

THE BREEDING SEASON OF THE OPOSSUM (*DIDELPHIS VIRGINIANA*) AND THE RATE OF INTRA-UTERINE AND POSTNATAL DEVELOPMENT¹

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NINE CHARTS AND FOUR HELIOTYPE PLATES

AUTHOR'S ABSTRACT

The breeding season of the opossum at Austin, Texas, begins in January, following a three months' anoestrous period. The modal point for ovulation days is reached in the third week. The rate of intra-uterine development was investigated chiefly by surgical removal of one uterus, noting the stage attained by the ova therein and allowing the surviving uterus to incubate its ova a precalculated period of time. Unique charts epitomize the results. The primitive-streak stage is completed, the medullary groove and chorda begin at seven and one-half days post coitum, seven days post ovulationem, leaving only five and one-half days' actual development of the embryo to birth. The rate of development is compared with Eutherian mammals.

The curve of postnatal growth has the shape of embryonic growth curve of higher mammals. The eyes and lips open, at about fifty days (young the size of mice). At this time the young leave the teat for the first time, but are not weaned for about thirty days more. Soon after weaning, the mother may become pregnant again. At ninety to one hundred days (young size of large rats), the young may begin to shift for themselves.

Since the study of the early development of the opossum was begun about 1913, protocols of over 800 females of *D. virginiana* have accumulated. This number includes, of course, every animal that was received, whether it contributed data of value or not. The animals were collected for other than biometrical purposes; hence the percentage of cases furnishing usable data of a statistical nature for the present paper is proportionately small. Nevertheless, a

¹The work on the opossum was begun at the suggestion of Dr. J. T. Patterson, Professor of Zoölogy, the University of Texas, about 1913. It was prosecuted intensively through the generous financial aid and moral support of The Wistar Institute, Dr. M. J. Greenman, Director, and with the assistance of Dr. C. H. Heuser, then fellow of The Wistar Institute. It is to the skill of Doctor Heuser that most of the photographs presented in the four plates accompanying this article and former papers of this series are due. Some of the photographs were taken from fresh living material in January and February of 1917 at Austin, Texas. The embryological investigations soon gave way in large measure to physiological studies in which the following generously aided: Mr. H. A. Wroe,

survey of the protocols yielded a sufficient amount of material to make its collation and publication seem desirable. These data, together with the writer's previous studies on the oestrous cycle of the opossum, should enable the embryologist and the physiologist to proceed in the collection and classification of material without the loss of time involved in pioneering.

This conclusion as to the extent of the data to be presented seems all the more justified when one considers the disappointing dearth of similar data concerning other mammals, even the laboratory rodents and the domestic animals that have been so much used for embryological studies. Thus, for example, of very few species do we know the stage attained at any given number of hours or days with any certainty. They can be counted on the fingers of one hand: mouse, rat, rabbit, guinea-pig, and domestic pig; and the data even in these forms are far from satisfactory. The opossum is, furthermore, the first wild form in which any considerable information as to its reproductive processes has been worked out. That it is also a marsupial, America's only pouched animal, lends further interest to the material under discussion.

The breeding season will be outlined in part I; part II will be devoted to the main topic, the intra-uterine development; part III of the postnatal growth.

I. THE BREEDING SEASON

In 1923, I outlined the breeding season of the opossum in the following words:

The breeding season of the Virginia opossum begins in January at Austin, Texas, and probably several weeks later in the North.

banker, and Mr. Herman Becker, merchant, Austin, Texas; the University of Texas, Department of Zoölogy; The Bache Fund of the National Academy of Science. I take this opportunity of reiterating my indebtedness to these sources of the necessary *nervus rerum* to carry on the work and for the spirit of helpfulness in which the grants were made. The Wistar Institute is the repository of most of the material collected and will supervise its study in the future. The present writer can promise only two more installments of these 'studies': one on the origin of the mesoderm and the chorda dorsalis, the other on pathological ova of the primitive-streak stage and earlier.

A few individuals may come into heat in the first week of the year, but more enter this condition in the second week. In the third week the season is at its height; hence the embryologist desirous of securing eggs and embryos would best time his collection during the last week in January and the first week in February. The prevailing weather seems to have no effect on the onset of the breeding season.

By the middle of February most females captured have young in the pouch. But late in the spring and the summer there is great irregularity in the condition of the females, so that one may capture females with small young in the pouch any time between May and September. Many reasons may be advanced for this variability: accidents to the mother resulting in the early loss of the young; variability in weaning age; condition of the female, the robust reproducing faster than the weak; age of animals, the very youngest 'yearlings' and the oldest multiparae being the latest to come into heat (p. 352).

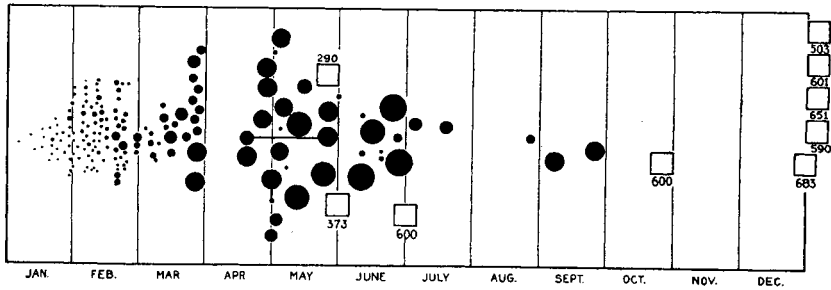
After studying the available data more intensively, I find these statements need but slight correction. They may now be amplified by further analysis of the old and additional details ascertained since 1923.

The progress of the breeding season for the first half of the year may be visualized from text figure A, in which the litters of pouch young are represented by black dots or discs, graduated in size roughly according to the size of the pouch young. Thus the largest circles represent pouch young about ready to leave the mother; the next size are weaned young still under the care of the mother; the third size are sucklings, but detached from the teats, and so on, to the smallest dots which represent young a week or less old. The absolute number of litters has no meaning, except for the early part of the year, since collection of animals by purchase was most brisk in January, February, and March, slackened greatly in April, and was accidental and sporadic after that. Only two years were any observations made in the summer, for vacation usually put an end to the field work until late in the fall.

An effort was made, by searching through the records of the United States Biological Survey, to determine the beginning of the breeding season in States farther North. In spite of the numerous records on file, an insufficient number of usable cases was found upon which to base safe deductions.

I leave this to future workers. The reason that most of the records are not useful for the present purpose is that the size of the pouch young is seldom indicated.

The opossum has no true hibernating season, but certainly an anoestrous period in the early winter. No pouch young, large or small, were ever taken in October, November, or December. In one case, ripe follicles were encountered in December (female no. 16, 455 mm., December 6, 1915). In one case, pouch young were born in the first week of January; in three cases, in the second week (text fig. B), hence these four exceptions constitute the only approaches to ovulation in December. All other females captured in October, Novem-

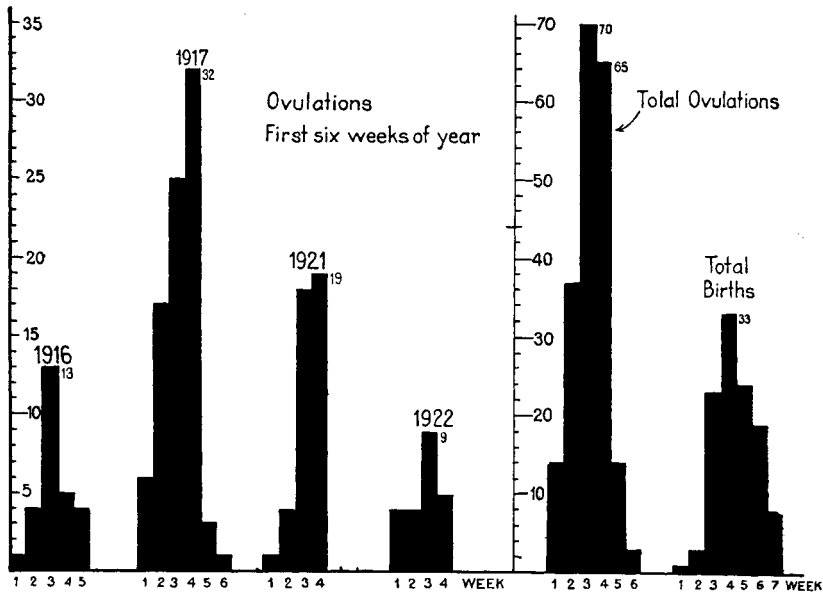


Text fig. A Chart to illustrate the progress of the breeding season. Each dot or disc represents a litter of pouch young, the size of the dot roughly corresponding to the size of the young (see text). Squares represent individual animals, with weight in grams (appendix D).

ber, and December were in the 'resting' condition absolutely, and usually no trace of a corpus luteum was to be found in the ovaries. Most females are in anoestrus in the first week of January also (text fig. B), hence the anoestrous period must be at least three months in length. It is of importance to note this point in endeavoring to determine whether the opossum bears two or three litters in a year.

The male seems to possess spermatozoa during the entire year. Painter ('22), however, found many spermatogonial, but few maturation divisions at the end of the calendar year and more maturation divisions in January. It would be interesting to learn more concerning the reproductive habits of the male opossum. It is quite probable that the male is capa-

ble of fertile coitus even though active spermatogenesis be in abeyance over several months, for the spermatozoa would remain viable in the epididymis over this period. Thus in bats the germinal epithelium of the testes undergoes atrophy during hibernation; however, in some bats, the epididymis is also involved in the general atrophy and is devoid of sperms (*Nyctinomus mexicanus*); in others the epididymis carries motile sperms all winter (*Myotis* sp.). There doubtless is

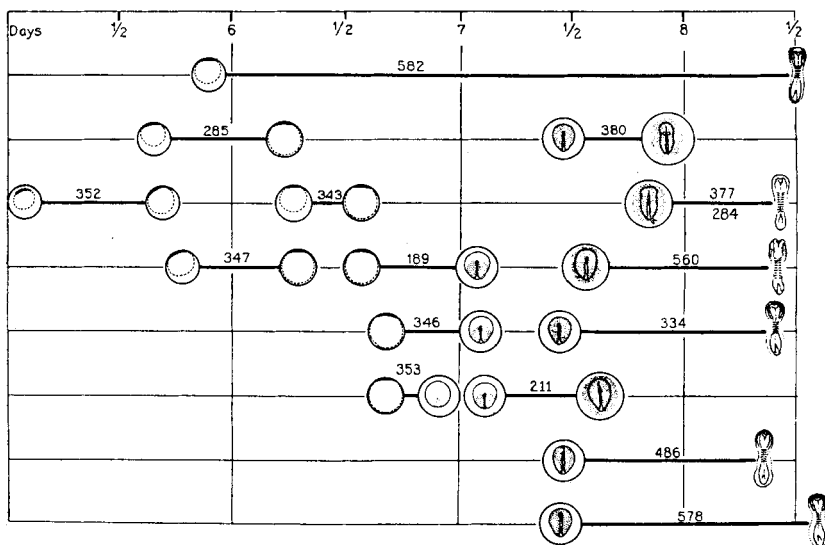


Text fig. B. Ovulation dates and birth dates plotted according to the week following January 1 of the calendar year for certain years, and totals for all years.

every gradation among mammals from almost complete anoestrous atrophy of the testes and accessory sex glands to continuous and unvaried sexual potency, as in primates.

The problem of determining the opening of the breeding season in January may be approached in another way. Since oestrus and ovulation constitute the obvious signs of sexual activity, this date was determined in as many cases as possible. More could have been added with an approximation to the truth by estimating the dates with the aid of text figures C and D on the rate of development. But this was

avoided for the reason that the results were to be used, if possible, to check the correctness of this chart itself. For it seemed reasonable that if a set of birth dates, based on an entirely different lot of material, could be prepared in addition to ovulation dates, then by calculation of the mean and the standard deviation in each of the distributions, the period of gestation (ovulation to birth) might be deduced by dif-



Text fig. D Chart 2. Similar to text figure C, shorter intervals involved. The scale is double that of text figure C, hence cannot be imposed upon the latter. The interested reader could photograph this figure $\times \frac{1}{2}$ and fit the photograph into its place in text figure C.

ference of the means, and the significance of this difference might be judged in the light of the variation of the distributions (v.i.). These two sets of data were prepared, 110 dates of birth and 203 of ovulation (table 1). They are grouped by weeks of the year. For the ovulation dates only those cases are used in which actual observation determined the date absolutely and those which fell, by calculation, near the middle of the week; the border-line cases were discarded. It is thought that in this way the warping of the data by

preconceived ideas on rate of development would be entirely avoided. The dates of birth were either determined by actual observation (some twenty cases), the remainder by the aid of the graphs of growth rate shown in text figures E and F,

TABLE 1

Ovulation and birth dates, by years and for the first six weeks of each year (compare graphs in text fig. B)

| YEAR | WEEK | JAN. 1-7 | 8-14 | 15-21 | 22-28 | 29-FEB. 4 | 5-11 | 12-18 | TOTAL |
|--------|------------|----------|------|-------|-------|-----------|------|-------|-------|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| 1914 | Ovulations | | | 1 | | | | | 1 |
| | Births | | | | | | | | 0 |
| 1915 | Ovulations | 1 | 8 | 3 | 3 | 5 | 1 | 0 | 21 |
| | Births | | | | | | | | 0 |
| 1916 | Ovulations | 1 | 4 | 13 | 5 | 4 | 0 | 0 | 27 |
| | Births | 0 | 1 | 1 | 0 | 4 | 2 | 4 | 12 |
| 1917 | Ovulations | 6 | 17 | 25 | 32 | 3 | 0 | 0 | 84 |
| | Births | 0 | 1 | 0 | 7 | 4 | 3 | 1 | 16 |
| 1920 | Ovulations | 1 | | | | | | | 1 |
| | Births | | | | 8 | 3 | 5 | 1 | 17 |
| 1921 | Ovulations | 1 | 4 | 18 | 19 | | | | 42 |
| | Births | 0 | 1 | 4 | 5 | 7 | 4 | 2 | 22 |
| 1922 | Ovulations | 4 | 4 | 9 | 5 | | | | 22 |
| | Births | 0 | 0 | 8 | 0 | 3 | | | 11 |
| 1924 | Ovulations | | | | 1 | 1 | 1 | | 3 |
| | Births | | | | | | | | 0 |
| 1925 | Ovulations | | | | | 1 | 1 | | 2 |
| | Births | | | | | | | | 0 |
| Totals | Ovulations | 14 | 37 | 70 | 65 | 14 | 3 | 0 | 203 |
| | Births | 1 | 3 | 23 | 33 | 24 | 19 | 8 | 111 |

which are probably quite accurate. All of the figures are given in table 1. The figures for the years 1916, 1917, 1921, and 1922 are expressed in the form of graphs shown in text figure B, which also contains graphs for all of the data for all years—one for the ovulation dates, one for the birth dates.

An inspection of the graphs shows that the third week of the year has the advantage in number of ovulations, though the fourth week is not far behind. After the fourth week, nearly all females brought in from the field may be expected to be pregnant or to carry pouch young. To secure the optimum results, the embryologist, desirous of securing early stages, should begin collecting not later than the end of the second week of the calendar year.

Reduced to figures, the means, or, mechanically speaking, the centers of gravity, of the four annual charts shown in text figure B, as calculated for me by Dr. H. L. Dunn, are as follows:

| | | | |
|-------|--------|-------------|------|
| 1916, | 26.315 | \pm 0.912 | days |
| 1917, | 25.259 | \pm 0.521 | days |
| 1921, | 26.667 | \pm 0.539 | days |
| 1922, | 21.636 | \pm 1.162 | days |

The figures argue for variations from year to year. Thus 1922 appears to be about five days earlier than the other three. I could find no conditions of temperature or rainfall from the weather reports of the preceding years to account for the early opening of the breeding season in 1922. On the other hand, 1925 was very late. In that year not one case of ovulation was encountered in the first two weeks, although a sufficient number of animals was on hand to make the observations significant. The peak came in the fifth week. Now, in central Texas, this collecting season was preceded by a severe drought which, acting through the lack of food, may have caused the delay in the accumulation of the sexual hormones at that season.

NUMBER OF LITTERS A YEAR

Barton ('23) and Meigs ('47) were both of the opinion that the opossum brought forth two litters a year, the first in January and the second in May. Audubon and Bachmann ('51) considered March, May, and July to be the dates for the three litters in the latitude of South Carolina. The latter, however, suggest (p. 124) that the July brood does not repro-

duce until the following May. But since these authors seem to be wrong about all of their calculations on the development of the opossum (v.i.), I should place no reliance upon their opinion on the breeding season.

There can be no doubt that there are at least two litters a year, for there are enough records to show that when one litter is reared another gestation follows (text fig. A). Hence it might at first be thought a matter of simple arithmetic; for if it takes three months to rear one brood (v.i.), three can be reared in nine months, leaving three months for the anoestrus. It is probable, however, that this is possible only to a few unusually vigorous or fecund individuals. If the time of the first oestrus of the year is variable (text fig. B), the time of the second must be much more so, since it depends upon a great many more factors: length of gestation, length of lactation, ovulation, and other physiological processes involved in reproduction in which there is great variability from one female to another. Hence, if one knew the facts and could construct ovulation charts for the early summer after the manner of text figure B, the curve would probably spread out over many weeks. Text figure A, meager as it is for May and June, seems to point in this direction.

An attempt was made to approach the problem statistically. In the lower portion of text figure F (v.i.) the weights of 192 females are plotted according to the nearest hundred grams weight. It is seen that there is a group of eleven females between 500 and 800 grams in weight; to these we may add the four cases around 900 grams. These fifteen females seem to be in a class rather sharply differentiated from the others. I suggest that these represent the August or September litters, the few third litters of the year. The details concerning these are given in appendix D. Nine of these cases are inserted as squares in text figure A. The 290-gram and the 373-gram females of June and the 600-gram female of July are either dwarfs, which is not probable, or exceptional late conceptions of the preceding year. To the same category belongs the litter of "two opossum young as large as small

kittens'' which Meigs found in February, 1845, presumably in the neighborhood of Philadelphia. On the other hand, numerous references of opossum young 'half grown' in the records of the Biological Survey range, so far as I have seen, from May to October, as is to be expected on the basis that breeding takes place only in the first half of the year.

SUMMARY

The breeding season begins at Austin, Texas, with the calendar year following a three months' anoestrus, or period of reproductive rest, in October, November, and December. There is a slight variation from year to year, though for practical purposes, as, for example, for collecting embryos, the variation is unimportant and the information contained in text figure B is probably a sufficient guide.

There are at least two litters of opossum young a year in the Southern States. There is no unequivocal evidence of a third, but it seems probable that a third litter is produced by a small percentage of unusually fecund females. The opossum should be bred in captivity and individuals observed over a period of years. Such observations would not only fill the gaps left in our knowledge of the breeding season, but also complete my growth curve (v.i.), determine the age of puberty and settle other points which the two following sections still leave open.

II. THE INTRA-UTERINE DEVELOPMENT

The effort is made in this section to determine time relation as accurately as possible, so that a given stage may be secured by future workers with a reasonable degree of certainty. The chief method employed is that used by Bischoff on the rabbit ('42) and, more especially, on the dog ('45), and since then only by Selenka ('87) and myself. The method consists of removing successively two stages from the same animal, the interval between the two operations being controlled to the minute.

In the present work, the favorable animal was selected by mammary palpation, the left uterus was removed by aseptic surgery, and the stage of its contained ova noted. The animal was then allowed to live such a period of time as seemed necessary, from previous experience, to secure the desired stage from the surviving uterus, which thus served as Nature's own incubator for the contained ova. Since the ova of both uteri are always in the same stage of advancement, ovulation being simultaneous from both ovaries, the rate of development may be accurately gauged. That the operation has no deleterious effect upon the remaining ova, I have set forth in 1919 and determined by numerous experiments since.

The results of our studies upon the rate of intra-uterine development of the opossum are epitomized in text figures C and D. Text figure C gives the longer intervals between copulation and a given stage, or between two stages recovered from the same animal after the manner above described. This chart is supplemented by text figure D, which covers the cases in which only a few hours were allowed to intervene between the removal of the left and the right uterus. The sketches by which the stages are to be identified, though semidiagrammatic, were made by Mr. James Didusch from life or from photographs. They are faithfully drawn by him and should offer the reader an easy and reliable method of identifying the stages in question. Four plates of twenty-nine figures in heliotype contain typical stages for the further orientation of the reader. The arrows of the chart indicate the exact end points of the intervals; that is, the sketches are placed immediately to the right of the arrow point or the time when the second specimen was taken. The exceptions are lines 1 and 2 of text figure C where the figures of the newly born pouch young were drawn to the left of the arrow points so as to retain the sketches within the limits of the chart.

The sketches require some further words of explanation. As is well known, the ovum proper or 'vitellus' of the opossum is surrounded by a thick layer of albumen, so-called, and

a thin, tough shell membrane (Hartman, '19). The individual ovum would therefore be represented by a dot in the center of a ring; the two-celled stage, by two dots, etc. When more than four blastomeres are present, it was found impracticable to show them by dots; hence the actual number is indicated by inserting the number itself. When the number of blastomeres has reached thirty-two, they are arranged in the form of a hollow sphere without evident polarity; here the vitellus is represented as a small circle in the center of the shell membrane, as, e.g., nos. 314 and 173 shown in the three-day column. Soon polarity begins to manifest itself by a thickening of the embryonic pole and a thinning out of the trophoblastic region (e.g., fig. 1), while the vesicle slowly grows in diameter at the expense of the albumen (nos. 344 to 931). The vesicle gradually encroaches upon the albumen by digesting and utilizing it, and this process occurs faster at the embryonic pole; hence there is left a shrinking crescent at the lower pole of the egg, the disappearance of the albumen being proportionate to the differentiation and to some extent the growth of the egg as a whole (Hartman, '19, pl. 13). At about the time the mesoderm begins to proliferate, the vesicle now having attained the diameter of 1.5 mm., the albumen has all but disappeared (no. 353, Hartman, '19, fig. 22, pl. 13). Up to this period the egg is represented as in a vertical section; from then on the surface view is shown as seen by transmitted light. The primitive streak, Hensen's node, medullary groove, outline of embryonic area, are shown by continuous lines, the spread of the mesoderm by stippling. Progressively larger circles indicate the growth of the ovum in diameter, but not in exact proportion to such growth. For relative sizes the reader is referred to the heliotype plates and the protocols (Appendix A). For lack of space, too, embryos only, without the chorionic vesicle, have been drawn from the later somite stages on (about eight days). The later embryos are represented in side view as contrasted with the dorsal view of the earlier embryos. The degree of development must be read off in certain landmarks, such as the head, heart, limbs, mouth, eye, ear, and the general form.

SELENKA'S AND HILL'S DATA

The studies of Selenka comprise the only pertinent data in connection with the present paper, if one except certain few findings of Hill ('10) on *Dasyurus*, the Australian 'native cat.' It is important to study Selenka's material, for with the proper correction his data are useful in checking my own. He secured a hundred Virginia opossums and had them sent to his laboratory in Germany. The animals yielded some excellent stages well described and illustrated by masterly drawings which are clear and unmistakable. He or his helper witnessed a number of copulations, hence he is able to give the copulation age of a number of specimens, though he fails to give the exact protocols. On the basis of copulation age, Selenka's data fit in well with my own, and I have incorporated them in line 2, text figure C. The copulation age is derived by adding five days to the age stated by Selenka. Thus his '8-hour' stage is actually five days eight hours!

The reason for this is as follows:

Selenka estimated the time of the beginning of cleavage as exactly '5 times 24 hours'; and this estimate is based on a single batch of eggs which he mistakenly considered normal cleavage stages. I have previously shown this batch of eggs to be made up of fragmenting ova or unfragmented but degenerating ova, as in the case of his figure 1, Tafel XVII. All of the eggs are in such a condition as one expects to find about five days after ovulation (compare Smith, '23). One of the same batch of eggs had arrived at the '20-celled' stage, and Selenka thought this normal also, although he states that ova are always in the same stage of development in a given female, in which contention he is substantially correct. Curiously, nine of the eleven eggs of this batch were still in the tube and several are reported as having spermatozoa in the perivitelline space. This batch of eggs is consequently hard to interpret, for usually the opossum's ova reach the uterus in the unsegmented condition. A second batch of eggs exhibited the same wide range of variation as the first, which did not seem to perturb the author in the least. None

of them appears to be quite normal; the one shown by him in his figures 1 and 2, Tafel XVIII, for example, has the earmarks of degeneration in the shrunken condition of the vesicle and its thick wall—a conclusion reached on the basis of numerous similar eggs in my possession. Selenka's earliest normal egg is therefore one which he considers ten hours old! (his fig. 3, Tafel XVIII). If five days be added to the ten hours, the time would be about correct, since it accords very well with my own no. 194 and fits well into the text figures C and D. We now know that ten hours is not too long an estimate for the performance of a single cleavage division, to say nothing of reaching the early bilaminar stage! Similarly, if five days are added to all of Selenka's estimates, the result is very nearly in accord with my own data, as may be seen by comparing Selenka's data (line 2, text fig. C) with the remainder of the chart. The sketches of line 2 are faithful copies from Selenka's own drawings.

In his studies of three Australian marsupials ('91), Selenka apparently estimates the age of the bilaminar vesicle, the primitive-streak stage, and the 6-somite embryo on the basis of the opossum previously studied, although he does not specifically so state. These three stages were removed fifteen, thirteen, and fourteen days, respectively, postcoitum. Similar variations are recorded by Hill ('10) in the case of ova removed from *Dasyurus viverrinus*. He states: "I obtained unsegmented ova from the uteri 4, 5, 6, 7, and 8 days after coitus, 2-celled eggs 6 and 7 days after, 4-celled eggs 11 and 18 days after. In one case the young were born 8 days after the last observed act of coitus, in another, 16 days after and in yet another 20 days after" (footnote, p. 3).

Now these observations of Selenka and Hill lead Hill to state that in *Dasyurus* and apparently in other marsupials "a very variable period intervenes between coitus and ovulation," and that, as a consequence "the obtaining of any desired cleavage or early blastocyst stage is largely a matter of chance." Since the postovulatory interval, for which Hill and O'Donoghue ('13) coined the term 'postoestrum,' con-

stitutes an important element in the problem of the rate of development in mammals, it demands more detailed discussion.

THE POSTOESTROUS PERIOD

From the above-mentioned observations of Selenka and of Hill a long postoestrous period would seem to be characteristic of marsupials in general, as distinguished from the condition in the Eutheria. On this point Marshall ('22) says: "In the mouse, the rat and the guinea pig ovulation occurs spontaneously during 'heat,' and generally, if not invariably, during oestrus" (p. 130). The same is said to be true of the dog and the ferret. Indeed, Marshall generalizes (l.c., p. 131) as follows: "There can be little doubt that in the great majority of mammals ovulation, as a general rule, occurs regularly during oestrus"; and he cites the bat as an outstanding exception. For in the bat it is now tacitly and generally accepted that mating takes place in the fall and conception in the spring. This tradition has recently been called in question (Hartman and Cuyler, '27), although we shall not allow a discussion of the merits of this matter to detain us here. Marshall's generalization, quoted above, needs revision for the sake of exactness, for the fact is that ovulation does not always occur during oestrus, but often follows oestrus and copulation. In other words, there is often a postoestrum in the Eutheria, albeit a short one. That this period is also a short one in at least one marsupial is made probable, if not established by more recent data on the opossum (Hartman, '24), as will be shown below.

That the data on marsupials are still insufficient for a final disposition of the problem is not surprising, in view of the fact that the relation of heat, copulation, and ovulation both as to time and physiological cause in any mammal except perhaps the rabbit is very unsatisfactorily known. Pertinent facts of this kind are hard to gather from the literature for any mammal, even the common laboratory mammals. For the rat and the mouse the matter is not answered, despite the attention that has been devoted to the embryology and physi-

ology of reproduction of these common laboratory rodents. An inspection of table 2, page 31, of Long and Evans' monographic study of the rat ('22) leads one to assume a delay of ovulation after oestrus, for this period is mostly past before the ova are very far down the oviduct. In the rabbit it has been known since Coste's beautiful work ('49) that ovulation occurs, without a great deal of variation, ten hours after coitus, and approximately the same thing is true for the guinea-pig (Loeb, '06). However, these two species differ from each other in that the former has no well-defined oestrous period, but will accept the male and ovulate over wide periods of time. In the cat (Longley, '11) and the ferret (Robinson, '18), as in the rabbit, ovulation usually depends upon copulation and occurs a variable number of hours, usually many hours, later. In the case of the ferret, Marshall states that ovulation occurs during oestrus. The statements of Marshall and Robinson with reference to the ferret are, however, not mutually exclusive, for a female may clearly be in heat and copulate successively over considerable intervals, both before and after ovulation. Whether copulation and fertilization shorten the heat period or not is not known definitely for any mammal. In the cow there seems to be no such influence, according to Küpfer ('20) and Hammond ('27); in the rabbit a positive answer seems to be necessary and Loeb ('11) states the same for the guinea-pig. In both *Dasyurus* (Hill) and the opossum the mated female will soon fight off the male, which might indicate that copulation cuts short the oestrous period.

It is thus seen that the correlation of events surrounding the ovulation period needs further experimental study. The common procedure of workers in this field is to mate their animals at convenient hours of the day, regardless of whether the animals are at the beginning or the end of heat. This must be a source of considerable error. There is no other way than continuous observation night and day, so that both the first and the last signs of oestrus may be taken into account. Results of intensive experiment along these lines should yield useful results (v.s.).

Further data on the time of ovulation is given by Keller ('09) in the case of the dog, in which ovulation occurs in the latter part of oestrus; in my opinion, however, evidence presented in the paper is far from conclusive. Better evidence is presented by Corner ('22) that the sow ovulates regularly toward the end of oestrus. But in the cow, contrary to the opinion of Krupski ('17), whose observations were made on slaughter-house material and who places ovulation at the end of oestrus, both Murphy ('26) and McNutt ('26) note a delay of one or two days after the end of heat before ovulation takes place. Küpfer ('20) does not insist on a constant relation between heat and ovulation. The observations of Murphy and McNutt may be regarded as reliable because these workers noted the exact time of rupture of the follicle by their technique of daily palpation of the ovary through the wall of the rectum.

These few citations illustrate the paucity of reliable information on the presence or absence of a post-oestrous period and the length of the period where it occurs. There doubtless is much variation from species to species and among individuals of a given species, but as yet there are no data upon which to calculate probabilities. What is clear, however, is that the method of dating embryos by copulation age is highly unsatisfactory, since this involves at least two variables, length of oestrus and of post-oestrus, which in different cases may be added together or subtracted, which increases the confusion. It is, furthermore, apparent that there is no real reason for considering marsupials fundamentally different from the higher mammals with regard to the physiological events centering about the process of ovulation.

With the reservations suggested by the foregoing discussion, the data of Selenka, Hill, and myself must be evaluated. An inspection of text figure C discloses some very considerable discrepancies. Thus, for example, no. 314 and no. 298 yielded identical stages three and one-half and six days, respectively, post-coitum. Nos. 838 and 343 also show less

development than is to be expected on the basis of averages deduced from a study of the chart as a whole. No. 314, rather than no. 298, seems to represent the more nearly normal case and agrees well with Selenka's data as reviewed above.

It remains for us now to mention the few cases in the opossum that tend to show that ovulation stands in closer relation to the oestrous period than I at first thought, on the basis of Selenka's as well as some of my own earlier observations (Hartman, '16). The five cases are set down in the lower part of columns 1 and 2 (first and second days, text fig. C). These cases have been considered previously (Hartman, '23) in connection with the viability of the opossum ovum after its discharge.

Female no. 566 was laparotomized at 5.00 P.M. January 12, 1921, and a suspension of *Ascaris* eggs placed under the fimbriae. Ovulation had just taken place, for not only did the fresh hemorrhages of the discharged follicles indicate this, but (which is a better proof still), there was later found an opossum ovum which had entangled in the meshes of its albumen one of the *Ascaris* eggs previously placed near the abdominal orifice of the fallopian tube. In twenty-four hours the ova had reached the uterus and were somewhat degenerated; and sixteen hours later palpably more so.

No. 521. This female had ripe, bulging follicles in the ovaries on January 21, 1922, when a laparotomy was performed; forty-two hours later, eggs considerably degenerated were found in the uteri.

No. 837. This female was seen to copulate at 4.00 A.M., January 23, 1923; at 3.00 P.M. the next day, seventeen eggs, much affected by degenerative changes, were removed from the left uterus and three eggs from the tube. In this case it is highly probable that copulation had taken place too late for fertilization.

No. 762. January 25, 1923, oestrus was indicated by a suspension of cornified cells in the vaginal smear. Copulation was not observed, however. The next day the cornified cells appeared in clumps, indicating that oestrus was past. The animal was then killed. The eggs removed from the uteri had the vitellus somewhat flattened, showing that degeneration had begun.

No. 798. January 25, 1923, the vaginal examination indicated that the animal was in oestrus. Killed the next day; eggs in beginning degeneration not much different from those of the preceding.

From these cases the deduction would seem justifiable that in the opossum ovulation is pretty well bound to oestrus, though not necessarily synchronous with it.

LENGTH OF GESTATION

Hill records parturition in *Dasyurus* 8, 16, and 20 days after the last observed act of copulation; Selenka, 13 days less 4 hours in the opossum. Michel ('50) observed copulation January 28, 1847, and 18 days later the birth. My own case of 13 days (exact hours not recorded) agrees with that of Selenka. This author, however, for reasons detailed above, places the actual gestation at 7 $\frac{5}{6}$ days. I am now convinced that 12 $\frac{1}{2}$ days marks very nearly the actual gestation not only because of the facts already stated with reference to the postoestrous period, but also from a study of text figures C and D as a whole, that is, after taking into consideration all of the facts there presented on the rate of development. These seem to me to be sufficiently numerous to justify the conclusion that at least 13 days is necessary for the development of the embryo from copulation to birth. No embryological facts known to me seemed to necessitate raising this estimate, and all the facts seemed to argue against lowering it.

At the suggestion of Dr. H. L. Dunn, of the Department of Statistics, Johns Hopkins University, School of Hygiene, the effort was made to check the data contained in text figure C statistically. For the necessary calculations I am also indebted to Doctor Dunn.

In part I of this paper two sets of figures are cited, one on ovulation dates (203 cases), the other on parturition dates (111 cases). The figures are given in table 1. It was thought that a difference between the means of these two sets of figures ought to reflect accurately the length of the period of gestation: from mean of the ovulation days to mean of the birth days. This presumes that no other factors have entered in to warp the estimation of the raw material (table 1). It is seen from the table that for only four years (1916, 1917, 1921, 1922) is there sufficient data concerning both ovulation

and birth days. The birth dates for 1922 are somewhat bizarre; but these were used, nevertheless, for the sake of fairness. The figures are scanty, but seemed sufficient—at least the difference in the means could be judged in the light of the variations of the cases.² The means, standard deviations, and the probable errors of the means were determined for the two sets of material. The differences between the ovulation time and the birth time for each year were computed in days following January 1st, and the probable errors of the differences calculated from the probable error of the means by the formula:

$$\text{PE diff. of means of ovulation and birth} = \sqrt{(\text{PE ovulation})^2 + (\text{PE birth})^2}$$

The experience of the four years was then summated by adding together the four differences and averaging them and the respective probable errors. The result tells us that the average difference is 10.557 days \pm 1.720 days. The period of gestation might, therefore, be anything from 8.84 days to 12.28 days. The calculated chances of the period being 12.5 days, as estimated according to text figure C, are 80 in 100. Had the data for 1922 been eliminated, the result would be considerably more favorable for the 12½-day period.

THE RATE OF CLEAVAGE DIVISION

Cleavage in the opossum egg may be considered as ending at about the thirty-two- to forty-celled stage when the blastomeres are arranged in the form of a hollow sphere (the blastocyst or vesicle) with cell margins contiguous. It is represented in text figure C as a small circle. Eleven cases from

² The calculation thus made illustrates the possibilities of a statistical treatment of biological raw material, even though in this instance the material is very meager. Much more material was actually collected, but measurements were not made at the time and valuable opportunities lost. This statement is made to emphasize the need for the accumulation of accurate biometrical raw material, which will find many uses in the hands of the statisticians of the future. Perhaps more important still, the study illustrates the value of the statistical treatment of meager material in order to learn its limitations for drawing fundamental theoretical conclusions.

the two-celled to the twenty-five-celled condition have been utilized in the chart. Since cases covering the ovulation-cleavage stage interval were lacking, the question arose, where in the chart the respective cleavage stages should be placed. In other words, the starting-point of these cleavage cases had to be determined theoretically on the basis of what is known of other animals.

The moment of ovulation is tentatively placed at a half-day after copulation on the basis of the rabbit, which in its developmental rate is almost identical with the opossum for the first 10 days. In 24 hours the opossum egg reaches the uterus in the pronuclear stage, in which condition it had probably been for some hours. The rat reaches this stage in 24 hours postcoitum (Huber, '15); the mouse in about 11 hours (Sobotta, '95); the rabbit in 14 hours (Hensen, '76) to 20 hours (Coste, '49), the latter being the correct time;³ the guinea-pig in 12 hours (Rein, '83) to 24 hours (Lams, '12), the sheep in 1½ days (Assheton, '98). In the rat the two-celled stage is reached in about 48 hours (Huber), in 24 hours according to Melissinos ('07). Coste, Assheton ('94), Nihoul ('26), and Rein all agree that the two-celled stage is reached in the rabbit in one day after copulation or 14 hours after fertilization. Further examples will be given below illustrating the agreement among authors as to the developmental rate in this species—further evidence as to the comparative reliability in the rabbit of estimating the ovulation period from the time of first copulation. In the guinea-pig Hensen found the two-celled stage in 24 hours; Lams, in 33 to 48 hours. In the pig Streeter and Heuser ('28) found two-celled ova in 2 days, 3½ hours postcoitum.

Taking these comparative studies into account, together with the fact that the opossum egg has a great deal of inert

³ I am permitted by Mr. P. W. Gregory, Research Fellow at the Bussey Institution, to report the following results of his painstaking work on the rabbit: Pronuclei are found up to 21 to 22 hours postcoitum; two-celled ova from 21½ to 25½ hours; four-celled ova from 26 to 31 hours. Thirty-two and one-quarter hours is the best time to secure the third cleavage in progress; the fourth cleavage begins around 41 hours.

yolk to expel during the first cleavage (which may or may not retard the first cleavage in comparison with the succeeding ones), I have set down the time of completion of the first cleavage at $2\frac{1}{4}$ days.

The succeeding divisions are, however, conceived to be more rapid, say $\frac{1}{4}$ day each, which is short in comparison with other forms.

For the rat Huber states that the second cleavage requires about a day (73-hour stage), which is twice as rapid as the first cleavage. The third division (89-hour stage) is still more rapid, say $\frac{2}{3}$ of a day, the fourth cleavage occupies only $\frac{1}{4}$ day. Hence, in the rat there is clearly a speeding-up of division rates with each successive cleavage, as the blastomeres become smaller. The same might well be true of the opossum, which casts off a variable amount of yolk with cytoplasm. The rabbit ovum divides still more rapidly, for in 72 hours it has reached the morula stage and consists of at least thirty-two cells (Nihoul, van Beneden ('80), Assheton, Coste). The guinea-pig seems to be a little slower (Spee, '01), the sheep and the pig still slower. Streeter and Heuser have recently observed some cases in the pig which show roughly that the second cleavage requires about a day, the third $\frac{3}{4}$ day. But a study of nine litters from the two-celled to the sixty-four-celled stage would seem to indicate about $\frac{3}{4}$ day to a cleavage division in the pig, without any acceleration in the later divisions.

Two experiments on the opossum would have settled the time required for passage from the four-celled to the thirty-two-celled stage (three divisions) unequivocally, but for the fact that the record in one case is defective. Thus, no. 203 yielded four-celled eggs at 8.40, January 28, 1917; the second operation was done at 11.45, but whether in the morning or the night of January 29th was not put down in the protocol. The cage notes, however, pointed to the night period, which would make the interval 39 hours instead of 27. That the latter period (27 hours) is probably the correct one, however, is borne out by an entirely similar case, no. 173, in which the interval is about a day, or about $\frac{1}{3}$ day for a single division.

Of interest in this connection are Bischoff's experiments on the rate of development in the dog, in which he removed surgically successive segments of the uterus, to note the development made in a 24-hour period in the same animal. Thus, in dog XV, the eggs passed in a day from the four-celled to the eight-celled condition; in dog XVI, from the four- to the nine- or ten-celled stage; in dog XVII, from five to seven cells to sixteen to thirty-two cells; in dog XIX, from nineteen to thirty-two or more cells.

THE EARLY VESICLE

We thus arrive in $3\frac{1}{2}$ days (no. 292, for example) at a stage when the entoderm mother cells are just beginning to proliferate. This stage corresponds roughly to the morula stage of the higher animals in which the segmentation cavity is beginning to appear. This is reached in the rabbit in about the same time as the opossum, namely, 3 days 10 hours (Coste, Assheton, Nihoul, van Beneden). In the guinea-pig, in 5 days (Spee); in the mouse, in $4\frac{1}{2}$ days (Sobotta) to 5 days (Jenkinson, '00). Six to seven days are required in the case of the sheep, according to Assheton, while the same stage is reached in the pig in 5 to 6 days, according to the same author. Streeter and Heuser find an early morula, mostly without segmentation cavity in 4 days $3\frac{1}{2}$ hours, with small segmentation cavity in 4 days 18 hours, and with well-developed cavity in 6 days $1\frac{3}{4}$ hours, and in 7 days $3\frac{1}{2}$ hours.

An examination of the chart (text fig. C) shows no. 314 to have arrived at the stage under consideration in the estimated time almost exactly; no. 173 also falls at that point. The discrepancy seen in no. 343 has already been alluded to, while the still greater discrepancy of no. 298 is to be explained on the basis of a later copulation than the observed one.

The further differentiation of the opossum blastocyst consists in a thinning out of the trophoblast and a continuance of entoderm formation at the embryonic area. This comes to have a more and more restricted proportion of the vesicle

which grows in size chiefly through the spreading out of the trophoblast. Thus litter no. 356, previously fully described (p. 63, pls. 8, 9, and 11, Hartman, '19) is placed in text figure C just in advance of the 4-day line. From the undifferentiated vesicle (nos. 193, 191) there is an easy gradation through nos. 344, 544 (fig. 1), 927, and 349 to the stage represented by no. 356. The time relations here are pure estimates based on a count of the number of cells in the ova as well as on a survey of the chart as a whole. Further experimentation is here needed.

The four-day stage (no. 356), with the exception of Rauber's layer which is characteristic of the eutherian blastocyst, corresponds very well with the 4-day rabbit vesicle as figured by Assheton, Hensen, van Beneden; with the 5½-day mouse egg (Jenkinson, Sobotta), the 6-day guinea-pig egg (Heuser, Spee, Rein), the 5- to 7-day pig (Assheton, Corner, Streeter and Heuser), the 9- to 10-day sheep (Assheton). The rabbit and the opossum are, therefore, in advance of other mammals so far as known on the fourth day of development.

THE BILAMINAR BLASTOCYST

The next landmark which we may select is the bilaminar blastocyst between 1.0 and 1.5 mm. in diameter, fully described by Selenka, and by Hartman ('16, '19). In both the rabbit and the opossum the vesicle at this stage is practically spherical (figs. 5, 9, 21), in the dog elliptical; in the rat, the mouse, and the guinea-pig it is more or less collapsed, while in the pig and the sheep it is beginning greatly to elongate. Bischoff found in the dog that 25½ hours were insufficient for the egg to pass through the bilaminar stage, vesicles of 2.0×2.5 mm. having in that interval grown to 3.5×4.5 mm., all still didermic. In the opossum the growth of the vesicle is at the expense of the albumen layer—a feature shown by the sketches in the charts (text figs. C and D). With the growth of the vesicle in diameter the entoderm spreads toward the lower pole of the egg. Thus in ova no. 352 (text fig. D and Hartman, '19) and 931 (text fig. C) the entoderm has not

yet reached the equator. Other batches of about this stage of advancement are nos. 931, 194, and Selenka's 10-hour' stage (actually 5 days 10 hours). It will be noted that copulation ages are known for both no. 194 and Selenka's case; no. 931 falls naturally on the 5-day line in comparison with other litters of the chart. Hence in text figure D, no. 352 is placed on the 5-day line to mark the beginning of the chain of stages there shown. This chart contains a sufficient number of cases to be a fairly reliable picture of what happens from the end of the 5th day to the completion of 8½ days.

The bilaminar stage is completed, that is, the entoderm has reached the lower pole of the egg in nos. 205, 352, and 347. A day and a half later (no. 353, figs. 22 and 23) the primitive-streak mesoderm begins to proliferate; hence it is apparent that the bilaminar stage in the opossum, as in the dog, persists for over a day. It marks the half-way stage in the actual period of gestation, and yet there is no embryo as yet, only what MacDowell, Allen, and MacDowell ('27) call the 'proembryo.'

Studying text fig. C as a whole, 6½ days may be set down as a fair average for the 1-mm. bilaminar blastocyst, which is exactly Selenka's finding as corrected (v.s.). Our 191 and 293, 285 and 347 accord with this estimate, while nos. 838, 343, and 306 fall 12 to 24 hours later. The first two have copulation data; hence it is possible that our estimate of 6½ days may need some revision in the future. One-half day later, the primitive-streak stage is well indicated.

THE PRIMITIVE-STREAK STAGE

This rather clear-cut stage has been described in a number of mammals, with copulation age in a few of them. In the rabbit Minot and Taylor ('05) find it 6½ days postcoitum. However, this seems an exceptionally early estimate, in view of the findings of other authors, as well as in consideration of the fact that Minot and Taylor themselves find but slightly more advanced stages 7½ and 8½ days postcoitum. Hence, we may accept Hensen's 7-day term as more nearly the modal

one. Sobotta thought the mouse reached this stage in 8 days or more, while MacDowell, Allen, and MacDowell find $7\frac{1}{2}$ days sufficient for the establishment of a primitive groove and the beginning of a head process. They state further: "Of twenty-one 7-day embryos from three litters, sectioned transversely or longitudinally, sixteen are sufficiently developed for a primitive groove, but none show a head fold." The mouse is thus almost exactly at the same stage as the opossum at 7 and $7\frac{1}{2}$ days. The guinea-pig appears to speed up at this point, if we are to accept Bischoff's time of 6 days 14 hours and Kölliker's 6 days 18 hours. But Kölliker also reports a bilaminar blastocyst at 7 days. These data are clearly at variance with Hensen's egg cylinders that have not yet any mesoderm at 11 days and Lieberkühn's areas with primitive streak and considerable mesoderm at 13 days and 13 days 20 hours. Hensen's data on rate of development between the inner cell mass stage of 6 days and the six-somite stage at 14 days likewise need checking up by future work. In the case of the dog the primitive-streak stage is reached in 16 days, according to Bonnet; in the sheep in about $14\frac{1}{2}$ days, according to both Keibel and Bonnet.

Of our own batches of eggs nos. 380 (figs. 15 and 16), 486 (fig. 17), and 578 are found to be in the same stage of development, although for the last-mentioned there are only a photograph of the eggs in salt solution and a verbal brief description extant; hence the eggs could not be reexamined for details, and their exact position in the chart is slightly problematical. The cue for the proper placing of these primitive-groove stages in text figure D is furnished by batch no. 211, in which the interval of $12\frac{1}{2}$ hours intervened between an early primitive-streak stage and one having a short medullary groove with a spread of mesoderm somewhat beyond the limits of the embryonic shield. Nos. 377 and 284 are somewhat in advance of no. 211. No. 380', with its single pair of somites just beginning (fig. 19) is still more advanced, though about the same as no. 349 of text figure C, which falls on the $7\frac{1}{2}$ -day line. No. 356' at $7\frac{1}{3}$ days and no. 298' at

9½ days (fig. 29) are midway between 377 and 380', their medullary grooves being about the same length as the primitive streaks and the notochord or head process half as long. No. 298' is, as already indicated, palpably out of place in the chart (text fig. C), as is the earlier stage secured from the same animal. The corresponding stages represented by Selenka (line 2 of text fig. C) are seen to be approximately in agreement with my own chart; the slight inconsistencies are, moreover, readily explained by the fact that the various stages came in part from different animals (v.s.).

For the dog, Bischoff records some further interesting experiments that are of unusual interest in connection with the stages shown in the latter part of text figure D. In his specimen XLV he removed a primitive-streak embryo corresponding to our no. 380 (fig. 15) or no. 486 (fig. 17) and 24 hours later a stage quite comparable to our nos. 298' (fig. 29) and 356', a rate of differentiation about half as rapid as in the opossum. Again, his no. XLVI yielded about the same stage as our no. 377, and in 24 hours a stage attained by no. 377' in 12 hours. This interesting comparison perhaps indicates a speeding-up in differentiation rather than growth in the opossum as compared with an animal whose birth is still far removed in time. There is need of more extensive work along this line in mammals, including the opossum. Certainly, such studies should carefully distinguish between growth in size and differentiation of parts. Counting mitotic figures in an embryo is an index of rate of growth, but not of differentiation.

THE EMBRYOS

Embryos with six to ten somites are to be expected in the opossum about 8½ days postcoitum. At this stage the brain vesicles may be made out and the pericardial cavity has the typical horseshoe shape about the head end of the embryo. The latter is, however, to be seen as early as the stage represented by no. 356 (Hartman, '19). One animal (no. 340) furnished two sets of embryos at about seven to ten somites

6¼ hours apart—an interval of time quite sufficient to add to the complexity of the brain, the number of somites, and the head fold.

With the 8½-day opossum embryo the rabbit again agrees very well. Thus Minot and Taylor found a two-somite rabbit embryo at 8¼ days, one of seven somites at 8½ days. Hensen found a three-somite embryo in 8⅓ days, one of more somites at 8½ days. Double this time is required in the pig (Keibel) and the sheep (Bonnet).

The older embryos are represented in text figure C by outlines that admit of the respective stages being made out with reference to the head and the limbs. In plates 1, 2, and 3 seven embryos are shown photographically. In the head the gill arches are indicated in the younger embryos (fig. 14); such a stage may be expected in both the rabbit and the opossum at 10 days. In the later embryos the optic pits may be seen. The mouth is at first wide open, almost closes later, and near term comes to be surrounded by the peculiar muzzle (Schnabelschild), as well described by Selenka. The fore limb is precocious, for near term the fingers are provided with claws adapted for crawling into the pouch (Hartman, '20) while the hind limbs are still embryonic paddles.

SUMMARY

In constructing our charts (text figs. C and D), we have assumed thirteen days for the entire period of gestation, from coitus to parturition, and have limited the outline of the chart with straight lines, as though the span were always invariably the same. This was done as a matter of convenience. There can be no doubt that the span of gestation is subject to enormous variations, as is well known for all mammalian animals and the human species as well. The opossum can be no exception. One might even expect a greater percentage of variation in the opossum because of the early birth, since the physiological processes that cause parturition at the appropriate time (still unknown) must be delicately adjusted and might be thought of as rather poorly established in the

presence of corpora lutea only twelve and one-half days old. Indeed, several cases among my records of two- to four-day intervals toward the end of gestation point rather significantly to an occasional period longer than thirteen days. Statistically, as shown above, the chances are against the assumption of a longer average period of thirteen days between copulation and parturition and twelve and one-half days between fertilization and birth.

It is apparent, moreover, that the first half of gestation is entirely concerned with the differentiation of the 'pro-embryo'; hence the actual intra-uterine development of the embryo itself comprises but five and one-half days, for at seven and one-half days the primitive streak is completed and the medullary plate begins to differentiate, the notochord to grow forward from Hensen's node. As one views text figure C, one must truly marvel at the complexity of even the visible changes which occur in the period represented by the latter half of the chart.

III. THE POSTNATAL GROWTH AND DEVELOPMENT

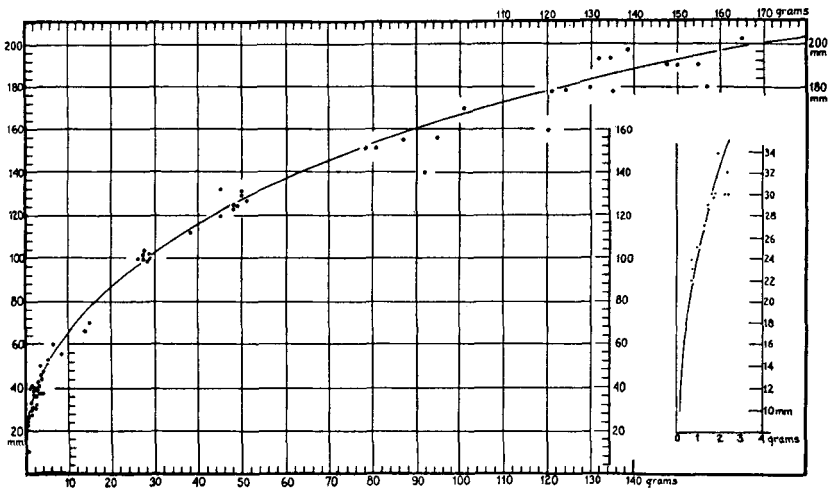
Though not as extensive as might be desired, the data upon which the postnatal development is based afford results which should prove of interest and value. For the opossum these new data stand alone, except for the four or five cases cited by Barton (1823), Meigs ('47), Michel ('50), and Audubon and Bachmann ('51). Of these the data given by the last on sizes at various ages are clearly wrong, unless I mistake the use of the grain as a unit of measure, which I take to be 0.0648 gram.

For the convenience of the reader, the new data have been reduced to the form of charts and growth curves, which may serve as a basis for discussion. The interested reader may refer for details to the appendices.

Before proceeding with the subject of growth, it is necessary to present some relations of body length to weight; for this information has been in part utilized in translating length to weight for text figure G and weight to length for text figure H in a few cases.

DATA OF LENGTH ON WEIGHT

The weights of the animals in grams is accurate, the measurements of length as accurate as the method employed. It was found best to use the snout-rump dimension, rather than crown-rump, for the latter depends upon the amount of curvature allowed the neck region. To secure the measurement, the dead animal, if large, is laid on its back, a rule is laid along the ventral surface, and the length from the tip of the snout to the junction of the tail with the trunk read off. A

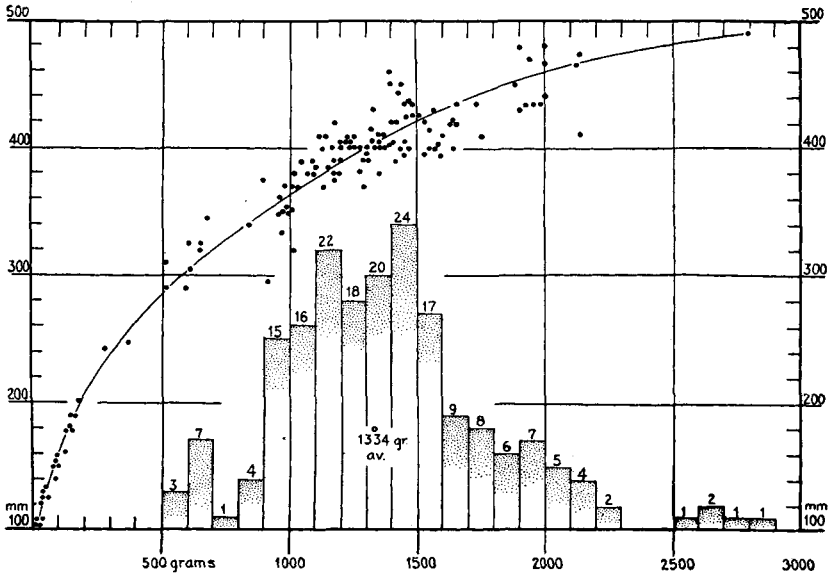


Text fig. E Curve of length in millimeters on weight in grams for sixty-eight pouch young of the opossum, both sexes.

small pouch young is held extended with the fingers of one hand and a rule applied to the ventral surface.

Some sixty-nine young animals of both sexes, still under the protecting care of the mother, if not actually sucking, were both measured and weighed. These range from about 10 mm. in length and a little over 0.1 gram in weight to about 200 mm. in length, 165 grams in weight. The plotted curve shown in text figure E has the usual shape of length-weight curves, as, for example, Streeter's curve of human material ('20).

Of adult or adolescent animals, females only were weighed and measured, a total of 115. An additional seventy-seven furnished weights only. The former were used to construct the curve in text figure F, to which has been added a portion of text figure E to complete the first part of the curve.



Text fig. F Curve of length in millimeters on weight in grams for 117 mature female opossums. At the beginning of the curve are added some prepuberty cases (under 200 mm.) from text figure E. Below the curve, graph of 192 female opossums arranged by weight and grouped according to 100-gram columns, from 5 to 600 grams up. 1334 grams, the 'center of gravity' of the graph, represents the 'average' female opossum capable of bearing young.

From text figure F it appears that somewhat over half of the females range from 1000 to 1500 grams (2.2 to 3.3 pounds) and measure 375 to 420 mm., and that this group is pretty evenly distributed about the point of intersection of the 400-mm. and the 1250-gram line. An average animal of such size I find is frequently designated in the protocols as 'medium' in size. Only one female weighed as much as 2800 grams (no. 429), the largest female ever weighed by us, though I have the impression that several were encountered

that struck one as slightly larger. Audubon and Bachmann must certainly have been mistaken when they described a full-grown female measuring $15\frac{1}{2}$ inches (394 mm.) as weighing 12 pounds.

The curve indicates, furthermore, that the opossum, like other animals, the rat, for example (Donaldson, '24), begins breeding long before she is full grown. The 115 dots in the chart represent not fully grown animals only, for many among them were seen to be palpably young; others are palpably old, of course, having permanently dilated pouches and enlarged nipple bases. The animals are simply random samples of females, mostly pregnant or at least approaching closely to their first oestrus, which happened to be unfortunate enough to be brought into the laboratory.

Of pregnant or pseudopregnant, and therefore sexually mature and breeding females, the following represent extremes in size.

No. 226, 660 grams, 345 mm.; had 10.5-mm. embryos (near term) February 11, 1915.

No. 234, 650 grams, 350 mm.; was pseudopregnant March 17, 1915. But this female was a black one and may have belonged to the species *D. marsupialis*; hence the preceding may be taken as the smallest breeding specimen of *D. virginianus* among my records.

No. 429 was the largest female, 2800 grams, 490 mm. She had pouch young about two weeks old, February 13, 1927.

No. 702, the next largest female, weighed 2720 grams, February 7, 1922, when young, unfertilized eggs were removed from the uterus.

Finally, attention may be called to certain descriptive terms for size applied to specimens whose records were being set down, such as 'tiny,' 'small,' 'medium,' 'large,' 'very large.' These terms were used along with other descriptive features, to make more certain the identification of experimental animals whose number otherwise was read off from a code of holes, slits, and notches in the ears in connection with cut-off stubs of vibrissae. Sometimes the animal was weighed or measured later. Hence, it was possible, by collating these cases, to define the general terms for size more precisely. The result is as follows:

| <i>Descriptive adjective</i> | <i>Weight (grams)</i> | <i>Length (mm.)</i> |
|------------------------------|-----------------------|---------------------|
| Tiny | 1000 or less | 355 or less |
| Small | 900 - 1300 | 350 - 390 |
| Medium | 1200 - 1400 | 375 - 410 |
| Large | 1300 - 2200 | 395 - 440 |
| Very large | 2000 or more | 430 or more |

THE GROWTH CURVES

Two charts are presented, one of weight on age (text fig. G), and the other snout-rump length on age (text fig. H). The former covers fifty-six days, the latter, thanks to data furnished by Doctor Langworthy, almost ninety days. Doctor Langworthy's cases are represented in text figure H by open rings, my own cases, as usual, by dots. Since Doctor Langworthy's cases were not weighed, those that come within the scope of the weight-age chart were translated into terms of weight with the aid of text figure E and entered in text figure G with open rings.

THE NEWBORN

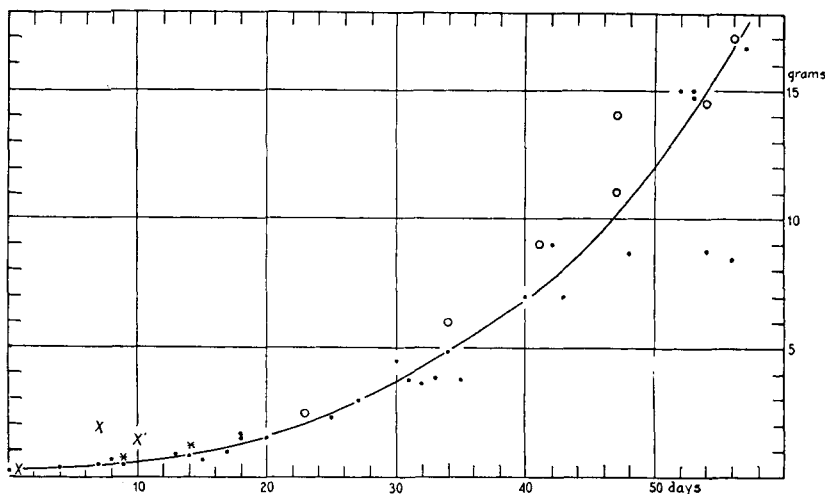
The newborn pouch young have been variously measured and weighed. Barton states that the smallest specimens of one litter weighed barely 1 grain (0.0648 gram); another specimen barely 2 grains (0.13 gram) and the remaining five, taken together, exactly 7 grains—an average of about 0.9 gram. Schwarz ('20) found sixteen young in a pouch; these filled a tablespoon and weighed 2 grains (0.13 gram) each. Meigs was not certain of the exact day of birth of his litter, one of which he found to weigh $3\frac{1}{2}$ grains (0.227 gram), which seems about right for a pouch young two days old. Audubon and Bachmann's birth weight of the opossum is still at variance with the other figures quoted and those of Meigs. These authors state that one embryo recovered by caesarean section weighed 4 grains, or 0.26 gram; others weighed $3\frac{1}{2}$ to $3\frac{3}{4}$ grains at birth. Incidentally, it may be stated that these authors' estimate of the seven-day pouch young at 30 grains (about 2 grams) and the ten-day pouch young at 22 grains (1.43 grams) are also rather far removed from my own curve; their cases are entered in text figure G at x and x' .

Selenka failed to weigh the newborn that came into his possession, but he measured one which he found to be 13 mm. in length. I also failed to weigh in the fresh state the young whose birth I witnessed in 1918 (Hartman, '18); but after storage for nine years in 80 per cent alcohol after fixation in Bouin's fluid the eleven remaining specimens out of the litter of eighteen weigh as follows: 0.125, 0.116, 0.112, 0.110, 0.108, 0.107, 0.105, 0.103, 0.1, 0.95, 0.09 gram. Dehydration causes shrinkage, of course; but it is probable that in life the embryos weighed in the neighborhood of 0.13 gram. Another female, stored in a shallow table drawer about 6 inches deep, overnight, was found the next morning to have given birth to ten young, of which only one was attached. This one weighed 0.16 gram; its stomach was filled with milk. The nine others that had perished weighed a total of 1.2 grams—an average of 0.133 gram.

In view of the aforementioned facts, it is safe to place the average weight of a newborn pouch young at 0.13 gram. If we assume that ten is an average litter born, the ratio of body weight of the litter to the mother is about 1:1000. Perhaps the bear is a close second to marsupials in the disparity between the size of the mother and the offspring at birth. This was first called to my attention by Dr. Francis G. Benedict, of the Carnegie Nutrition Laboratory, Boston, who presented me with the photographs of a pair of cubs measuring 210 mm. in snout-rump length and weighing about a half-pound each. The ratio in this case would be one to four or five hundred. Brehm states in his 'Tierleben' that the polar-bear cubs weigh 750 grams each, or about 3 pounds for the pair. If we take the maximum weight of a polar-bear female at 1500 pounds, we get the ratio of 1:500. Probably the other extreme in the mammalian series is found in the bat, where the single fetus may weigh fully one-third as much as the mother (!)⁴

⁴I am indebted to Dr. C. Hart Merriam for information (private communication) which indicates that in bears the ratio of the weight of the cubs to that of the mother is considerably greater than 1:500. Thus a Yellowstone Park

The further growth in weight is plotted in text figure G; in length, in text figure H. In the latter, Barton's sixty-day young, weighing 35 grams, is entered at x and Meigs' seventy-four-day young, weighing 27.8 grams, is entered at x' , after



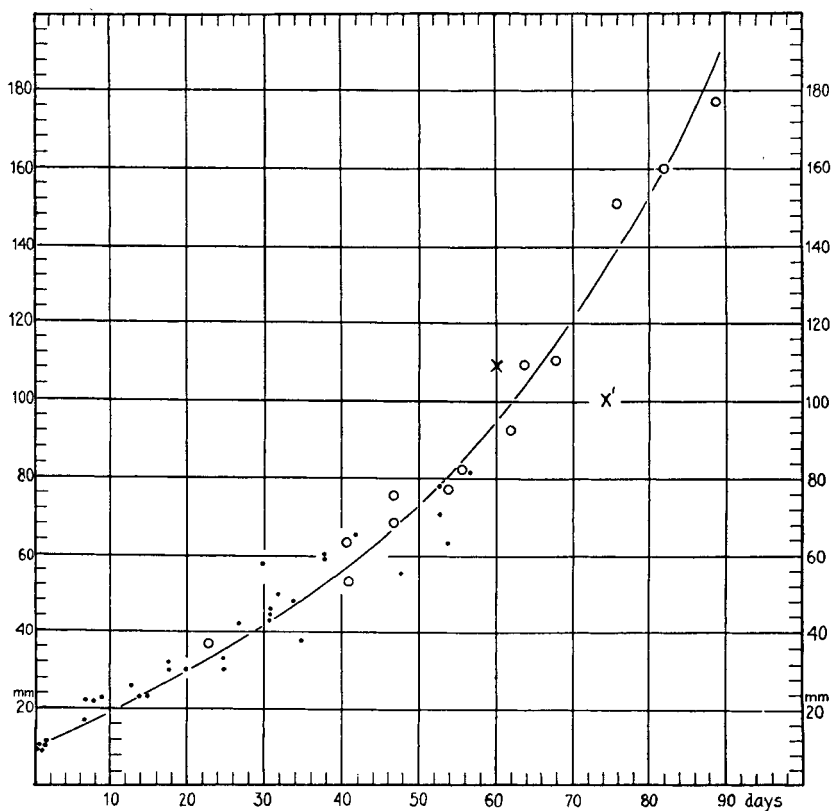
Text fig. G. Growth curve of the opossum pouch young, weight on age, for the first fifty-six days. Each dot represents the average of a litter (details given in appendix C). xx , Audubon and Bachmann's data, apparently wrong. $**$, data by Meigs, nine days, fourteen days (corrected).

these weights were reduced to length equivalents (107 and 100 mm., respectively) by the aid of text figure E. No other data on rate of postnatal development in the opossum are known to me.

It will be seen that the growth curve thus far closely parallels the first part of the growth curve of other mammals and

grizzly in the National Zoological Park at Washington, D. C., gave birth to two or three cubs (number uncertain), January 13, 1913. One cub died at five days of age; it weighed $9\frac{1}{7}$ ounces. The mother weighed 390 pounds the year before. A Yellowstone Park black bear at the same Zoo. lost one of its two cubs the day after its birth, when it weighed 9 ounces (Baker, Smithsonian Misc. Coll., vol. 45, p. 178, January 7, 1904). A European brown bear, *Ursus arctus*, at the Philadelphia Zoological Park, thirty-six hours after its birth, weighed $12\frac{3}{4}$ ounces (Forest and Stream, p. 84, Feb. 4, 1899).

man.⁵ In other words, the opossum is still long after birth, to all intents and purposes, an embryo, although leading, as Doctor Meigs has grandiloquently put it, "a chylopoietic, warm-blooded, oxygenating, innervating, and free-willing life."



Text fig. H Growth curve of the opossum pouch young, length on age, for the first ninety days. Dots represent averages of litters. Circles are individuals measured by Doctor Langworthy, who kindly turned the data over to the writer. x' , Meigs' case: seventy-six days, 100 mm., calculated length equivalent; x , Barton's case: sixty days, 107 mm., calculated length equivalent.

⁵ Chapters 1 and 2 of Pearl's "The biology of population growth" gives a good summary of "How things grow." It contains numerous normal growth curves for various plants, animals, and populations.

THE FIRST APPEARANCE OF HAIR

The opossum young are born naked as tiny red flesh-like masses. In several days the color changes to a healthy pink, or typical flesh color. My earliest record of 'hairy' young concerns the pouch young of no. 116, forty-two days old, weighing 9.0 grams and measuring 65 mm. However, examining the alcoholic specimens of litter no. 593 a, less than 5.0 grams in weight, thirty-four days old, I find that these are already provided with down easily visible under the hand lens. The hair is barely visible in the pouch young of no. 861, thirty-one days old. The 15-gram pouch young of no. 883, fifty-three days old, have hair that is distinctly pigmented with the color characteristic of the species and the color phase of the animal.

PERIOD OF ATTACHMENT TO THE TEAT

Barton states that the young are continuously attached to the teat for fifty to fifty-two days, when they are about the size of a common house mouse. In the case of the female kept under observation by Meigs, the young remained 'undetached' for seventy-four days (two days are added to the figure given by the author); but two days later, one embryo, with eyes slightly open, was crawling on the body of its dam. Audubon and Bachmann state that in four weeks the young relax their hold and peep out of the pouch; in five weeks they crawl on their mother's back. It is needless to add that here again these naturalists are mistaken in their estimates of age.

Between the estimates of the last-named authors and Meigs there is a 100 per cent discrepancy; between the observations of Meigs and Barton, 40 per cent. The latter I regard within the limits of normal variability, if I may judge, for example, from my own data on the time the eyes open. In certain crucial cases, no. 905, 907, and 1007, the notes fail to state whether the young were attached or not. However, the young of no. 876, fifty-seven days old, weighing 12.5 to 18.3 grams, were still attached; the young of no. 150, about the size of mice (25 grams, 95 mm.), were running freely about on the mother.

In general it may be said, then, that the young begin to leave the teat for the first time when about the size of a house mouse and between fifty-two and seventy-four days old. This relates to animals kept in captivity.

The question is sometimes asked whether the pouch young, if removed from the teat by the experimenter before the time intended by nature, can find their way back and get hold again. This must be answered in the affirmative. Audubon and Bachmann tested this out by experiment and found that not only a mother's own young, but even the larger young of another female would attach themselves anew. I removed newly born young and found they could reattach themselves; indeed, one young found the teat three successive times. On one occasion the two large, hairy young of one female were found missing; one was crawling about in the cage, the other had found a foster mother. The free individual was placed on its mother teat, but would not take hold while held by the observer's hand, hence it was dropped into the pouch. An hour later, it was found attached to the teat.

OPENING OF EYES AND MOUTH

The mouth of the embryo is at first a wide cleft which gradually closes toward the end of gestation until only a circular orifice is left for the reception of the teat. Later the lips form and separate like the eyelids and about the same time. But there is great variation in this respect, as suggested above. An inspection of the data presented in Appendix C will make this clear. Thus, for example, litter no. 883, weighing about 15 grams, is made up of individuals with eyes open, closed, or one eye open, the other closed. The individuals of litter no. 1007 have the eyes still closed, although they were over 27 grams in weight—nearly double that of the preceding whose eyes were in part open. Within a single litter there may be great discrepancies in both size and differentiation, as, for example, in litter no. 876, in which the smallest member, weighing 50 per cent less than its fellows, had its eyes and mouth open, whereas the largest member had its eyes still closed, its mouth open on one side only.

WEANING AND INDEPENDENCE

After leaving the teat, the pouch young does not yet leave the mother, but clings to her fur and makes periodic visits to the mammae for nursing. At the approach of danger the young scamper into the pouch, wherein the mother encloses them with the sphincter as well as possible, in consideration of the size of the litter and the individuals comprising it. Lactating young are sunned at times by the mother and may be seen, as I have, crawling about the mother while she lies curled up in sleep. Doctor Barton stated that even after the mother gives birth to a new litter she "does not withdraw from them (the older litter) her useful attention and assistance." Audubon and Bachmann believed that the young are only two months with the mother, but continue in the vicinity till autumn. "In the meantime," they say, "a second and often a third brood is produced and thus two or more broods of different ages may be seen, sometimes with the mother, and at other times not far off." Parenthetically, attention may again be called to these authors' low estimate of the rate of development in the opossum. They give no specific cases to warrant the exaggerated statement quoted above. They cite an interesting case, however, of a female that had in her pouch five very small young, while seven others, about the size of full-grown rats, were detected peeping from under rubbish. Other similar cases might be cited as follows:

Barton secured on May 14th a female that had five young as large as a two-thirds-grown rat; seven days later, she gave birth to a new litter of seven young. I doubt not that the older litter had been weaned some time before the birth of the second litter. This case is paralleled by my own no. 1001, which on May 1, 1925, was encumbered with a litter of six young the size of rats (140 to 155 grams in weight), while in the pouch was a newly born young and signs of the former presence of others. The older litter had been weaned some time before, for the teats had greatly retrogressed and returned to a size consistent with the reception of the new litter. In this connection the specimen reported by Meigs is

of interest. In 1833, he captured at Camden, New Jersey, a female with five young, each as large as a half-grown rat and still unweaned. He saw them "take the teat, and creep on the mother's back and muzzle, and hold on by means of the prehensile tail, wrapped round her ear, or round her leg."

From the above-cited cases it seems clear that weaning takes place a considerable time after the young attain the size of mice, and some time after they reach the size of a half-grown rat. I should add that this happens when the young are at least two and one-half months old.

Another case should be mentioned in this connection, that of female no. 150 (Appendix C). April 17, 1915, she had young about 95 mm. long, running freely about her. Thirty-eight days later, the young had attained the size of rats; their estimated age at this time was one hundred days. It is probable that in a state of nature these young would have begun an independent existence. Certainly, the young of no. 939, measuring 250 mm. in snout-rump length (about 350 grams in weight), had been independent for a week when killed. Such litters keep together ('den-up' together, as hunters would say) for some time, as Audubon and Bachmann described them. The records of the Biological Survey contain a number of just such references. A photograph of such a young opossum may be seen on page 276 of the National Geographical Magazine, vol. 52, no. 3, September, 1927. According to the legend, there were five of these young opossums together, the other four having escaped, which renders it likely that in this case also the litter was held together as a family by the mother.

SUMMARY

The newborn opossum weighs about 0.13 gram. An average litter might weigh one one-thousandth of the body weight of the mother. The growth curve for weight is given for fifty-six days, for length for ninety days, and is, for the period covered, the shape of an embryonic growth curve of higher mammals and man.

The eyes and mouth open as early as the fiftieth day, when the pouch young are as large as small mice or may remain closed considerably longer—until the size of a large house mouse. They are weaned when about eighty days of age. Soon thereafter the mother may again become pregnant with a second litter, but the weaned young still remain with the mother for some time. It probably takes at least ninety days to raise a litter of young to a state of independence.

DISCUSSION

In studies of the kind here undertaken one is struck by the extent of the variations in rate of development involved, which are, of course, only expressions of the heterozygosity of germ cells. Rate of development differs from one race to another and, since individuals of no large litter are ever exactly alike, the rate differs from one individual to another. Note, for example, the variation within litters of pouch young as given in the second part of this paper. Hence, data on rate of intra-uterine development will never be quite 'satisfactory' in the sense of being exactly predictable, or enabling the collector to secure with certainty the precise stage that he may desire. The greatest source of error, however, in making a Normen-tafel for the mammal lies in the difficulty thus far experienced in determining the exact hour of conception or fertilization of the ovum.

As pointed out, embryos are usually dated from the time of copulation. But the heat period may last days or even weeks; so that between the first and the last copulation as many as seventeen days have been recorded (Bischoff, '45). What a percentage of difference this may make in calculating the age of a two-day embryo! But this percentage may be very high in the case of the younger stages and the physiological events that center around ovulation, even in those animals that exhibit brief heat periods. The method has proved so elusive that numerous authors have timed their specimens from the hour of parturition in forms (the laboratory rodents) in which ovulation is known to follow close onto

parturition. But the relation of heat or oestrus to ovulation is itself not fixed and is subject to variations common to all physiological processes, and these time relations vary between large limits.

In this connection I may hazard three suggestions as to how this problem may in part be met. In the first place, the species used for the experimental work should be favorable. If it is selected on the basis of availability and the anatomy and physiology of the reproductive organs, the rabbit, rat, mouse, guinea-pig, and opossum are the only species worthy of consideration at present. The rabbit has the advantage of ovulating almost exactly ten hours after copulation, as has been known since Coste began his excellent work in 1834; and this explains the agreement among workers on this species. The rabbit differs from most mammals in that the female will accept the male and is able to ovulate over long periods, for the ripe graafian follicles appear to remain quiescent and 'ready' for a very considerable portion of the oestrous cycle. The rat, the mouse, and the guinea-pig, as well as the opossum, have definite oestrous periods easily studied by Stockard and Papanicolaou's vaginal-smear method now in universal use; by this method the events of the cycle, including ovulation, may be approximately determined and the next step greatly facilitated. These rodents, furthermore, ovulate and accept the male soon after parturition.

The second step suggested consists of verification by laparotomy; if necessary, by successive laparotomies. These need not seriously affect the reproductive capacity of the animal (Hartman, '19; MacDowell, '27; Corner, '27). And finally we must return to the method of Bischoff, described in part II of this paper, namely, of surgically removing a uterus or portions of the uterine horns and noting the progressive development in the surviving egg chambers. Furthermore, in any mammal two cleavage stages might be secured, with accurate intervals, by successive removal of the fallopian tubes. It is believed that such material, timed to the minute, is more valuable than the same quantity or more

material based on copulation ages of different animals. It is this method that has furnished the greater part of the data on which the intra-uterine development of the opossum, as above set forth, is based.

Finally, it has been shown that intensive experimentation within closely defined limits is needed on the early ontogeny of mammals, such, for example, like the recently published study on the growth curve of the mouse by MacDowell, Allen, and MacDowell.

SUMMARY

In the first part of this paper the optimum time for collecting pregnant opossums at Austin, Texas, is discussed. The breeding season begins in January after a three months' anoestrous period, and in the third and fourth weeks the modal point for the number of conceptions is reached. After this period, most of the animals captured in the field are pregnant or have pouch young.

The rate of intra-uterine development (part II) was investigated chiefly by the method of surgical removal of one uterus, noting the stage of eggs or embryos which it contains, and allowing the surviving uterus to incubate its ova a precalculated period of time. Unique charts (text figs. C and D) allow the reader to see the development of different intervals at a glance. Thirteen days is probably near the truth for the average period from copulation to birth; twelve and one-half days for the actual prenatal development. Comparison is made with other mammals concerning the time when certain well-defined stages are reached—information which has been brought together here probably for the first time. It is shown that the rate of development of the opossum for the first ten days, at least, is most like that of the rabbit, representative of one of the most primitive orders of Eutheria. The primitive-streak stage is reached in seven and one-half days, which leaves but five and one-half days for the actual development of the embryo itself to the time of birth.

The curve of postnatal development has the shape of the embryonic growth curve of the higher mammals, for birth is so early in ontogeny that it does not constitute the momentous physiological event as in the higher mammals. The pouch young begin to open their eyes and lips when about fifty days old and when they have attained the size of a full-grown house mouse. At this time they let go of the nipples for the first time, but continue to nurse for thirty days more. Even then they take advantage of the protecting care of the mother until they attain the size of the full-grown Norwegian rat, when they are ready to shift for themselves. The mother again becomes pregnant soon after weaning the first brood. It therefore requires quite all of three months to rear one brood of young. It is doubtful if a given female can rear more than two broods a year, except in rare instances, since the last three months of the calendar year constitute the anoestrus.

APPENDIX A

Extracts from the protocols of animals furnishing data for the construction of charts 1 and 2 (rate of intra-uterine development) and not mentioned in my paper of 1919 (*Jour. Morph.*, vol. 32, pp. 8-16). It was deemed unnecessary here to repeat the protocols previously published. The new material here presented was collected since 1919 or concerns only stages from the primitive-streak stage on. ('Chart 1' = text figure C; 'chart 2' = text figure D.)

No. 114. Copulation observed 10.50 to 11.00 A.M., February 6, 1915. February 11th, 2.30 P.M., a laparotomy was performed; no eggs were found. February 15th, 10.00 A.M., 11-somite embryos (chart 1).

No. 134. Two sets of embryos, sixteen hours apart, were removed from this female, the first litter nine days after copulation. A representative of the second litter is seen in figure 20.

No. 207. January 29, 1916, five vesicles about 4.2 mm. in diameter and five unfertilized eggs. Sixty hours later, three vesicles, 11 mm. in diameter, and seven dead vesicles or unfertilized eggs. The normal vesicles contain 4-mm. embryos, according to the notes. These specimens are not entered in the charts.

No. 211 (*Didelphis marsupialis* from San Benito, Texas). February 2, 1916, thirteen blastocysts in early primitive-streak stage, no unfertilized eggs. Measurements are as follows:

| | | | | | | | | | | | |
|------------------|-----|-----|------|------|------|------|------|-----|------|------|------|
| In salt solution | 2.5 | 2.5 | 2.36 | 1.78 | 2.3 | 2.0 | 2.6 | 2.3 | 2.26 | 2.24 | 2.13 |
| In fixing fluid | 2.8 | 2.6 | 2.50 | 1.75 | 1.95 | 1.86 | 2.45 | 2.3 | 2.28 | 2.15 | 2.05 |

Twelve and one-half hours later, vesicles with pear-shaped embryonic areas and short medullary grooves. They measure as follows (chart 2):

| | | | | | | | | |
|------------------|-----|------|------|------|------|------|------|------|
| In salt solution | 3.6 | 3.52 | 3.52 | 3.46 | 3.37 | 3.37 | 3.26 | 2.8 |
| In fixing fluid | 3.3 | 3.2 | 3.16 | 3.16 | 3.06 | 3.06 | 3.02 | 2.65 |

No. 284. January 13, 1917, four vesicles, measuring 4.5 to 4.7 mm. Fourteen and one-half hours later, ten vesicles, measuring 9.5, 9.0, 9.0, 8.9, 8.7, 8.7, 8.7, 5.5, 4.0 mm. The former set are comparable to 298', 344, and 356'; the latter contain embryos with 8 to 10 somites. These specimens are not entered in the charts.

No. 298. Figure 29 is introduced to show the stage of advancement reached three and one-half days after the 60 to 120-celled blastodermic vesicle (Hartman, '19, p. 13, for protocol). (Chart 1.)

No. 314. Figure 13 shows an embryo about nine days after an observed copulation (chart 1).

No. 334. January 27, 1917, eleven vesicles, 2.75 to 3.0 mm. in diameter, slightly elliptical embryonic areas, late primitive-streak stage, no medullary groove, mesoderm has reached the anterior margin of embryonic area. Twenty hours later, one defective 5.0-mm. vesicle with embryo of 6 somites and two unfertilized eggs. The vesicle is small in proportion to the differentiation of the embryo (chart 2).

No. 337. Figure 26 shows one of the larger eggs photographed fresh in utero and shown in figure 10, Hartman ('19). The mesoderm has almost reached the anterior margin of the formative area (the light streak is free of mesoderm). This represents four and one-half days' development after the sixteen-celled stage (chart 1).

No. 340. Six and one-quarter hours' development represents, in two batches of vesicles from this female, an increase of about 2 somites (twelve to fourteen), and an easily recognizable increase in differentiation of brain contour; the otic vesicles also appeared in the interval. These specimens are not entered in the charts.

No. 344. Figure 28 is introduced to show the spread of the mesoderm, the primitive streak, and short medullary groove, a stage reached three and one-sixth days after the early proliferation of entoderm, as detailed in Hartman ('19, p. 15) (chart 1).

No. 346. See figures 21 and 24; also Hartman ('19, p. 15).

No. 349. The notes given for this specimen, Hartman ('19, p. 16), need correction in so far as the vesicles at the second operation (three and one-third days after the first) measured 8 mm. The eggs are no longer extant for examination. The embryos had only a few somites (chart 1).

No. 353. See figures 22 and 23; also Hartman ('19, p. 16).

No. 356. January 30, 1917, young vesicles almost identical with those of no. 544 (fig. 1) and fully described in Hartman ('19). Three days three and three-quarter hours later, the ova measure 4.9, 4.6, 4.25, 4.4, 3.25, 1.5 mm. (the last two moribund). The stage is almost identical with no. 298', shown in figure 29.

No. 360. January 30, 1917, bilaminar blastocysts, about 1.5 mm. in diameter (Hartman, '19, pl. 8). Three days later, 5.75-mm. embryos like that shown in figure 14. This interval has been added to that of no. 306 of chart 1, for no. 306' is almost identical with no. 360.

No. 377. February 1, 1917, the 5- to 6-mm. vesicles; medullary plate only a little less developed than in no. 356' and 298'. Thirteen hours later, the embryos have about 10 somites and brain with optic pits (chart 2).

No. 380. February 3, 1917. 3.0- to 3.5-mm. vesicles with pear-shaped formative areas; the mesoderm has reached the anterior border of the area (fig. 15). Ten and three-quarter hours later, the formative area is slipper-shaped, the medullary plate is longer than the primitive streak; the first somite is in process of differentiating (fig. 19). Figure 16 shows this batch of eggs photographed fresh in utero (chart 2).

No. 486. January 29, 1921, eleven vesicles recovered, the normal ones measuring about 3 mm.; the primitive-streak stage, formative area pear-shaped, no medullary plate (fig. 17). Twenty and one-half hours later, embryos are in the 6-, 7-, or 8-somite stage. One of these is reproduced in figure 18 (chart 2).

No. 544. January 22, 1921, nineteen eggs removed, of which several are shown in figure 1. The vesicles are just a little earlier in development than those of no. 356. Seven days later, "large fetuses, 3 slightly hemorrhagic and one dead." These are no longer extant for examination. A photograph of the uterus with hernia of the mucosa pushed out by a fetus speaks for embryos of about the same stage as no. 549', shown in figure 2 (chart 1).

No. 560. This repeats the story of no. 486 almost exactly. The nine vesicles removed January 24, 1921, are slightly more advanced than those of no. 486, the mesoderm having spread beyond the limits of the formative area and the medullary groove having just begun (fig. 27). An eighteen-hour interval brought the embryos to the identical stage attained by 486' (fig. 18) (chart 2).

No. 561. January 24, 1921, eight eggs in the sixteen-celled stage (fig. 3, pl. 1). Seven and one-half days later, embryos with 7- to 9-mm. allantois, about two days of term. If in our chart the sixteen-celled stage is correctly placed at three and one-half days after ovulation, this period added to the interval of seven and one-half days gives us eleven days, or two days short of a thirteen-day gestation period for the embryo shown in figure 4 (chart 1).

No. 578. January 26, 1921, six vesicles, about 3.5 mm. in diameter removed. These have pear-shaped embryonic areas and long primitive streaks. Twenty-four and one-half hours later, the embryonic area was slipper-shaped, the embryo contained 6 somites (chart 2).

No. 580. January 27, 1921, eight vesicles, 4.0 to 4.5 mm. in diameter, primitive-streak stage. Both ovaries were removed at this time for experimental purposes. Four days nineteen hours later, the embryos had attained a stage approximately like those of no. 841. The specimen was not used in the charts.

No. 582. Nine 0.7-mm. blastocysts recovered, January 27, 1921. Considerable albumen is still left between the trophoblastic portion of the vesicle and the shell membrane (fig. 9). Both ovaries were removed at this time. Two days eighteen hours later, the vesicles (practically normal still) shown in figures 10 and 11, 8.4 to 9.0 mm. in diameter, embryos with about 6 somites were removed (fig. 12) (top of chart 2).

No. 585. January 28, 1921, young bilaminar blastocysts, 0.75 mm. in diameter, but slightly in advance of no. 582 and about the same as no. 293', were found. Left uterus and right ovary were removed. Five days less three hours later, 7.5-mm. embryos were recovered (fig. 6). Because of the similarity of the first

stage of this specimen and the second stage of no. 293, this interval has been added to that of 293 in chart 1.

No. 829. January 20, 1922, nine embryos with 2-mm. allantois. Both ovaries were removed at this time. Four and one-half days later, four embryos, slightly hemorrhagic, near term. This specimen is not utilized in the chart.

No. 838. April 1, 1922, oestrus was recognized in this animal for the third time. She was kept with a male. April 8th, thirty eggs were removed from one uterus, thirty-six from the other—a total of fifty-six—mostly excellent bilaminar blastocysts nearly 1.0 mm. in diameter. This is probably the record for normal ovulation in the history of mammalogy! In this case seven days' development postcoitum seems to be pretty nearly correct.

No. 841. January 21, 1922, removed left ovary and right uterus. Uterus contained fifteen ova (fig. 7) in late cleavage stages. Eight days fifteen hours later, four normal embryos within about two days of term (fig. 8) (chart 1).

No. 927. January 31, 1924, eggs in about the same stage as no. 544 (fig. 1): small vesicles with early polar differentiation. Both ovaries were removed with the left uterus. Eight days later, embryos with 3-mm. allantois. They are no longer extant for exact examination, though most like litter no. 549, from which the sketch shown in chart 1 was made (fig. 2). The embryos were still alive when removed, but may have been a little retarded because of the ovariectomy.

No. 931. January 29, 1924, removed right ovary and left uterus. From this eggs about like litter no. 294 were recovered (compare Hartman, '19, fig. 2, pl. 6, and fig. 1, pl. 11). Seven days later, embryos near term. The sketch in the chart was made from another litter near term, however, since embryos no. 931 were given away and no record of measurement could be secured from the recipient.

No. 969. February 7, 1925, ova in about the sixteen-celled stage. Seven days later, young embryos like those of no. 360 shown in figure 14.

APPENDIX B

PROTOCOLS OF ANIMALS WHOSE RECORDS FORM THE BASIS OF TEXT FIGURES G AND H

The protocols given in this appendix are presented in order that a definite idea may be given of the character of data upon which the first ninety days of postnatal growth is based. In no case, except no. 443, was a birth actually observed; but in a number of cases the birth was known within a few hours. A few cases are included in which an estimate of a week or ten days was made on the age of the pouch young when first discovered, and this involves some error, inasmuch as I had but a hazy notion as to the age of pouch young when this compilation was undertaken in 1927, two years after making the last collection of opossums. However, such pure estimates are only included in which the percentage of error finally is very slight, that is, the error of a day or two at the beginning of a long interval really constitutes an unimportant proportion of the total

length of time under consideration. Lengths are from snout-rump measurements. Where no sex is indicated, none had been recorded.

No. 116. February 1, 1916, pouch young just born. February 11th, eight young in pouch, two removed. March 2nd, removed a male pouch young (49 mm.; estimated weight, 4.5 grams) and a female (48 mm.; estimated weight, 4.5 grams); age, thirty-one days. March 14th, young measure 65 mm. (estimated weight, 7.0 grams). Young are hairy, testes well descended; age, forty-three days.

No. 517. Nine pouch young put down in the notes as ten days old, January 20, 1921. One measures 17.0 mm., another, 20.0 mm. The estimated weight of these is about 0.45 gram.

No. 528. Large female received January 22, 1921; by mammary palpation she was diagnosed as in late stage of pregnancy. July 9th, nine pouch young are found, of which six weigh 10.1 grams, or 1.7 gram each; length, 30 mm. The young are fifteen to eighteen days old. The age is plotted as of eighteen days, which seemed, all points considered, the probable age.

No. 552. Received January 25, 1921, in early stage of pregnancy. February 1st, one pouch young, about a day old. February 12th, young weighed 0.851 gram. Age of pouch young estimated at thirteen days.

No. 569. January 24, 1921, near oestrus; February 1st, pregnant. February 4th, one pouch young found, size not recorded, probably less than day old. February 19th, male pouch young weighed 0.65 gram; fifteen days old.

No. 591. February 5, 1921, pouch young 'about week old.' February 23rd, four young weighed: ♀, 2.35 grams; ♀, 2.20 grams; ♂, 2.29 grams; ♂, 2.29 grams; average, 2.3 grams; age, twenty-five days.

No. 593a. February 5, 1921, six pouch young, estimated at a little better than two weeks old. February 23rd, the young, all males, weighed 5.05, 4.97, 4.87, 4.87, 4.55, 4.43 grams. In alcohol they measure about 43 mm., perhaps equivalent to 45 mm. in life. Age, about thirty-four days.

No. 608. February 1, 1921, pregnant. Night of February 4th to 5th, parturition. February 10th, pouch young, a week old, observed, but not measured. March 11th, five young are left; these are killed and their heads removed for special brain fixation. Their preserved bodies are carefully compared with intact specimens and are found to be nearly the same size as batch no. 633, which weighed in the living state as follows: ♀, 3.16 grams; ♂♂, 3.52, 3.27, 3.21, 3.20, 2.92 grams; average, 3.22 grams. Five males measured about 37.5 mm.; age, thirty-five days.

No. 614. February 1, 1921, very small, red pouch young, not over a day old. February 10th, three 'good-sized' pouch young observed. February 20th, pouch young are dead; one weighed 1.5 gram. Age, twenty days.

No. 619. Large female, received February 2, 1921. Mammary palpation showed the animal to be in early stage of pregnancy, about ten days before parturition. February 20th, nine pouch young are seen; March 7th to 23rd, seven only; March 24th, the young, now forty days old, were killed. The weights were as follows: ♂♂, 7.15, 6.95, 6.51 grams; ♀♀, 7.60, 7.57, 7.01, 6.68 grams.

No. 624. February 4, 1921, received with pouch young at least a week old; February 16th, nine large young present. March 5th to 25th, six only left; March 26th, pouch young lost with the exception of one dead and one living. The latter weighed 8.5 grams; this was at least fifty-seven days old.

No. 636. February 5th, ten pouch young at least a week old. March 24th, the ten young weighed: ♂♂, 8.45 and 6.05 grams; ♀♀, 9.37, 9.25, 8.81, 8.44, 8.14, 7.89, 7.59, 7.19—an average of 8.8 grams for fifty-four-day-old pouch young.

No. 648. February 10, 1921, six pouch young about four days old. March 10th, the six young weighed 21.4 grams altogether—an average of 3.57 grams; length, 50 mm. Both mother and pouch young seemed rather lean. Age, thirty-two days.

No. 747. Received January 14, 1922. January 22nd, one pouch young two days old; January 28th, aged eight days; it weighed 0.64 gram.

No. 749. Pouch young born January 19, 1922. January 28th, at age of nine days, two were removed weighing 0.45 and 0.5 gram, respectively. February 2nd, at age of fourteen days, three pouch young weighed 0.81, 0.735, 0.61 gram; average, 0.722 gram. One female measured 23 mm.

No. 788. Received January 9, 1922, having been trapped, foot lacerated. Animal in early stage of pregnancy. January 16th, she was placed in a table drawer about 6 inches deep, where she gave birth during the night to a litter of ten young. Only one succeeded in attaching to a nipple; it weighed 0.16 gram. The other nine were dead and weighed together 1.2 gram, or an average of 0.133 gram each.

No. 824. January 20, 1922, received when in early stage of pregnancy. January 30th, mammary glands very thick and turgid, as when near term. March 24th, three pouch young, fifty-two days old, measure 68 mm. each and weigh 15.9, 14.9, 14.4 grams; average, 15.1 grams.

No. 861. January 22, 1922, pouch young two days old. February 20th, their weights and measurements were as follows: ♀, 4.23 grams; ♀, 4.02 grams; ♂, 3.98 grams (46 mm.); ♂, 3.95 grams; ♂, 3.52 grams; ♀, 3.33 grams; and two others, sex not recorded, each 3.9 grams in weight; average of all, 3.82 grams; age, thirty-one days.

No. 863. January 22, 1922, pouch young seventeen days old. February 1st, one pouch young, aged twenty-seven days, weighs 3 grams.

No. 876. Pouch young born January 30, 1922. March 24th, at the age of fifty-three days, one young was removed; its weight was 15.1 grams. At fifty-seven days (March 28th), five others were removed, weighing: ♂, 18.3; ♂, 18.0; ♂, 17.5; ♂, 16.6; ♀, 12.5; average, 16.6.

No. 883. Young born January 17, 1922. March 6th, at age of forty-eight days, two were removed; weights, 8.85 and 8.50 grams; length, 55 mm. At fifty-three days of age (March 18th), the following were removed: ♀, 15.6; ♀, 15.4; ♀, 14.9; ♂, 14.4; ♀, 13.4 grams. They measured 66 to 70 mm.

No. 884. January 24, 1922, eight pouch young were about five days old. Removed them February 4th. Their weights were as follows: 0.96, 0.94, 0.91, 0.91, 0.91, 0.9, 0.9—a very uniform series. Average weight, 0.92 gram; age, seventeen days.

APPENDIX C

Data concerning opossum pouch young 15 grams or more in weight, arranged in order of size.

No. 148. Length, 63 mm.; estimated weight, 15.0 grams. March 30, 1915.

No. 1002. Large litter, May 1, 1925. One young weighed 12.5 grams, a female, 15.0 grams.

- No. 883. Fifty-three days old, March 18, 1922. Average weight, 14.7 grams.
1. ♀, 13.4 grams. Eyes closed; lips separated further on left side than right.
 2. ♂, 14.4 grams. Eyes?; lips separated.
 3. ♀, 14.9 grams. Both eyelids and lips separated.
 4. ♀, 15.4 grams. Right eye open, left closed; mouth open.
 5. ♀, 15.6 grams. Eyes closed; mouth open.
- No. 824. Fifty-two days old, March 24, 1922. Average weight, 15.1 grams.
1. ♂, 14.1 grams. No description.
 2. -, 14.9 grams, 68 mm., both eyes open in slits, lips separated.
 3. ♂, 15.9 grams. One eye open, lips separated.
- No. 876. Fifty-seven days old, March 28, 1922. Average weight, 16.6 grams.
1. ♀, 12.5 grams. Eyes open (slits); mouth open.
 2. ♂, 16.6 grams. Eyes closed; mouth open.
 3. ♂, 17.5 grams. Eyes closed; lips partly separated.
 4. ♂, 18.0 grams.
 5. ♂, 18.3 grams. Eyes closed; mouth open on one side only.
- No. 139. 75-mm. pouch young (estimated weight, 16 grams), March 14, 1915.
- No. 905. July 2, 1923, 85-mm. pouch young (estimated weight, 21 grams).
- No. 907. July 17, 1923. Weights: 20.4, 21.1, 22.6, 23.5 grams. Eyes still closed, lips separated.
- No. 150. April 17, 1915, 95-mm. (estimated weight, 25 grams) pouch young running freely about the mother. May 25th, as large as rats. Estimated age, one hundred days.
- No. 998. March 25, 1925, pouch young the size of small mice, 95 mm. (estimated weight, 25 grams). Definitive hair color indicated.
- No. 1007. May 14, 1925. A very uniform series. Eyes not yet opened, although the weights are much greater than others that had eyes open, as, e.g., nos. 824 and 876. Females: 27 grams, 102 mm.; 27 grams, 102 mm.; 28 grams, 104 mm.; 27.5 grams, 100 mm. Males: 26.5 grams, 100 mm.; 27.2 grams, 100 mm.; 27.5 grams, 100 mm.; 28.0 grams, 100 mm.; 28.0 grams, 103 mm. Average weight, 27.4 grams.
- No. 1005. May 2, 1925. 30 grams. Eyes open.
- No. 1003. May 1, 1925. 31 grams. Eyes open.
- No. 940. April 27, 1924. A litter of eight, 45 to 51 grams (average, 48 grams) and 120 to 132 mm. (average, 126 mm.). This litter had been supplemented in nature by three larger young belonging to another female. The larger young weighed 101 grams and measured 170 mm.
- Nos. 66 and 67. End of March, 1915. 138-mm. pouch young (estimated weight, about 55 grams).
- No. 681. Caught May 25, 1921, in possession of two litters, her own of ten and a 'borrowed' one of about fourteen. Of the latter, two were said to have still had their eyes closed. This smaller litter escaped. The former litter ranged from 61.5 to 70 grams in weight; average, 66.6 grams. The mammary apparatus of the mother weighed 61 grams.
- No. 1006. May 3, 1925. Weights and measurements as follows: 78.75 grams, 151 mm.; 80.5 grams, 152 mm.; 87.0 grams, 155 mm. The young were weaned, the mother's nipples greatly retrogressed. Two young wandered away from the mother and joined female no. 1005, which had 30-gram young.
- No. 895. May 5, 1922: 1) ♂, 95 grams; 2) 95 grams, 158 mm.; 3) 157 mm.

No. 1009. June 14, 1925. The only litter of as many as fourteen pouch young that I have personally ever seen. With the mother, but no longer suckling. 1) 132 grams, 193 mm.; 2) ♀, 134.5 grams, 193 mm.; 3) ♂, 138.5 grams, 197 mm.; 4) ♂, 147.5 grams, 190 mm.

No. 1013. May 11, 1925. A litter of eight, average size, 145 grams, 185 mm.; except for one 'runt' of 92 grams, 140 mm. Range of the normal seven: 121 grams, 178 mm. to 165 grams, 203 mm.

No. 1001. This animal is remarkable for several reasons. She was a rather lean black female which, May 1, 1925, had six young the size of rats clinging to her and one small red pouch young, a day or two old, attached to a teat in the pouch; other nipples indicated the recent attachment of other newly born young that had been lost. The weight of the three large young: ♂, 140 grams; ♀, 155 grams; ♂, 155.2 grams. The color of the young was as follows: four were gray, the common phase of *D. virginiana*; one black, like the mother; one black, with a light sprinkling of white overhairs.

No. 939. February 15, 1924, caught by boys when the pouch young were 'the size of a pecan,' estimated by me to be at least twenty-one days old. When independent of the mother, the young were allowed to run free in the woods, but returned to the kitchen for food. April 28th, one of the young was brought to me, a male, 250 mm. in snout-rump length, aged not less than eighty-eight, nor more than ninety-five days.

No. 1008. Male, captured May 25, 1925; 290 grams, 244 mm.

APPENDIX D

Data concerning adolescent females, 920 grams or less in weight, in part entered in the chart, text figure A

| PROTOCOL NO. | WEIGHT (GRAMS) | SNOUT-RUMP LENGTH | REMARKS |
|--------------|----------------|-------------------|--|
| 226 | 660 | 345 | 10.5-mm. embryos, February 11, 1915. |
| 234 | 650 | 350 | Pseudopregnant March 17, 1915. |
| 275 | 503 | 310 | Prepuberty, January 5, 1917. |
| 276 | 601 | 325 | Prepuberty, January 5, 1917. |
| 278 | 651 | 320 | Prepuberty, January 5, 1917. |
| 281 | 590 | 290 | Prepuberty, January 5, 1917. |
| 321 | 900 | 375 | Fetuses, January 26, 1917. |
| 337 | 840 | 340 | Fertilized eggs January 28, 1917. |
| 339 | 900 | 350 | Fertilized eggs January 28, 1917. |
| 375 | 885 | 350 | Fetuses, February 1, 1917. |
| 474 | 867 | — | First oestrus, February, 1921. |
| 476 | 683 | — | First oestrus, end of February, 1921. |
| 497 | 920 | 297 | Early prooestrus, January 10, 1921. |
| 548 | 850 | — | Immature, January 23, 1921. |
| 671 | — | 'tiny' | 7-gram pouch young, March 24, 1921. |
| 684 | 373 | 245 | Immature, May 31, 1921 (estimated age, 4½ months). |
| 901 | 608 | 305 | Immature, January 20, 1923. |
| 1008 | 290 | 244 | Immature, May 25, 1925. |

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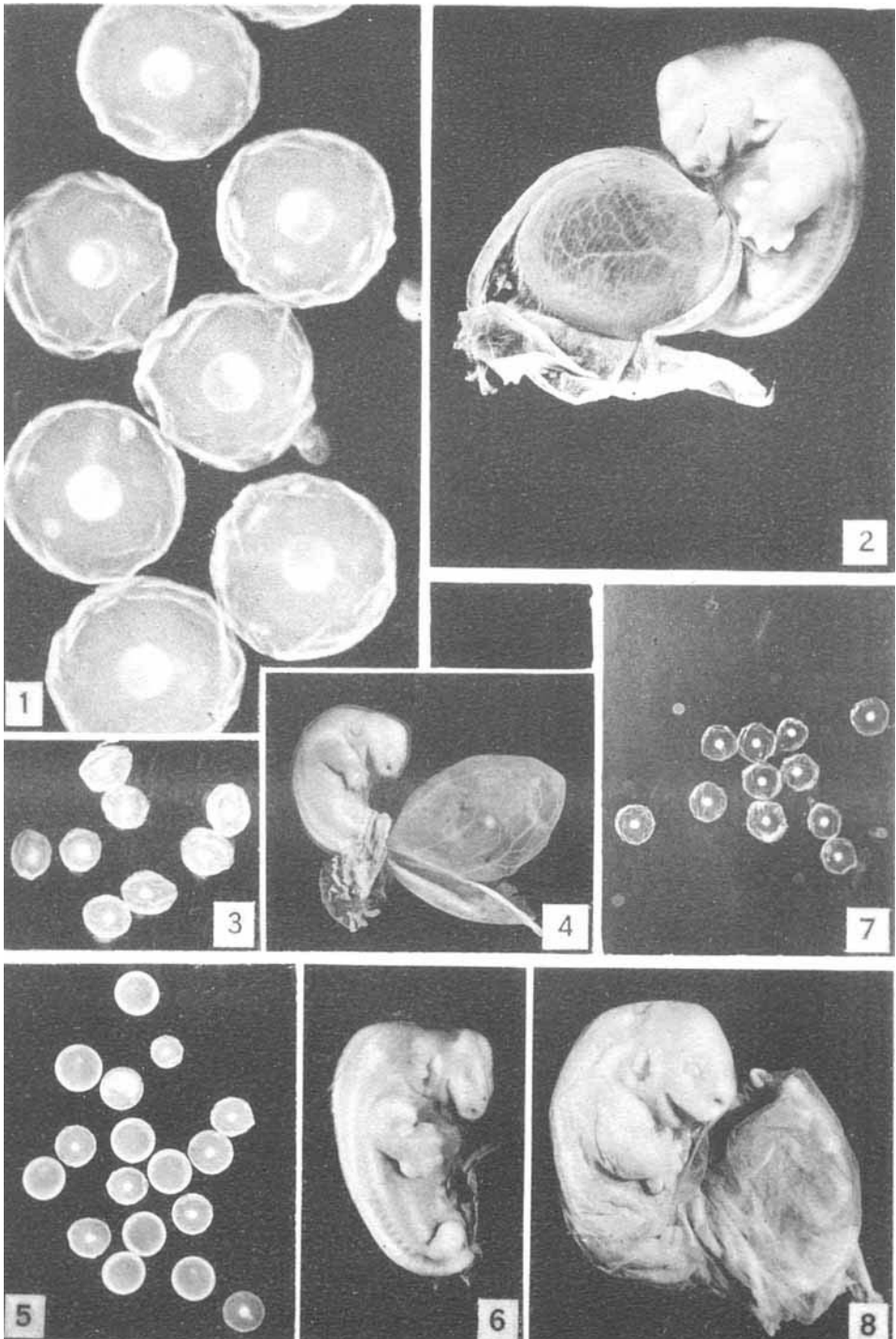


PLATE 1

EXPLANATION OF FIGURES

The eight figures in this plate illustrate four different developmental intervals as ascertained by successive removal of the two uteri of the same animal. In each of the four cases the embryos attained nearly the same stage of development at the end of the intervals. Figures 3 and 7 are cleavage stages of about sixteen cells ($\times 8$); figure 1, small vesicles with evident polarity, early entoderm formation ($\times 40$); figure 5, bilaminar blastocysts ($\times 8$).

- 1 and 2 No. 544, seven days' development.
- 3 and 4 No. 561, seven and one-half days' development.
- 5 and 6 No. 585, five days' development.
- 7 and 8 No. 841, seven and five-eighths days' development.

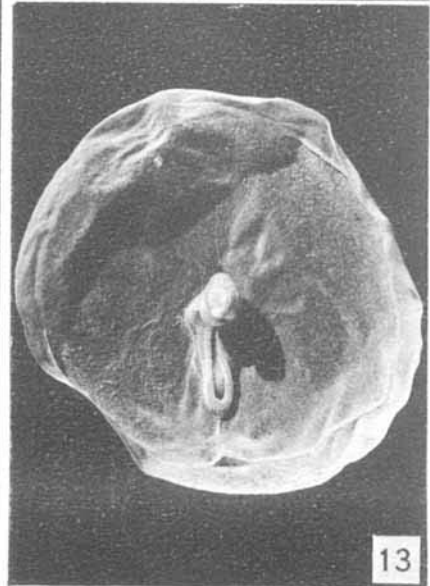
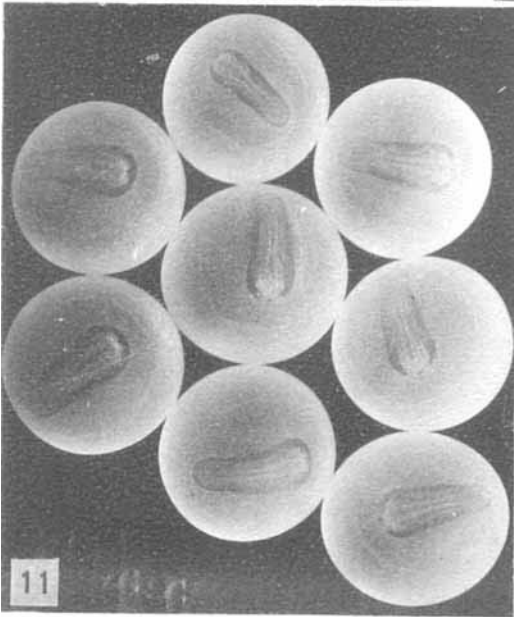
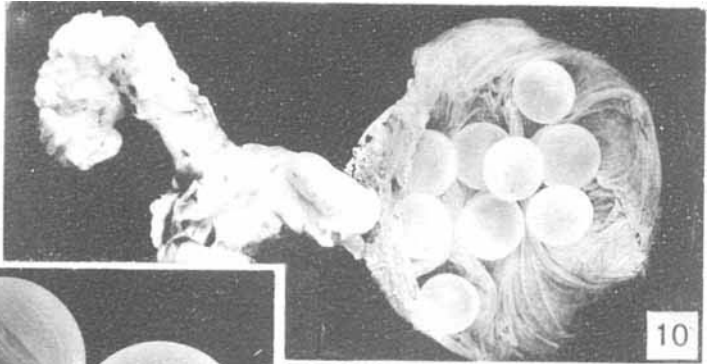
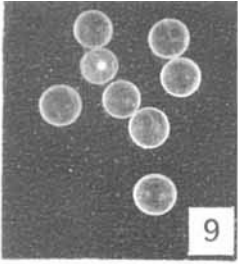
PLATE 2

EXPLANATION OF FIGURES

9 to 12 Relate to no. 582. Figure 9, bilaminar blastocysts, $\times 8$. Figure 10, the vesicles of the surviving uterus two and three-quarter days later, natural size; photographed alive in salt solution. One of the embryos with six somites is shown in figure 12, photographed by transmitted light in alcohol after cutting the lower hemisphere away ($\times 16$). Figure 11, ova of no. 795 of the same stage as 582', showing the embryos on the surface of the vesicles as they appear alive in salt solution ($\times 3$).

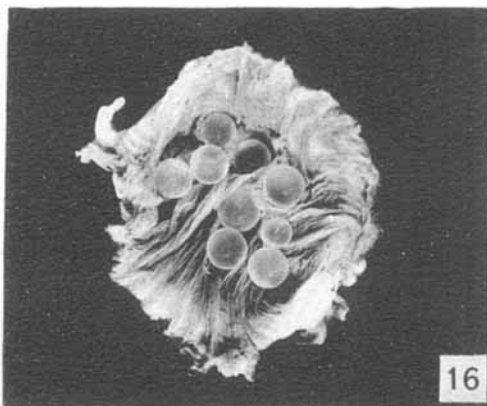
13 An embryo of no. 314', looking into the chorionic vesicle ($\times 4$). The head of the embryo is flexed as in figure 14, protruding into the vesicle. Nine days postcoitum, five and one-half days after the early vesicular stage.

14 An embryo of no. 360, three days after the 1-mm. blastocyst stage. The proamnion has been removed to the vitelline vein, which can be seen just caudad of the fore-limb bud. The heart and pharyngeal clefts are well shown. No allantois is as yet visible.

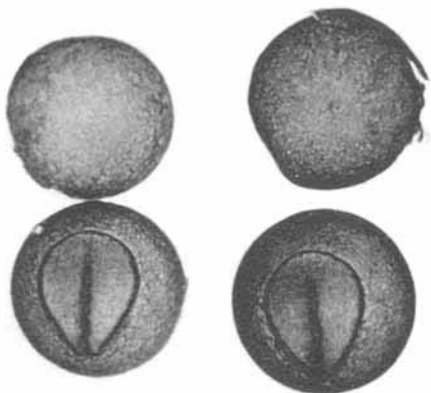




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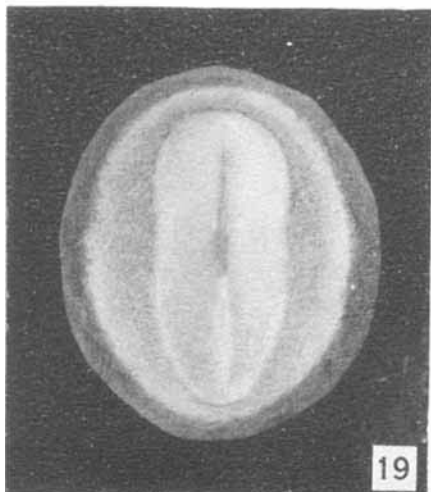
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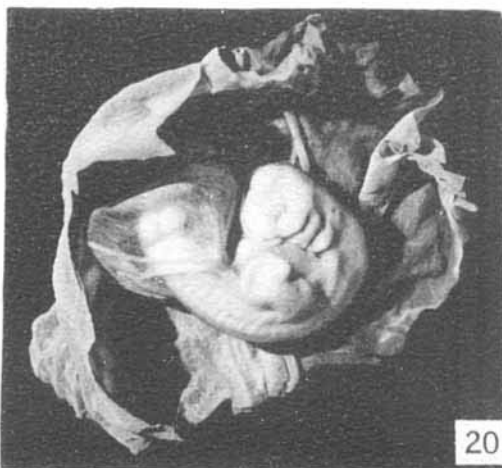
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PLATE 3

EXPLANATION OF FIGURES

15, 16, and 19 relate to no. 380. Figure 15 is one of the eggs recovered at the first operation ($\times 16$, photographed in alcohol by transmitted light). The egg represents the end of the 'proembryo,' the beginning of the actual embryo (beginning of the medullary plate). Yet in five and one-half days the embryo is destined to be born! In twenty and one-half hours the medullary groove has exceeded the primitive streak in length, as seen in figure 19. The embryo is slipper-shaped ('shoe-sole' embryo) and the first somites are about to appear. The photograph was made by reflected light. This egg is one of the litter shown in figure 16, photographed alive in the open uterus, natural size.

17 Two ova, formative and trophoblastic halves, of litter no. 486. $\times 16$.

18 The stage reached twenty and one-half hours later ($\times 8$). The limits of the mesoderm, the heart rudiment, somites, neural groove, and brain are well shown. The pair of lobes on the sides of the brain are strands of head mesoderm (compare Selenka, '87, Fig. 4, Tafel XX).

20 Embryo of no. 134', within its chorion. The anterior limb bud is well differentiated. A portion of the yolk sac covers the small allantois, ten days postcoitum.

PLATE 4

EXPLANATION OF FIGURES

Figures 21 to 29 represent altogether about one day's development, that is, from the seventeenth hour of the seventh day to the eighteenth or nineteenth hour of the eighth day (text fig. D). Text figure C gives the intervals involved in the eggs shown in figures 26 to 29. All photographs were made by transmitted light with the eggs in alcohol, the non-formative half of the eggs removed. $\times 16$.

21 and 24 No. 346. In figure 21 egg 346(1) is still in the bilaminar condition; in figure 24 the egg, 346'(2), has the mesoderm occupying an elliptical area about the primitive streak; the embryonic area is beginning to elongate.

22 and 23 Eggs 353'(3) and 353'(5). The former has twenty-seven mesodermal cells; the latter about 125, seen as a cloud in figure 23.

25 Egg 338(9). The spread of the mesoderm is well shown. No time relations are known for this egg.

26 Egg 337'(1), four and one-half days after the sixteen-celled stage. A small mesoderm-free area still persists at the anterior margin of the embryonic area.

27 Egg 560(1). Mesoderm has spread beyond the margin of the embryonic area; medullary groove is hardly begun.

28 Egg 344'(2). A short medullary groove is seen. This stage was attained (text fig. C) nearly three days after a late cleavage stage.

29 Egg 298'(3). Eighty-six hours after the early vesicular stage, i.e., after cleavage had just ended. Note the limits of the mesoderm, the embryonic area, the primitive streak, the medullary groove (light area), and the notochord (shadow under the medullary groove).



26



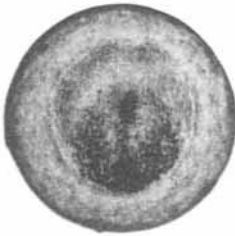
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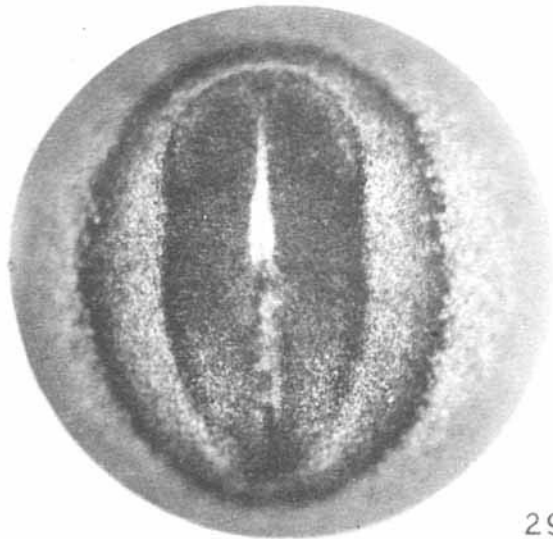
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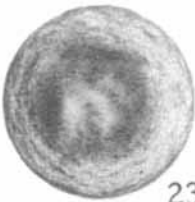
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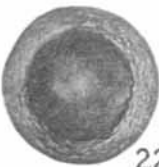
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