilogarithmic paper as are the mouse averages in Fig. 1. This means either that the rat and mouse follow essentially different laws of prenatal growth, or that the two sets of data are not equally reliable. Although these rat data have become familiar in the literature, their

<sup>1</sup> Brody (1926-27), Fig. 9, p. 650.

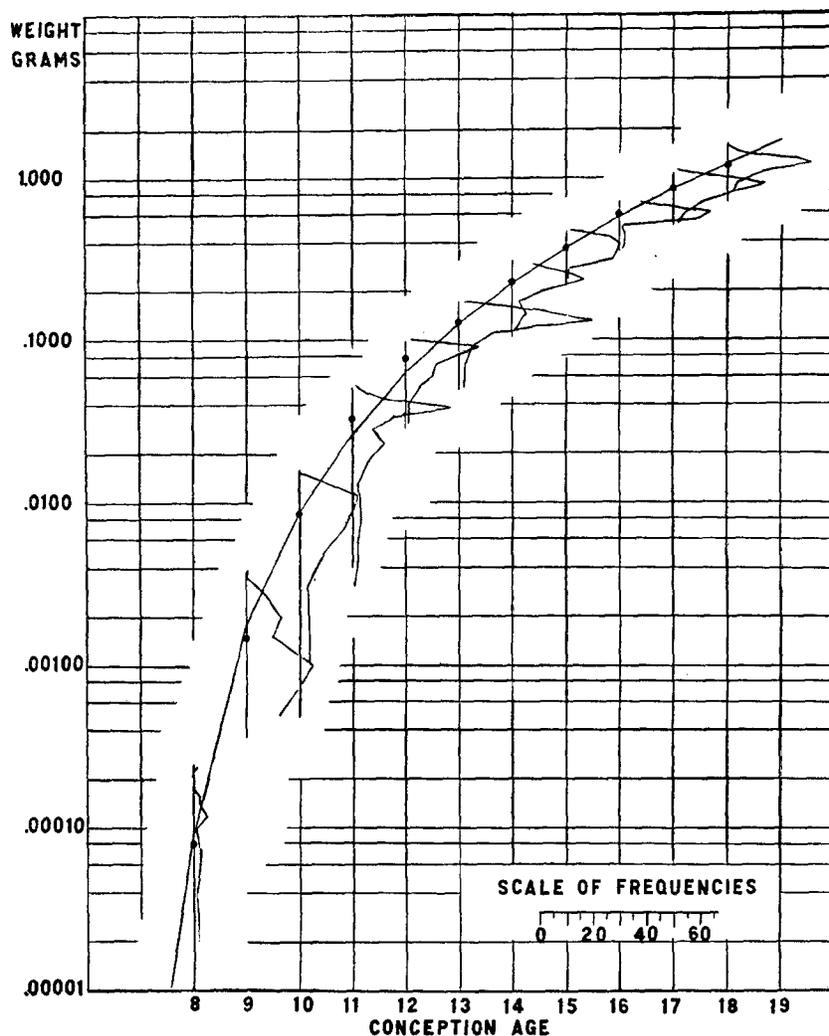


FIG. 1. Logarithmic distributions of classified wet weights of individual mouse embryos 8 to 18 days from conception. Ordinates, in logarithmic ruling, give actual weights in gm.; abscissæ in arithmetic ruling give age and number of individuals. On each day the range of individual unclassified weights is shown by a vertical line which is used as the base line for the frequency distribution of classified individual weights. The number of cases in the distribution is shown by the distance to the right of points connected by light lines; note scale of frequencies at bottom of chart. The means, weighted by the number of individuals in each litter, are shown by dots on the vertical base lines. The theoretical means for each day are calculated from the formula

$$\log W = 3.649 \log [10 (t - 7.2)] + \bar{8}.6587$$

and are connected by a continuous line. The mean for the 8th day includes ten litters, although the frequency distribution includes only two litters, as the others were weighed in groups.

significance has not been discussed. Besides satisfying Brody's exponential function, these data are presented by Robertson (1923)<sup>2</sup> as demonstrating that weight is an autocatalytic function of age. The data are obviously not sufficiently critical to distinguish between these two formulæ. Altogether they include only thirty-eight litters, divided among ten age groups, with ten to forty-four embryos in each group. Some litters were killed as much as 2 hours after the designated 24 hour interval. They were collected over a period of 6 years during which time the diet of the colony was radically changed. With the data being collected at the approximate rate of six litters a year, the question may be raised as to the genetic comparability of the animals used, and also as to the probability of strictly uniform technique in dissection during this long period. These various limitations indicate the desirability of additional data for the rat, and furthermore they provide an explanation of the marked difference between the

FIG. 2. Average weights for embryos of mouse, guinea pig, and chick plotted on logarithmic rulings against embryo age given in units of 1/10th day. Mouse, data of the present authors; sources of other data are indicated on the figure. The straight lines are the graphs of the following equations; velocity constant for the mouse was determined by the method of least squares; for the guinea pig and chick it was obtained graphically.

$$\begin{aligned}\log W \text{ (mouse)} &= 3.649 \log [10 (t - 7.2)] + \bar{8}.6587 \\ \log W \text{ (guinea pig)} &= 3.987 \log (t - 12) + \bar{5}.1839 \\ \log W \text{ (chick)} &= 3.436 \log [10 (t - 0.5)] + 7.626.\end{aligned}$$

Draper data: each point represents the average of one litter as recalculated from the records for individual embryos in Draper (1920, Table I, pp. 385-386). Ibsen data: each point represents several litters; frequencies of embryos, going up from 8 days embryo age as follows: 17, 15, 23, 16, 24, 18, 17, 17, 54. Von Hensen data: each point is an average of individual embryos from two or more litters, in frequencies as follows: starting with 9 days embryo age: 3, 3, 4, 4, 8, 4, 4. Murray data include the following number of embryos, starting from 4.5 days embryo age: 200, 45, 91, 42, 48, 48, 27, 15, 35, 15, 12, 10, 35, 29, 30. Needham data include the following number of embryos, starting with 2.5 days embryo age: 85, 77, 80, 78, 83. Schmalhausen data include the following number of embryos (frequency of first point not stated): 12, 16, 15, 12, 10, 9, 10, 7, 5, 6, 6, 5, 2, 2, 3, 2, 2. Data beyond minimum prenatal period not included in any case.

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<sup>2</sup>Robertson (1923), p. 56.

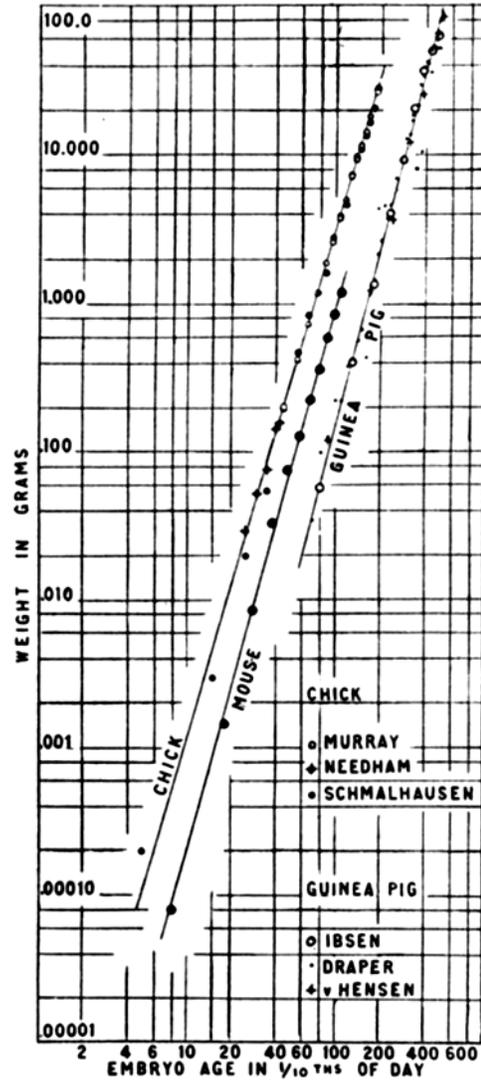


FIG. 2.

semilogarithmic curves for the rat and the mouse that is more reasonable than the supposition of a real difference in the processes of growth.

When the mouse averages were plotted on logarithmic paper the higher averages approached a straight line, but the lower ones bent markedly downward away from this line. By subtracting different amounts from conception age and replotting each time, it was found that all the data were brought most clearly into a linear relation when 7.2 days were subtracted. The averages for the mouse in Fig. 2 are plotted on this basis. The straight line is the graph of the equation

$$\log W = 3.649 \log [10 (t - 7.2)] + 8.6587$$

in which  $W$  is the weight and  $t$  conception age, which is reduced to units of 1/10 day to facilitate the calculations with logarithms.

Our histological evidence agrees with this time relation. Embryos 6 days old show no mesoderm. Of twenty-one 7 day embryos from three litters, sectioned transversely or longitudinally, all but two show mesoderm in varying quantities, sixteen are sufficiently developed for a primitive groove, but none shows a head fold. Of six from a litter of  $7\frac{1}{2}$  days, five have reached the primitive groove stage and one has the beginning of the head fold. While there is great variation in our 8 day embryos, very few do not have the head fold and many show somites (up to six). Further embryological data will be published later, but from the evidence here presented it would seem that the primitive streak first appears on the 7th day, and that usually the anlage of the embryo proper is not actively differentiated until the early part of the 8th day. Sobotta (1911) records mesoderm in an embryo at the end of the 7th or the beginning of the 8th day after conception.<sup>3</sup>

This statistical method of determining embryo age has been applied to the data for the guinea pig and the chick.

*Guinea Pig.*—Probably the most accurate data previously published on prenatal growth of a mammal are those for the guinea pig, Draper (1920), von Hensen (1876), and Ibsen, who has most kindly permitted the use of his averages in advance of their publication. In the guinea pig the extended period of gestation, which is over three times as long

<sup>3</sup> Sobotta (1911), Taf. XIV, Fig. 3.

as that of the mouse and includes the infantile stage that is postpartum in the mouse, gives an excellent test for the applicability of the power function and embryo age as a description of growth *in utero*. Fig. 2 shows that when embryo age is taken as  $t-12$ , the logarithms of the average weights plotted against the logarithms of embryo age fall remarkably close to a straight line. This line is defined by the equation

$$\log W = 3.987 \log (t - 12) + 5.1839.$$

As in the mouse, the age modification required to fit a logarithmic straight line very closely approximates the time that elapses before the first differentiation of the embryo proper. Von Hensen (1876) figures 11 day egg cylinders without primitive streaks and 14 day embryos with as many as six somites. Bischoff (1852) states, "Am 13 Tage aber beginnen nun in dem angeschwollenen freien Ende des Eizapfens die merkwürdigsten Veränderungen, die rasch fortschreitend, binnen 48 Stunden den Embryo in seinen meisten Hauptteilen in Dasein rufen." Lieberkühn (1882)<sup>4</sup> presents a surface view of an embryonic disc aged 13 days, which shows the primitive streak with a well developed area of mesoderm. Another drawing<sup>5</sup> of an embryo 13 days 16 hours old shows a primitive streak nearly twice as long and a much larger area of mesoderm.

Given the uniform conditions of uterine life, the same velocity constant describes the growth of the guinea pig from the first organization of the embryo proper through the long range of developmental stages, even including those which correspond to postnatal infancy in many mammals. This suggests that the variations of the curves after parturition in animals with short gestation are not due to the innate constitution of the embryos, but rather to the changed method of life. It is further suggested that the cycles found by Read (1913) at one age and at another by Brody and Ragsdale (1922-23)<sup>6</sup> in the data of Minot, reveal the inaccuracy of their data (weights of pregnant females in both cases) rather than the autocatalytic nature of growth.

*Chick.*—Murray (1925-26) has shown that the equation  $W = .665t^{3.6}$ ,

<sup>4</sup>Lieberkühn (1882), Fig. 3.

<sup>5</sup>Lieberkühn (1882), Fig. 3a.

<sup>6</sup>Brody and Ragsdale (1922-23), Fig. 6, p. 211.

in which  $t$  is incubation age, gives an accurate description of his own extensive series of embryo chick weights as well as those of Lamson and Edmond, and Hasselbalch.<sup>7</sup> The weights given by Schmalhausen (1926) also agree with this equation as far down as 5 days, but earlier than this his averages and those of Needham (1927) fall distinctly below the line of Murray's equation. While incubation and embryo age are so very nearly the same that they serve equally well for the larger embryos, the averages for the very early embryos reveal the difference by bending away from the line based on incubation age. All these data are brought into fair agreement with the straight line whose equation is

$$\log W = 3.436 \log [10 (t - 0.5)] + 7.626$$

in which incubation age  $t$  is reduced by half a day and multiplied by 10 to reduce the unit of time to 1/10th day. Fig. 3 shows the averages up to 5 days in comparison with the values given by this formula, by Murray's formula, and by an exponential extrapolation of Murray's averages given by Needham.

Our graphic estimate of embryo age is closely borne out by the embryological data. Duval (1889), whose timing of chick embryos is usually taken as a standard, figures the earliest primitive streak with mesoderm in the neighborhood of the 10th hour of incubation.<sup>8</sup> He shows 16 hour blastodiscs with the primitive streak varying from no primitive pit to the development of a chorda dorsalis. Jenkinson (1913)<sup>9</sup> states, "—and soon the first sign of the embryo appears (about the 12th hour of incubation) in the form of the primitive streak, . . . ." This is shown in his Fig. 105*a*.

Other descriptions of the weight-age function for chick embryos have appeared. Schmalhausen shows<sup>10</sup> that the cube roots of weight plotted against age roughly approach a straight line. This is the same power function used by Murray but expressed in a different way with a less accurate velocity constant. In this same figure Schmalhausen demonstrates that the logarithms of weight against age form a

<sup>7</sup> Murray, Fig. 5, p. 45.

<sup>8</sup> Duval (1889), Plate III, Figs. 47 and 62.

<sup>9</sup> Jenkinson (1913), p. 172.

<sup>10</sup> Schmalhausen (1926), Fig. 1, p. 339.

smoothly graded curved line. Brody (1926-27) draws a series of straight lines through corresponding exponential curves for different series of chick data, including those of Murray, and concludes that

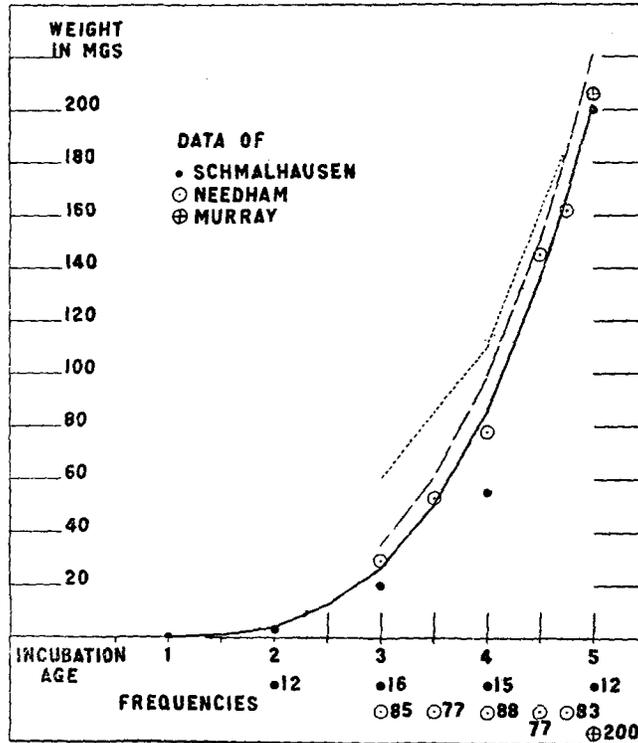


FIG. 3. Average weight for chick embryos, 1 to 5 days incubation age. Solid line is the graph of the equation based on embryo age given, under Fig. 2, for the chick; broken line is the graph of the formula given by Murray. The dotted line is from figures given by Needham (1927, p. 261, Table I, Column 5) as an extrapolation of Murray's averages based on the assumption that embryos grow at the same rate before 5 days as between 5 to 7 days,—that is, an exponential function. (Needham's Fig. 1 gives a curve which does not entirely agree with the numbers given in his table.)

growth rate does not decline continuously, but by abrupt drops between periods of uniform rate. Since any curve can be approximated by a series of straight lines, the critical significance, both of the specific

number of straight lines used and of his general conclusions, seems somewhat questionable.

#### CONCLUSIONS.

1. The general course of prenatal growth in the mouse, the guinea pig, and the chick can be expressed by straight line relations between the logarithms of the weight and age only when age is counted from the beginning of the embryo proper.

2. This is interpreted as showing that the manner of growth before the beginning of the embryo proper is essentially different from that after this time.

3. The velocity constants for the animals mentioned are similar; the major differences in their curves depend on the amount of tissue involved in the first organization of the embryo proper and in the length of prenatal life.

4. Growth of different animals may be compared more accurately if, instead of either birth age or conception age, embryo age is used.

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