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THE EGGS OF MAMMALS

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THE EGGS OF MAMMALS

BY

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This Book Is Dedicated to

W. E. Castle and W. J. Crozier

PREFACE

I should like to express my appreciation to Dr. J. B. Collip, Dr. H. Selye, Dr. D. L. Thomson, and Dr. W. J. Crozier for their kindness in reading the manuscript of this book before publication. Their comments have been taken advantage of in a manner for which I, not they, am responsible. I am indebted too to Dr. F. H. A. Marshall and Mr. John Hammond of Cambridge University for encouragement and interest which led to the undertaking of this monograph, and to my friend and collaborator Dr. E. V. Enzmann who actively assisted in a number of the investigations herein described. The National Research Council Committee for Problems of Sex and the Josiah Macy Jr. Foundation provided grants making possible most of my own work, and the preparation of the monograph itself is due in no small measure to their assistance. To the editors and publishers of the following journals I am indebted for permission to reprint the various tables and figures indicated in the text: the *American Journal of Anatomy,* the *American Journal of Physiology,* the *Anatomical Record, Archives de Biologie,* the *Biological Bulletin,* the *Carnegie Institution of Washington Publications in Embryology,* the *Journal of Anatomy,* the *Journal of Experimental Biology,* the *Journal of Experimental Medicine,* the *Journal of Experimental Zoölogy,* the *Journal of Morphology,* the *Quarterly Review of Biology,* and the *Proceedings of the Royal Society.*

I ask the understanding of the reader if this account of the development of mammalian eggs seems at times to deal in summary fashion with some of the voluminous literature on this subject. The investigative aspects are what interest and intrigue me. I emerge confessedly with the impression that at best a qualitative basis for future work has been established, and since I am possessed by the belief that

accurate quantitative observations afford the means for elucidating the nature of biological processes, I feel that this is a book of interrogation, not explanation. If it does indeed create curiosity its major objective will be attained.

GREGORY PINCUS

CAMBRIDGE, MASS.
July, 1936.

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THE EGGS OF MAMMALS

CHAPTER I

INTRODUCTION

The behavior of mammalian eggs from the time of their genesis in the ovary to their implantation in the uterus is the subject matter of this book. The attempt has been made to include experimental investigations of the growth and development of ova rather than morphological descriptions. This is not an easy task, because an acute morphologist may make deductions about the nature of his material which are far more illuminating than those of an eager but inexpert experimenter. Furthermore, except for certain notable investigations of ovarian dynamics, there has been no extensive inquiry into the physiology of living mammalian ova. It has been tacitly assumed, for example, that the reactions involved in the activation of non-mammalian ova occur also in mammalian eggs. Until quite recently no attempt has been made to test even this assumption. Since the middle of the last century a controversy has raged about the possibility of ovarian parthenogenesis. Almost every observer of mammalian ovaries has contributed an opinion, but no one has tried to see if ovarian eggs can be induced to develop parthenogenetically. Experimentation has lagged presumably because of the difficulty of handling living ova.

It is interesting to note that the discovery of the mammalian egg by von Baer in 1827 led initially to extensive observations of living ova. At first the exact morphology of the egg and its membranes was a matter of some debate (see Wagner, 1836; Jones, 1837, 1838, 1885; Barry, 1838;

Bischoff, 1842). Following Barry's (1839) initial observation of cytoplasmic cleavage there ensued a long series of observations on the developmental history of fertilized eggs. Attention gradually shifted from living eggs to fixed specimens, chiefly employed for the determination of the exact cytology of fertilization and the histological changes occurring during differentiation. This resulted in the publication of numerous detailed descriptions of the early embryology in various classes of mammals (Bischoff, 1845, 1852, 1854; Bonnet, 1884, 1891; Caldwell, 1887; Hartman, 1916, 1919; Heape, 1883, 1886; Hensen, 1876; Hill, 1910, 1918; Hill and Tribe, 1924; Huber, 1915; Hubrecht, 1912; Jenkinson, 1900, 1913; Keibel, 1888, 1894, 1899, 1901, 1902; Lams and Doormè, 1908; Lams, 1910, 1913, 1924; Melissinos, 1907; Minot, 1889; van Oordt, 1921; Reichert, 1861; Rein, 1883; Robinson, 1892; Sakurai, 1906; Selenka, 1883, 1884, 1887; Sobotta, 1893, 1895; Tafani, 1889; Van Beneden, 1875, 1880, 1899, 1911, 1912; Van Beneden and Julin, 1880; Weil, 1873; Wilson and Hill, 1907). The living egg was neglected presumably because no technique was developed for preserving it intact *in vitro* long enough for any extensive experimentation to be performed. Nor did the possibility of experimental manipulation of ova *in vivo* receive more than passing attention (see Grusdew, 1896; Novak and Eisinger, 1923).

Since the publication of Stockard and Papanicolou's (1917) and Long and Evans' (1922) exhaustive accounts of the oestrus cycle of the guinea pig and rat respectively, a new era in the study of sexual physiology has been initiated. Enormous strides have been made in the discovery and purification of the hormones regulating the activities of the genital tracts of mammals. The ovarian control of the various phases of the sex cycles in the female has received exhaustive attention, and the control of gonad function by the anterior pituitary has been investigated in detail. Despite the enormous accumulation of data on the endocrine regulation of the ovarian and oviduct environment of ova, the ova them-

selves have received relatively little attention. The study of the hormonal control of ovarian function has centered upon the relation of hormone activity to the development of follicle and corpus luteum. The ovary has been largely considered as a sort of diphasic machine geared for hormone production by certain specialized follicle components. Its primary function as a producer of gametes has been relatively neglected. The endocrine control of the proliferative, secretory and contractile activities of the oviducts themselves is known in detail, and it is tacitly recognized that all these activities have as their end and aim the nutrition and protection of the developing egg. Yet the exact nature of the dependence of the ovum upon these activities is still problematical. We are now provided with the sort of knowledge that should certainly make profitable *in vivo* experimentation with eggs.

Brachet (1912, 1913) did indeed take advantage of the development of a tissue culture technique in order to investigate a specific stage of development in rabbit ova. But neither the availability of the technique nor Brachet's suggestive discourse led to any active investigation until 1929 when Lewis and Gregory published their account of the cinematography of rabbit ova developing in culture. Since then a number of workers associated with Lewis (Gregory, 1930; Squier, 1932; Lewis and Hartman, 1933; Lewis and Wright, 1935) have conducted a fairly intensive examination of living ova, chiefly with the object of culturing fertilized eggs. In addition to these investigations and similar work undertaken by Nicholas and his coworkers (Nicholas and Rudnick, 1933, 1934; Defrise, 1933), the physiological properties of developing ova have been examined from quite different angles. So there exists a measurable body of work of recent origin which is properly experimental. Wherever possible the factual data of this work have been presented in the hope that these, speaking for themselves, may stand side by side with any interpretation herein presented.

It is the earnest belief of the writer that these experimental inquiries represent a small fraction of the work that should and will be done. The enormous variety and richness of mammalian material that is available and untapped should provide an extraordinary temptation to exploitation now that a beginning has been made in the development of technical facilities for the manipulation of this material. I emphasize that only a beginning has been made. This book is a beginning.

CHAPTER II

THE ORIGIN OF THE DEFINITIVE OVA

A long-lived controversy concerns itself with the origin of the definitive germ cells. Do they arise *de novo* from somatic tissue in the sexually mature adult, or are they segregated as primordial precursors early in embryogeny? Weismann's theoretical considerations (1883, 1904, also Nussbaum, 1880) on the continuity of the germplasm led initially to the active investigation of this problem. In the light of modern theoretical genetics the strict interpretation of the Weismannian dogmata is probably no longer necessary. For, since the data of genetics indicate that every normal nucleus in the organism contains the full complement of genes and that somatic segregation of genes is a rare and exceptional phenomenon, it is no longer necessary to postulate the transmission of a special, unimpaired germ tissue. The problem of the origin of the germ cells thus properly becomes one concerned with the dynamics of embryonic differentiation and peculiarly one of regeneration. In fact most of the recent experimental approaches have been concerned with the probability of the regeneration of germ cells from somatic tissues. Able reviews of the general problem are contained in the paper of Heys (1931) and the monograph of Harms (1926).

Since we are concerned specifically with the origin of the definitive ova of mammals the question that we may set is concerned less with general theory and more with pertinent fact. We want to know what processes are responsible for the emergence in the ovary of the functional eggs.

We may at once distinguish two types of investigation. The first, essentially descriptive, is concerned with the development of the ovary and its germ cells from early em-

bryonic life through sexual maturity. The second is concerned with varying the conditions of ovarian growth by experimental means and deducing from the derived data the nature of the factors concerned in the production of functional eggs. We shall assume that these two types of observations are distinct, and consider them separately as: (1) the morphogenesis of egg cells and (2) the experimental investigation of the growth of egg cells.

The Morphogenesis of Egg Cells

Thanks to the Weismannian controversy we have available a fairly detailed description of oögenesis in embryonic life. It is unnecessary here to enter into a detailed description of the embryogeny of the mammalian ovary (see Jenkinson, 1913, de Winiwarter, 1901, de Winiwarter et Sainmont, 1909, Brambell, 1927 and esp. 1930). Our interest lies in the so-called "primordial" germ cells of the embryo, since it is to these cells that a number of observers trace the origin of the definitive ova.

The general opinion seems to be that large wandering cells originate from the entoderm of the gut before or at the time of the formation of the genital ridges (Nussbaum, 1880; Fuss, 1911, 1913). These primordial germ cells migrate to the gonad site and enter the genital ridges. The ridges are first seen as thickenings of the peritoneal epithelium between the base of the mesentery and the Wolffian duct on the ventral side of the developing mesonephros. The thickened peritoneal epithelium becomes the germinal epithelium and the primordial germ cells complete their migration when they become arranged beneath this epithelium which then proliferates medullary tissue into the germ cells. The underlying mesenchyme forms connective tissue trabeculae in the medulla and also the primitive tunica albuginea which separates the medulla from the germinal epithelium.

There are among investigators various opinions about the rôle of the primordial germ cells. A number maintain that these are the only germ cell precursors. The increase in

number of these cells is by mitosis only, and no new cells are recruited from somatic tissue. This view is set forth at some length by Hegner (1914, also Vanneman, 1917). It leads naturally to the conclusion long maintained as a biological truism that by the end of embryonic life or shortly thereafter the complete quota of future eggs is attained (*cf.* Waldeyer, 1870 and 1906; Felix, 1912 and Pearl and Schoppe, 1921). The calculations of Aschner (1914) indicating the presence of some 400,000 ova in the human ovary at birth furnishes an apparent statistical substantiation. Furthermore, meiotic phenomena are observable in these primordial germ cells during embryonic and prepubertal life (Cowperthwaite, 1925) but not thereafter, and the assumption is made that typical meiosis is necessary for the formation of definitive ova.

This conception of a large early store of future ova is scarcely controverted by a second group of investigators who admit the primordial germ cells as precursors of the future ova, but who claim that additional egg cells are supplied by proliferations from the germinal epithelium. Brambell (1927) in a careful study of the developing gonads of the mouse finds that the primordial germ cells persist throughout embryonic life and undergo maturation stages, but declares that additional cells from the germinal epithelium must be responsible for the large increase of cortical cells found in the gonad before the formation of the tunica albuginea in ten and twelve day embryos.

Perhaps the largest group of observers consists of those who also consider post-pubertal production of new egg cells non-existent or negligible but who find that the primordial germ cells degenerate and are replaced by secondary proliferations during embryonic or prepubertal life. Thus Rubaschkin (1908, 1910, 1912) decided that the large differentially staining primordial germ cells with their prominent attraction spheres degenerate in the early guinea pig embryo and are replaced by two successive proliferations from the germinal epithelium. De Winiwarter and Sainmont

(1909) describe a degeneration of the primordial germ cells in the cat ovary and their replacement by ingrowths from the germinal epithelium from three and one-half to four months after birth (*cf.* Kingsbury, 1913 and 1914*a*; Foulis, 1876 and Balfour, 1878). De Winiwarter (1910) observed the same phenomena in human ovaries. In the rat embryos Firket (1920) observed a secondary proliferation following degeneration of the first generation of germ cells. Kingery (1917) in a detailed study of oögenesis in the mouse found that the definitive oöcyte arose from secondary proliferation begun at three to four days before birth and lasting until thirty-five to forty days post partum. He found no evidence for oögenesis after puberty. In the rabbit Buhler (1894) also found only prepubertal ovogenesis.

Simkins (1923 and 1928) questions the validity of the term primordial germ cells, going so far as to state that in the human embryo they are not large wandering cells at all but large liquefied areas surrounding degenerating nuclei. He attributes complete autonomy to the genital ridge. Kohno (1925) recognizes primordial germ cells in the human embryo but declares their origin is in lateral plates of the mesoderm whence they reach the gonad via the gut epithelium and mesentery. Hargitt (1925) also denies the peritoneal origin of the germ cells in rat embryos declaring that large differentially staining cells are found throughout the embryo in the epithelium, mesoderm, ectoderm, gut entoderm and extra embryonic tissues. The disappearance of these cells he attributes to division, not to migration into the genital ridge.

A number of more recent investigators have observed a more or less continuous proliferation of ova from the germinal epithelium throughout life. The chief modern protagonists of this view are Robinson (1918), Arai (1920*a* and *b*), Allen (1923), Papanicolou (1925), Butcher (1927), Swezy (1929*a*, 1933*a* and *b*) and Evans and Swezy (1931). Their histological studies are essentially confirmations of earlier observations on post natal ovaries (Pfluger, 1863—cat;

Schron, 1863—cat and rabbit; Koster, 1868—man; Slawinsky, 1873—man; Wagener, 1879—dog; Van Beneden, 1880—bat; Harz, 1883—mouse, guinea pig, cat; Lange, 1896—mouse; Coert, 1898—rabbit and cat; Amann, 1899—man; Palladino, 1894, 1898—man, bear, dog; Lane-Claypon, 1905, 1907—rabbit; Fellner, 1909—man) save that the work of Allen and those who follow takes advantage of recent discoveries of the nature of the oestrus cycle, and presents observations made upon ovaries taken at definite times during the cycle. Since the embryogenesis of the primordial germ cells and the germinal epithelium are separate and distinct it follows from the findings of these observers that the definitive ova of adult life do not arise from the primordial germ cells at all. Most of the earlier workers observed evidences of growth and thickening of the germinal epithelium or even extensions of germinal epithelium into the ovarian cortex. In some cases these signs of activity were associated with the period of heat.

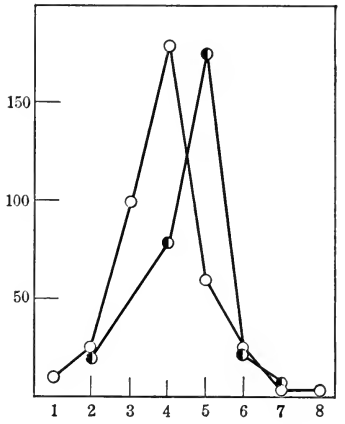

FIG. 1. The frequency of mitoses in the germinal epithelium of mice. From the data of Allen, 1923. Open circles indicate combined data on semi-spayed mice. Half circles indicate normal unoperated controls. Abscissae are stages of oestrus cycle; 1, early proestrus; 2, late prooestrus; 3, proestrus to oestrus; 4, early oestrus; 5, oestrus; 6, early metoestrus; 7, metoestrus; 8, dioestrus. Ordinates are average number of mitoses per mouse. (From the *American Journal of Anatomy.*)

Allen (1923) whose investigations are perhaps pioneer to the most recent developments distinguished four stages in the behavior of the germinal epithelium of the adult mouse during the oestrus cycle. The first, characterized by extensive mitotic activity occurs just before and during oestrus (see Figure 1). The second is marked by a fairly abrupt decrease in mitosis frequency, and a position of the daughter epithelial cells one cell layer below the germinal epithelium

due to the plane of cell division (Figure 2). In the third stage the daughter cells extend two cell layers below the epithelium. And by the fourth stage, occurring during dioestrus, several hundred young ova surrounded by a few follicle cells are found just beneath the epithelium (Figure 3).

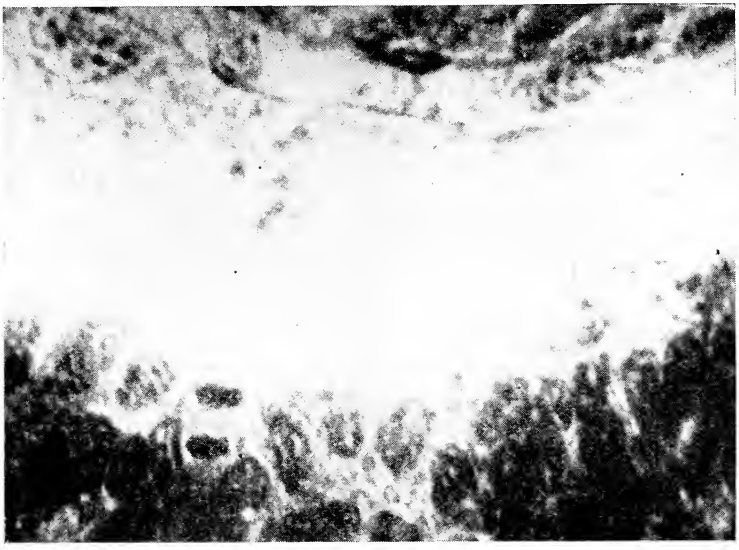

FIG. 2. A late anaphase in the germinal epithelium of the mouse. The plane of division is nearly parallel to the surface of the ovary. (From the *American Journal of Anatomy.*)

According to Allen the tunica albuginea forms "from connective tissue ingrowth during the absence of ovogenetic proliferation of the germinal epithelium." Allen notes a relatively intact tunica in animals that have had a long period of dioestrus and also a complete or an almost complete absence of young follicles.

Cowperthwaite (1925) has criticized Allen's data on the grounds that he gives no demonstration of the presence of meiosis in these presumable new ova. Typical meiotic phenomena in adult ovaries have, in fact, rarely been observed. De Winiwarter (1920) noted oöcyte formation in the region of the hilum in ovaries of cats shortly after puberty but no such process in the remaining tissue, and Gerard

(1920) observed typical meiotic prophases in nests of young oöcytes in the adult ovaries of Galago. On the basis of these observations and the presence of typical oöcytes in certain undescribed adult ovaries of Loris (material of Prof. J. P. Hill and Dr. A. Subba Ran), Brambell (1930) inclines to the belief that these primate oöcytes derive from primitive oögonia, not the germinal epithelium.

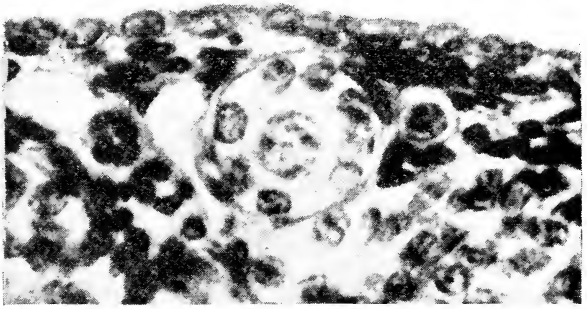

FIG. 3. A stage 4 ovum (see text) in the mouse. Note complete layer of follicle cells. (From the *American Journal of Anatomy.*)

In rodents, however, such typical meiotic prophases have never been described. Here the observations of Swezy (1929*a*) and also of Evans and Swezy (1931), are very much to the point and apparently resolve the mystery. Swezy found the classical meiotic stages in the oöcytes of rat embryos and young rats up to five days post partum (Plate I, Figs. 1–5), but she noted definite degeneration of all these ova by the 15th day post partum. By the 10th day definitely atypical synizesis and pachytene stages occur (Plate I, Figs. 6–13) and in 15 day old rats (Plate I, Figs. 14–16) synizesis stages are rare or missing, the pachytene modified to a chromatin aggregation much less sharp than in typical stages, and the diplonema chromosomes also less distinct. On twenty day old rats (Plate II, Figs. 17–22) nuclear growth of oöcytes involves essentially similarly modifications, and in the adult the new ova derived from the ger-

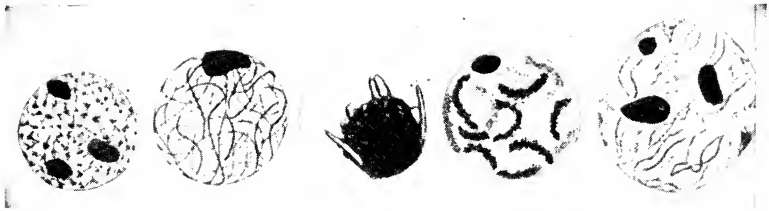

FIG. 1 FIG. 2 FIG. 3 FIG. 4 FIG. 5

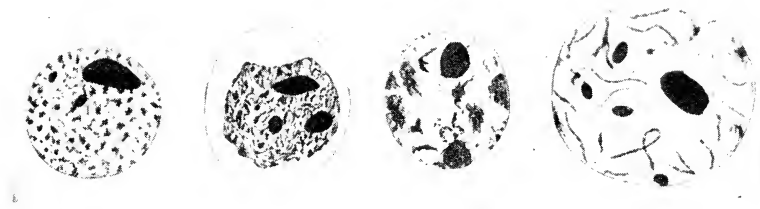

FIG. 6 FIG. 7 FIG. 8 FIG. 9

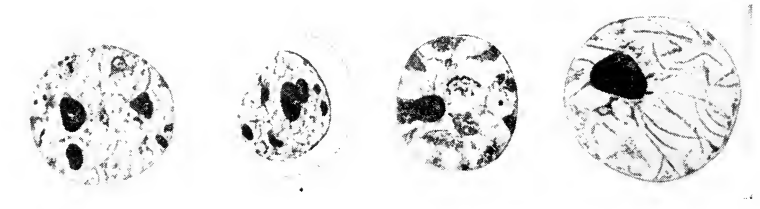

FIG. 10 FIG. 11 FIG. 12 FIG. 13

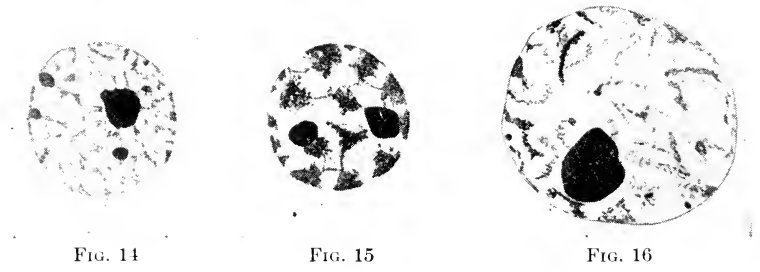

FIG. 14 FIG. 15 FIG. 16

PLATE I. (From the *Journal of Morphology*)

Figs. 1–5. Nuclei of ova from ovary of rat 5 days post partum. 1, Deutobroch nucleus in germinal epithelium. 2, Leptotene nucleus. 3, Synizesis. 4, Pachynema. 5, Diplonema.

Figs. 6–9. Nuclei of ova from ovary of rat 8 days post partum. 6, Deutobroch nucleus. 7, Synizesis. 8, Stage following 7, evidently modified pachynema. 9, Diplonema.

Figs. 10–13. Nuclei of ova from ovary of rat 10 days post partum. 10, Deutobroch nucleus. 11, Synizesis. 12, Modified pachynema. 13, Diplonema.

Figs. 14–16. Nuclei of ova from ovary of rat 15 days post partum. 14, Deutobroch nucleus. 15, Modified pachynema. 16, Masses of chromatin changing into loose threads.

FIG. 17

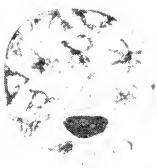

FIG. 18

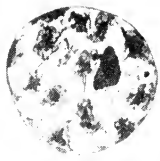

FIG. 19

FIG. 20

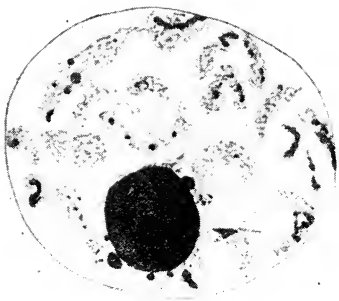

FIG. 21

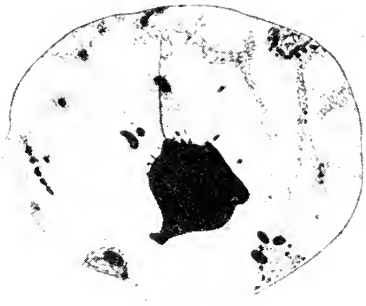

FIG. 22

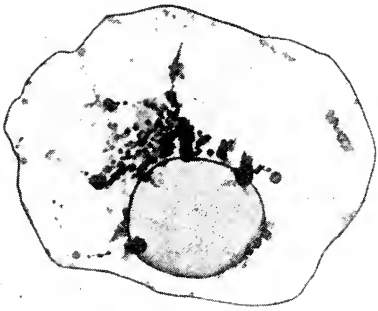

FIG. 23

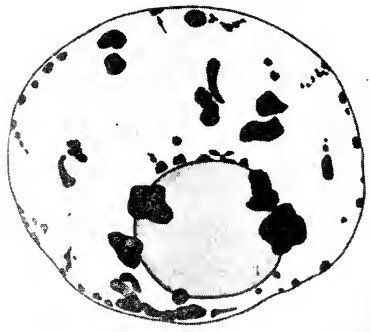

FIG. 24

PLATE II. (From the *Journal of Morphology*)

Figs. 17–22. Nuclei of ova from ovary of rat 20 days post partum. 17, Deutobroch nucleus. 18, Beginning of the formation of clumps shown in next figure. 19, Modified pachynema. 20, Later stage showing characters of diplonema. 21, Nucleus toward the end of the growth period. 22, Final stage in twenty-day rat.

Fig. 23. Nucleus in mature follicle from adult rat. Fig. 24. Nucleus from ripe follicle from adult rat.

minal epithelium contain mature nuclei (Plate II, Figs. 23–24) in which the modification presaged in the younger animals attains culmination. These definitive ova show then a modified type of meiosis which involves essentially the disappearance of leptotene and synizesis, and the formation of an atypical pachynema and diplonema. Evans and Swezy (1931) obtained confirmation of these findings in the guinea pig, cat, dog, monkey and man. They point out that instead of being long-lived, the egg cells of mammals are subject to heavy mortality and exhibit a very short life cycle, correlated apparently with the length of the normal ovarian rhythm. In those animals in which the oestrus and ovarian cycles coincide (*e.g.*, rat, mouse, guinea pig) the length of the oestrus cycle is a measure of the lifetime of the ovum in the ovary.

These rather straightforward histological findings seem to indicate, on the whole, that the definitive ova originate from the germinal epithelium. All our recent knowledge of the rhythmic activity of the ovary with its periodic production of large numbers of young ova (Allen, Kountz and Francis, 1925) militates against the assumption of a single large initial store of ova gradually being exhausted throughout sexual maturity.

The Experimental Investigation of the Growth of Egg Cells

Any attempt to analyze the experimental data pertinent to the problem of the origin of the definitive ova encounters two difficulties. First of all many of the experiments are concerned with the simple Weismannian problem and ignore certain now obvious endocrinological implications. And secondly, the difficulty of experimental treatment of mammalian embryos makes for a hiatus in our knowledge that can only be bridged by indirect deduction.

The information that we do have at hand is derived from experiments concerned with the effects resulting from (1) bilateral ovariectomy, (2) partial ovariectomy, (3) ovarian

transplantation, (4) the irradiation of ovaries with x-rays, (5) hypophysectomy, (6) the injection of gonad-stimulating hormones and (7) the transplantation of embryonic gonad rudiments.

Bilateral ovariectomy has been extensively employed in order to determine whether ovarian tissue and eggs can be derived from somatic cells. It is a common experience that ovariectomized animals apparently regenerate ovarian tissue some time after the operation. Thus Davenport (1925) observed as many as 64 per cent of bilaterally ovariectomized mice with apparently functional ovarian tissue appearing within a few weeks to several months after the ovariectomy. Such data may be explained as due either to: (1) regeneration of germinal tissue *de novo* from somatic cells or (2) the presence of accessory gonadal tissue distinct from the ovary and not removed during the operation or (3) the incomplete removal of ovarian tissue so that fragments remaining hypertrophy and attain dimensions sufficient to permit the manifestation of ovarian function. If the first alternative is accepted then it follows that neither germinal epithelium nor, presumably, primordial germ cells are necessary for the production of ova. The two latter alternatives exclude the first but scarcely affect the problem of origin via germinal epithelium or primordial germ cell though careful observation of the process of hypertrophy may yield pertinent data. Even if the first alternative is acceptable and may thus very well settle the ghost of germplasm continuity, it does not necessarily inform us about the normal process of egg production.

In rodents accessory gonadal tissue is rarely, if ever, present. On the other hand, it is known that fragments of ovarian tissue, remaining after incomplete extirpation of the ovaries, will hypertrophy to such a remarkable degree that a completely normal ovary will be reëstablished from which fertilizable ova are liberated (*cf.* Haterius, 1928; and Pincus, 1931). Furthermore it is quite possible to fail to extirpate small fragments of the irregularly lobed encap-

sulated rat and mouse ovaries, or even after careful excision to drop very small crushed fragments. A number of investigators have therefore repeated Davenport's experiments using extreme operative precautions, in some instances going to the trouble of making serial sections of the extirpated ovaries in order to be certain of the completeness of removal.

In practically every instance the per cent of animals showing return of oestrus symptoms or of detectable ovarian

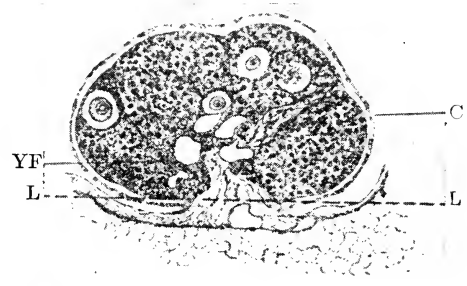

FIG. 4. Section through ovary of young rat showing small, compact ovary. YF, young follicle; C, ovarian capsule. LL, line of excision. (From the *Quarterly Review of Biology.*)

tissue has been much below that reported by Davenport. Pallot (1928) found return of vaginal cornification in three out of twelve ovariectomized rats within six to six and one-half months after operation, and ovarian tissue was found in two of these. Parkes, Fielding and Brambell (1927) detected oestrus symptoms after operation in eleven out of one hundred and twenty-one mice, identifying ovarian tissue in eight of these eleven. Haterius (1928) also found apparent regeneration in 10 per cent of the mice he ovariectomized, and attributed the regeneration to incomplete extirpation. Pencharz (1929) reported return of oestrus in only three of 118 ovariectomized rats and mice, and demonstrated by serial sections of the ovarian region that incomplete removal had been made in the case of these three.

Heys (1929 and 1931), in an extremely careful analysis of

a series of double ovariectomies in the rat, has demonstrated the presumable source of regenerated tissue in animals with apparently completely extirpated ovaries. In an initial series of 105 double ovariectomies she found germ cells at the ovarian site in eight cases, and observed that all eight

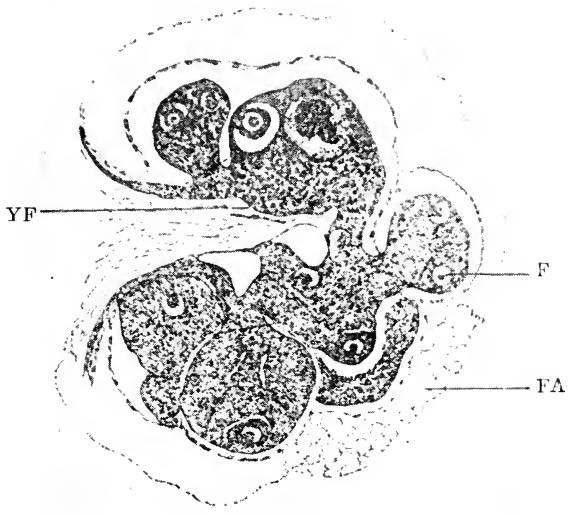

FIG. 5. Section through the ovary of mature rat showing the lobed condition. YF, young follicle; F, follicle; FA, fatty tissue. (From the *Quarterly Review of Biology.*)

occurred in the sixty animals over forty days of age. She noted that in females under forty days of age the ovary is relatively smooth and compact and not very heavily embedded in fat (Figure 4), whereas in older animals the ovary is lobed and surrounded by a larger amount of fat (Figure 5). She accordingly ovariectomized a second set of animals consisting of eighty-five females under forty days of age and twenty-three older females. Three of the older animals regenerated germ cells but none of the younger ones did. In several of the positive cases serial sectioning of the removed ovaries gave no detectable indication of lost fragments, but Heys believes that certain narrowly constricted lobes of

ovarian tissue might very well be lost and the loss not noticed upon serial sectioning (see Figure 5). Heys' results can scarcely be due to chance alone, the difference in regeneration incidence between the young and older rats being 3.43 times the standard error of the difference, *i.e.*, the odds are over 3000 to 1 against this being a chance difference.

It is clear, therefore, that regeneration of ovogenetic tissue from somatic tissue is improbable in mammals. And certainly the definitive ova are normally not recruited from somatic cells. We must turn to other experimental procedures to obtain some insight into the processes that lead to the birth of ova in normal functional ovaries.

The simple observation that unilateral ovariectomy or incomplete total ovariectomy leads to a compensatory hypertrophy of the remaining tissue has led to a long series of researches which, often incidentally, form the basis for our modern knowledge of the elements of ovarian dynamics. The fact that such hypertrophy occurs was originally established both clinically (Robertson, 1890; Gordon, 1896; Sutton, 1896; Morris, 1901; Doran, 1902; Kynoch, 1902; and Meredith, 1904) and experimentally (Kanel, 1901; Bond, 1906; Carmichael and Marshall, 1908). An almost exact doubling of weight in the remaining ovary of unilaterally ovariectomized rats has been reported by Stotsenburg (1913) and Hatai (1913, 1915) and the number of eggs shed is demonstrably equal to the number normally produced by two ovaries (see Lipschütz, 1924; Hanson and Boone, 1926; Crew, 1927; and Slonaker, 1927). In the opossum Hartman (1925) has reported a tripling of the weight of the remaining ovary and a similar threefold increase in the number of eggs shed. In the rabbit (Asdell, 1924; Hammond, 1925; Lipschütz, 1928) and the cat (Lipschütz and Voss, 1925) a single remaining ovary or even small ovarian fragments produce the typical adult number of ripe follicles and eggs, but an exact compensatory hypertrophy of ovarian tissue is not so evident. Emery (1931) in a large series of unilaterally ovariectomized rats found not a doubling in weight,

but a one and one-half times compensatory hypertrophy when careful comparison with a control series was made. It is significant that in Emery's material about 50 per cent of the rats were found at autopsy to have large ovarian cysts. Similar cystic formations were observed in about half of the semi-spayed females in Wang and Guttmacher's (1927) series, and Williams (1909) reports that such cysts are commonly found in ovarian fragments left after incomplete ovariectomy.

Arai (1920*b*) found definitely that the compensatory hypertrophy in the rat is due exclusively to an increase in the number of large follicles and corpora lutea. Semi-spaying before puberty when the formation of corpora lutea does not normally occur led to a 40 per cent increase in ovarian weight, whereas semi-spaying after puberty led to a 100 per cent increase. Furthermore, by careful counts he established that the *total* number of follicles in the ovary does not increase after semi-spaying. In this Arai was confirmed by Allen (1923) who found that in semi-spayed mice the number of ova differentiating from the germinal epithelium during stages 2 and 3 (*vide supra*) was scarcely larger than normal whereas the average number of mature ova formed was normal. The implication from these studies is that the germinal epithelium produces a large more or less constant number of young ova, that some extra-gonadal factor is responsible for the ripening and maturation of a limited number of follicles, and that the maturing crop of ova are chiefly involved in the compensatory hypertrophy. It is now well established that an enormous atresia of young follicles occurs during the course of a single oestrus cycle. Thus in swine 14 per cent of the visible follicles less than 3 mm. in diameter become mature (Allen, Kountz and Francis, 1925) and in the rat of the ova less than 20 μ in diameter only 0.8 per cent attain a diameter greater than 60 μ (Arai, 1920*a*). This extensive destruction of young ova and follicles is particularly striking in the dog and cat (Evans and Swezy, 1931) where all the new eggs (except

those ovulated) formed in the metoestrum and anoestrum preceding ovulation are completely degenerated by the time of ovulation.

That the germinal epithelium is the source of new ova formed in hypertrophying ovarian tissue is demonstrated by the behavior of transplanted ovarian tissue. Among those who have observed the histological development in such tissue only Marshall and Jolly (1907, 1908) report complete disappearance of germinal epithelium with retention of function. Lipschütz (1928) notes a decrease in the number of primary oöcytes in small fragments of rabbit ovaries in incomplete ovariectomy when comparison is made with similar sized fragments isolated from the ovaries in unovariectomized controls. But it is notable that his protocols describe a partially preserved or "flattened" (degenerating?) germinal epithelium in the experimental group whereas the germinal epithelium in the control fragments is apparently much better preserved. Tamura (1926) examining a series of ovarian transplants made onto the kidneys of male mice found the presence of primary follicles and many young ova associated with an actively mitotic germinal epithelium. Where the degree of activity of the germinal epithelium is less and more varied, small and medium sized or various sized follicles are present. Apparently the activity of the germinal epithelium is largely conditioned by the pressure of overlying connective tissue growths since its activity is greatest at free surfaces. Nonetheless, Tamura claims a rhythmical proliferation of ova from the germinal epithelium, but assigns a length of ten days to the ovogenetic cycle which is twice the length of the normal five-day oestrus cycle. Schultz (1900) and Voss (1925) also observed the persistence of functional germinal epithelium in their series of transplantations, but offer no such detailed an analysis as Tamura. Butcher (1932) has examined the nature of ovogenesis in ligated ovaries and in autotransplantations of ovarian fragments and observed that the development of young ova is definitely associated with the activity of the

germinal epithelium. Furthermore, in the ligated ovaries the follicles become necrotic and new ova are proliferated from the germinal epithelium which is relatively unimpaired. Athias (1920) has described proliferation of new ova from the germinal epithelium of transplanted guinea pig ovaries. No attempt has been made to make a quantitative study of the relation between the number of new ova formed and the amount of functional germinal epithelium in transplanted or fragmented ovarian tissue, but it seems evident that the formation of new ova in such tissue occurs in the germinal epithelium. Thus in Tamura's material the few cases of degenerated transplants were marked by a complete absence of germinal epithelium.

It is possible, however, to preserve an intact germinal epithelium with total disappearance of follicles in x-rayed ovaries (Parkes, 1926, 1927*a*, *b* and *c;* Brambell, Parkes and Fielding, 1927*a* and *b*). Parkes and his coworkers have described in some detail the replacement of degenerated follicular tissue by cellular proliferations from the germinal epithelium in the irradiated ovaries of mice. These proliferations never give rise to ova, however, though the ovaries seem to retain their hormone-producing capacities as evidenced by the continuance of oestrus cycles of normal length in the irradiated animals. In the ferret (Parkes, Rowlands and Brambell, 1932) x-ray sterilization is also marked by an obliteration of the follicles and oestrin secretion, whereas in guinea pig ovaries (Genther, 1931, 1934) a transformation to luteal tissue usually occurs with only occasional follicle formation. Brambell (1930) inclines to the belief that the destruction of primordial ova is responsible for the lack of ovogenesis, but it is equally likely that the x-rays affect differentially the ovogenetic and hormone-producing capacities of ovarian tissue. It is notable therefore that the proliferation of new tissue from the germinal epithelium in x-rayed mice resembles the production of anovular follicles. Hill and Parkes (1931) have attempted to induce germ cell formation in mice with irradiated ovaries by means of in-

jections of pituitary and pregnancy urine extracts, but no ova were ever produced in the injected animals.

That the early stages of ovogenesis in adult ovaries are scarcely under the control of pituitary hormones is abundantly evident from observations made upon the ovaries of hypophysectomized animals. Smith (1930) noted that in completedly hypophysectomized rats no new large follicles or corpora lutea develop, but the proliferation of young follicles goes on unimpaired for many months after hypophysectomy. Swezy (1933*b*) has presented quantitative measures of the rate of ovogenesis in hypophysectomized rats, and her data indicate that a larger number of young ova may be produced in hypophysectomized females than in normal non-pregnant animals. In Table I is presented a summary of her findings.

TABLE I

NUMBERS OF OVA, FOLLICLES AND CORPORA LUTEA IN A SINGLE OVARY OF THE RAT DURING THE OESTRUS CYCLE, PREGNANCY AND PSEUDOPREGNANCY, AND AFTER HYPOPHYSECTOMY AND THYROIDECTOMY. (From Swezy, 1933*b*)

STAGE	NUMBER OF RATS	DAY OF CYCLE (OR DAYS AFTER OPERATION)	AGE, DAYS	AVERAGE NUMBER OF OVA AND PRIMARY FOLLICLES	AVERAGE NUMBER OF LARGER FOLLICLES	AVERAGE NUMBER OF CORPORA LUTEA	TOTAL
Oestrus cycle	5	2nd(1), 4th(4)	206–208	1809	171	27	2007
Pregnant and pseudopregnant	10	5 to 22	98–224	3857	311	16	4184
Hypophysectomized	8	12 to 90	95–202	4164	—	20*	4184
Thyroidectomized	3	36 to 42	403	1371	193	15	1579

* Persisting old corpora.

Swezy concluded from these data that there is a basic rate of ovogenesis which is observed in hypophysectomized animals. That the increased number of ova in hypophysectomized animals is due to an increased rate of production and not merely to accumulation is proven by the absence of any unusual number of degenerated ova. This rate is

decreased when the hypophysis is secreting active maturity hormone as in non-pregnant females. The maturity hormone is concerned with the ripening of large follicles, ovulation and corpus luteum formation. During pregnancy and pseudopregnancy maturity hormone is secreted only in subthreshold amount, as evidenced by cyclic ovarian changes

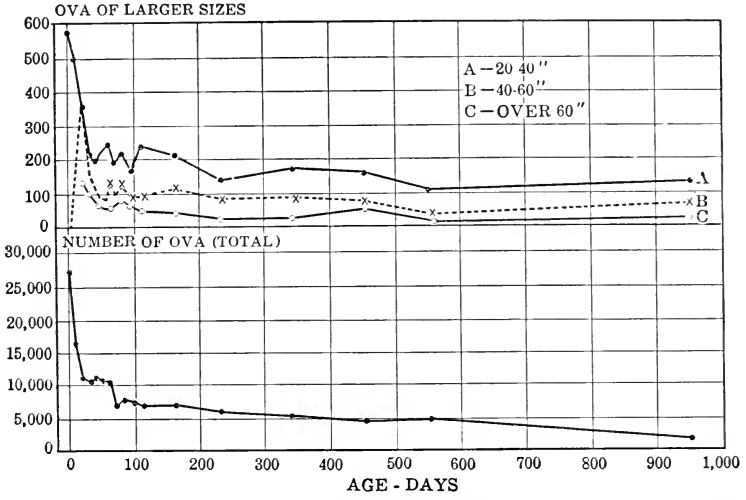

FIG. 6. Showing the total number of ova as well as the number of ova of different sizes in the albino rat at different ages (condensed). (From the *American Journal of Anatomy.*)

in the ovary during pregnancy (Swezy and Evans, 1930), so that the hypophysectomized level is attained. During the normal non-pregnant ovogenetic cycle that portion marked by the presence in the ovary of ripe follicles and fresh corpora lutea is always associated with a minimum of small, newly formed ova. The pituitary secretions, then, are concerned with promotion of ovulation and luteinization and presumably inhibit ovogenesis to a certain extent. The factor controlling ovogenesis is unknown although the effects of thyroidectomy indicate that the thyroid *may* promote ovogenesis to a certain extent. It should be pointed out, however, that the thyroidectomized rats were much the eldest of the lot and Arai (1920*a*) has demonstrated a small

decline of ovogenesis with age in adult females (see Figure 6).

The experiments of Engle (1928) demonstrate adequately that pituitary secretions are responsible for the later stages of maturation. He injected anterior lobe tissue into normal and semi-spayed rats and found that the per cent of hypertrophy due to pituitary stimulation was approximately equal in the two groups of animals. We have already noted that in compensatory hypertrophy the increased ovarian weight is due to the doubling of large follicle and corpus luteum number, the number of primary follicles being the same in a single ovary whether the second ovary is present or not.

Swezy (1933*b*) also determined the effect of various pituitary hormone preparations upon ovogenesis in adult and immature rats. Her data are collected and summarized in Table II.

Immediate verification of the conclusions deduced from Table I is found in the data derived from the injection of rat hypophyses into adult and immature rats (columns [1], [10] and [11]). Rat hypophyses are notably rich in gonad stimulating hormones (Smith and Engle, 1927), and their administration results in a decrease in the rate of ovogenesis, and an increase in total ovarian tissue. The data on the immature rats are particularly striking, for a few days of pituitary administration results in a halving of the total number of ova. Arai (1920*a*) found that the average total number of ova in prepubertal rats was about 10,000 and approximately 6000 in post-pubertal animals.

Beef hypophyses, on the other hand, are relatively poor in maturity hormone and rich in growth hormone. Evans and Simpson (1928) have demonstrated an antagonism between the growth and gonad-stimulating hormones of the anterior pituitary. The increase in follicle number following beef hypophysis administration (column 2) might then be interpreted as a neutralization of the intrinsic maturity hormone effect by the growth hormone of the beef pituitary.

TABLE II

THE NUMBER OF OVA, FOLLICLES, CYSTS AND CORPORA LUTEA IN SINGLE OVARIES OF RATS SUBJECTED TO VARIOUS HORMONE TREATMENTS. (From Swezy, 1933*b*)

TREATMENT	NO. OF RATS	AGE OF RATS (DAYS)	DAYS OF ADMINISTRATION	OVA AND PRIMORDIAL FOLLICLES	LARGE FOLLICLES	CORPORA	CYSTS	TOTALS	WEIGHT MGMS.
(1) Rat hypophysis	5	153–182	9–20	1436	155	58*	12	1661	227*
(2) Beef 1/8 c.c. hypophysis	4	153–168	9	4017	228	20	3	4268	42
(3) Beef 1/8 c.c. hypophysis plus rat hypophysis	1	154	9	1476	295	4	none	1813	97
(4) Pregnancy urine	2	172–174	10	3322	216	20	12	3570	59
(5) 1/8 c.c. theelin	6	181–190	18	3574	245	22	none	3841	—
(6) 21–34 c.c. follicular fluid	6	183–254	10–14	2183	203	18	none	2404	26
(7) 1/4–1 c.c. growth hormone	6	255	35–97	4996	144	3	—	5143	sub-normal (3) and hypophysectomized types
(8) 1/4–1 c.c. growth hormone	2	255–408	60 and 394	2277	190	60	—	2527	76 maturity type
(9) 0.5 c.c. growth hormone	2	139 and 141	9	1952	300	31	—	2283	35 (mixed type)
(10) Control	1	24	—	7225	—	—	—	7225	9.5
(11) Rat hypophysis	5	24–26	2–8	3664	—	present in some	—	3664	67†

* Varied with amount of hypophysis.

† Average of three.

Simultaneous injection of beef and rat hypophysis tissue results in inhibition of ovogenesis (column 3).

When, however, examination was made of the ovaries of animals receiving injections of growth hormone extracts various results were obtained. In six of the ten animals observed (column 7) the expected result was obtained, namely an inhibition of ovarian growth and a rise in the rate of ovogenesis. Two animals (column 8) with normal, good sized ovaries exhibited a normal rate of ovogenesis, and two animals (column 9) with somewhat decreased ovarian weight gave no indication of increased ovogenesis. Two interpretations of these data are possible: (1) the growth hormone preparations may in some instances have contained sufficient maturity hormone to overcome the typical growth hormone effect or (2) there may have occurred in some of the injected animals a conversion of growth hormone to maturity hormone (*cf.* Evans, Meyer and Simpson, 1932; Evans *et al.*, 1933). It should be pointed out that Reiss, Selye and Balint (1931*a*, *b*) have obtained from the pituitary extracts free of growth hormone which also inhibit the action of maturity hormone. Swezy's extracts are not made in a manner that would free her preparations of such materials. Obviously the use of highly purified extracts and carefully timed injections should assist in resolving the situation.

Pregnancy urine extracts (column 4) seem to increase ovogenesis to some extent. It is known that pregnancy urine is only partially effective as a maturity hormone (Engle, 1929; Evans and Simpson, 1929).

Prolonged oestrin injection is known to reduce ovarian growth (Doisy, Curtis and Collier, 1931; Leonard, Meyer and Hisaw, 1931; Spencer, D'Amour and Gustavson, 1932; Pincus and Werthessen, 1933), presumably by inhibiting secretion of maturity hormone from the anterior pituitary (Meyer, Leonard, Hisaw and Martin, 1932). One would expect therefore that the data of columns 5 and 6 should show an enhanced ovogenesis. It is interesting to note

that this seems to be the case when relatively light oestrin doses are injected (column 5), but not with heavy doses (column 6). The theelin-injected animals received about 6.25 r.u. per day, and while continuous vaginal cornification resulted, an apparently normal cycle of uterine changes occurred and the ovaries appeared relatively unimpaired. It is possible that in the animals receiving light doses the ovogenesis inhibiting capacity of maturity hormones was impaired but not the follicle stimulating capacity. The heavier dosages may have caused the hydropic degeneration of the germinal epithelium described by Doisy, Curtis and Collier (1931) and so prevented maximum ovogenesis, although Swezy makes no note of such degeneration. Swezy, noting that normally during the oestrus cycle there is a drop in the production of new ova at the period just succeeding the period of maximum oestrin production (*e.g.*, ovulation), is inclined to attribute this drop (and therefore the results in her oestrin-injected animals) to a factor other than the "suppression" of hormone secretion from the pituitary.

Recently Hisaw and his collaborators have advanced an explanation of the oestrus rhythm which involves a separation of the maturity principle of the pituitary into two hormones (Fevold, Hisaw and Greep, 1934; Lane and Hisaw, 1934; Hisaw, Fevold, Foster and Hellbaum, 1934; and Lane, 1935). One hormone is follicle stimulating, the other luteinizing and a chemical separation of the two has been attained (Fevold, Hisaw and Leonard, 1931; Fevold and Hisaw, 1934). These investigators report an increase in the total number of follicles in rat ovaries on administration of follicle stimulating hormone to prepubertal rats but no increase when luteinizing hormone is administered. Their count of "total follicles" includes only ova in definitely formed follicles. Swezy (1933*b*) attributes the ovogenesis inhibition to the luteinizing hormone. It is possible, therefore, that in addition to the ovogenetic activity which is independent of the hypophysis (*e.g.*, the ovogenesis seen in hypophysectomized

animals) a stimulation to ovogenesis may be engendered by the follicle stimulating hormone. Hisaw and his collaborators find that corporin (the hormone of the corpus luteum) exerts effects on the ovary like those of the follicle stimulating hormone while oestrin decreases the secretion of follicle stimulating hormone and stimulates luteinizing hormone production from the hypophysis. Pregnant and pseudopregnant animals may therefore exhibit an increase in ovogenesis due to direct action of corporin from their corpora lutea, whereas animals in oestrus and those receiving oestrin injections show reduced ovogenesis perhaps because of the action of the induced luteinizing hormone secretion.

It is obviously not possible to arrive at any final decision concerning the factors governing ovogenesis until additional pertinent data are available. The most concise summary of the evidence indicates that ovogenesis occurs from the germinal epithelium at a typical intrinsic rate which may be reduced by the action of a hormone or hormones from the anterior pituitary. But even this deduction requires further verification in the form of careful quantitative estimates of ovogenesis in its relation to atresia, and particularly an inquiry into the nature of the atresia of young ova and follicles. We are completely unaware of the intimate nature of the intrinsic proliferative capacity of the germinal epithelium. How does it compare with the mitotic index of tissues generally? Is it a self-perpetuating phenomenon in the sense that the atresia of its products releases substances stimulating cell division? We shall see for example that the atresia of maturing follicles is often accompanied by the formation of mitotic spindles and it is well known that cytolized cell products (trephones) promote cell division. An extraordinary variety of problems suggest themselves. Patience and the formation of substantiated hypotheses will result in their solution.

In summating the evidence relating to the normal ovogenetic processes in prepubertal and post-pubertal animals little doubt remains that the definitive ova are proliferated

from the germinal epithelium. What then is the rôle of the primordial germ cells of the embryo? Are they essential structures or merely incidental? There are practically no illuminating experimental data on the development of embryonic gonads. The experimental manipulation of mammalian embryos is dependent upon the elaboration of techniques now in the process of initiation. Certain investigations of gonadogenesis in amphibian and chick embryos offer provocative suggestions, but their applicability to mammals has yet to be proven.

In the chick a gonad or gonad-like organ may form free of primordial germ cells. This can be demonstrated by removal or destruction in three to nine somite embryos of the anterior crescent in which the primordial germ cells originate. The embryos nonetheless develop small gonad rudiments (Reagan, 1916; Benoit, 1930). Willier (1932, 1933*a* and *b*) has excised the germ cell crescent and transplanted the entire blastoderm and found a sterile gonad developed in the transplant. In the frog (Kuschakewitsch, 1910) sterile gonads free of germ cells develop from the genital ridge when delayed fertilization prevents germ cell migration. Humphrey (1928), on the other hand, finds that in Amblystoma gonads form in grafted tissue only when a sufficient number of primordial germ cells are located beneath the coelomic epithelium which gives rise to the germinal epithelium.

It is notable that in all instances gonads arising free of primordial germ cells are sterile. Thus Domm (1929) found in the fowl that if the large functional left ovary is removed prior to the time of the disappearance of the germ cells from the small rudimentary right gonad the latter develops into a testis which produces sperm. If excision of the left ovary is delayed until the time when the germ cells of the right gonad are no longer present (the germ cells normally disappear by the third week after hatching) a sterile testis develops.

Willier (1933*a* and *b*) has demonstrated by means of

chorio-allantoic grafts of the gonad-forming areas of chick gonads that germ cells remaining outside the germinal ridge area do not differentiate into oögonia or spermatogonia, whereas those that become situated under the germinal epithelium develop as typical sex cells. On the basis of this and other evidence he agrees with Witschi (1929) that the cortex (*e.g.*, the cortical sex cords) of the gonad acts upon the germ cells as a specific organizer of female sex cells, and the medulla as organizer of spermatogenetic tissue. In the free-martin of cattle, which is a female twin developing *in utero* under the influence of the hormones of its male partner, a sterile testis-like organ develops. It is notable that while typical male sex cords are present, germ cells are absent (Chapin, 1917; Willier, 1921). Perhaps in the case of the free-martin (as in the frogs with delayed fertilization) a spermatogenetic tissue is not formed because primordial germ cells do not reach the gonad.

If these data are generally applicable to mammals it would seem that although ovogenesis takes place from the germinal epithelium the formation of a functional ovary is dependent upon the primordial germ cells. We have seen, in the case of x-rayed ovaries, that an ovary with morphologically normal germinal epithelium may be incapable of forming ova. A necessary mechanism is lacking. It may be that the primordial germ cells are the precursors to this mechanism in normally developing ovaries.

The evidence from the free-martin and recent data on the transplantation of embryonic gonad rudiments indicates that, as in amphibia and birds, the development of an ovary in embryogeny is dependent upon the formation of a cortex in the developing gonad. Normally in ontogeny the gonads of both sexes are morphologically indistinguishable for some time. The genital ridge, as already noted, consists of germinal epithelium overlying primordial germ cells. At about the 10 mm. stage in both the pig (Allen, 1904) and cat (Sainmont, 1905) and at the 12th day post coitum in the mouse (Brambell, 1930) the germinal epithelium begins to

proliferate the primary sex cords from its inner surface. During the formation of these cords (or nest of medullary cells as in man [Felix, 1912]) the gonad is still morphologically indifferent. Morphological differentiation may be considered as initiated when these primary cords become isolated in the medulla by the formation of the primitive tunica albuginea under the germinal epithelium in the male gonad and the proliferation of a second set of cortical sex cords from the germinal epithelium in the female gonad. In the embryonic ovary the medullary cords persist for some time but are rarely found after birth; the cortical cords break up to form primitive follicle cells surrounding the primordial ova.

Buyse (1935) has transplanted rat gonads in the morphologically indifferent stage onto the kidney of adult rats of both sexes. Over 60 per cent of the transplants developed as testes, 16 per cent as ovaries and the remainder were bisexual gonads or gonads of undetermined sex. A small percentage of the gonads classified as rudimentary testes seemed to be transformed ovaries. It will be seen that if these are included in the group of gonads other than testes the normal sex ratio is approximated. Since the type of gonad developed was not correlated with the sex of the host Buyse concludes that adult sex hormones do not affect sex differentiation. The differentiation was then dependent on the history of the sex cords in the transplanted tissue. Presumably the clear cut segregation of testes was due to the presence of formed primary sex cords, *e.g.*, the testis organizers, whereas various types of zygotic ovaries were obtained dependent on the probability of formation or partial formation of the cortical sex cords.

CHAPTER III

THE GROWTH OF THE OVUM

We have seen that the production of ova from the germinal epithelium may proceed in the absence of the hypophysis. But does the formation of mature ova depend upon hypophyseal hormones? It is clear that ovulation and particularly the number of follicles that liberate ova is dependent upon hypophyseal hormones. Does this dependence involve merely a maturation of the follicular apparatus or is the actual growth of the ova also concerned? In fixed material cells distinguishable as primary ova are in the mouse a little less than 7 microns in maximum diameter (Pincus, unpublished data), in the rat 8 microns (Arai, 1920*a*). They eventually attain maximum diameters of 65 to 70 microns. What are the factors governing the growth of these ova to maximum size?

While direct measurements are unavailable it seems obvious that in hypophysectomized animals the ovum attains the maximum size. Smith (1930) notes that the primary follicles in hypophysectomized rats "continually are undergoing development, but invariably undergo atresia not later than the stage of cavity formation." Swezy (1933) notes the presence of a follicle having a diameter of 270 microns in a rat ovary 90 days after hypophysectomy and mentions follicles with diameters of 200 microns. It is evident from the figure in Selye's (1933) paper that follicles with antra occur in 43 day old rats hypophysectomized at 18 days of age. In the dwarf mouse the largest follicles are about 200 microns in diameter and contain antra (Pincus, unpublished data).

Now it has been demonstrated (Brambell, 1928) that in the mouse the diameter of the follicle when the ovum is

fully grown is 125 microns and in the rat (Parkes, 1931) the maximum diameter of the ovum is attained when the follicle is 160 microns in diameter. Full growth of the ovum, then, is attained just before the time of antrum formation which begins in rats and mice in follicles having diameters of about 200 microns. We may therefore deduce that the ova of hypophysectomized animals attain the dimensions of the mature ova in ovulating animals, and that the growth of the ova (and early follicular growth) is independent of the hypophysis.

This conclusion is supported by various independent lines of evidence. Arai (1920*a*) found that ova over 60 μ in diameter appear in the ovaries of rats between the 15th and 20th days of age. Engle (1931*a*) found pseudomaturation spindles, which appear only in ova of full size, first evident in 16 day old mice and no follicles more than 180 μ in diameter in 14 day old mice. Smith and Engle (1927) found that 10 day old mice treated with gonad-stimulating pituitary implants had to have daily implants for 5 days in order that full ovarian response should be attained, whereas 17 day old mice showed full response in 36 hours to 3 days. Corey (1928) found practically no ovarian response to pituitary extracts in rats until after the 15th day, and Selye and Collip (1933) found no follicular maturation in 6 to 12 day old rats treated with anterior pituitary-like hormone (see also Zondek, 1931). In rabbits (Hammond and Marshall, 1925) the antrum develops later than the 10–11th week of life. Hertz and Hisaw (1934) were able to obtain definite follicular response to follicle-stimulating and luteinizing hormones only in juvenile rabbits (12 to 13 weeks old), not in infantile rabbits. Casida (1935) reports that pig ovaries show definite response to pituitary hormones only when antrum-containing follicles are present.

Nonetheless, fully potent pituitaries are present in 5 to 8 day old rats (Smith and Engle, 1927; Lipschutz, Kallas and Paez, 1929) as judged by their effects in transplantation to immature recipients. It would seem, then, that the at-

tainment of a certain degree of follicle maturity and full ovum size is necessary before activation of the pituitary hormones can be attained in developing animals. It is to

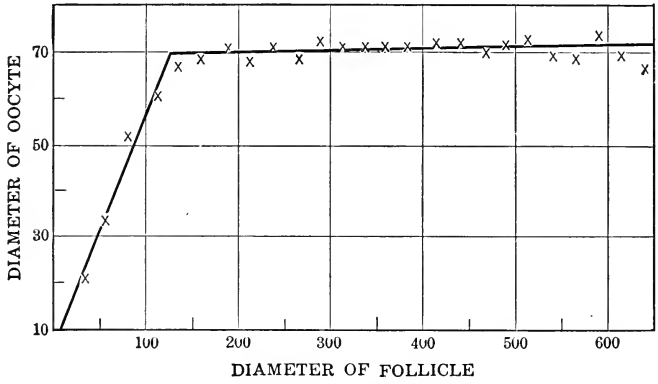

FIG. 7. Showing the relation of ovum growth to follicle growth. Data on the mouse. (From Brambell, 1930, courtesy of The Macmillan Company.)

be remembered, however, that the release of substances from the normal gland *in vivo* and the injection of excised

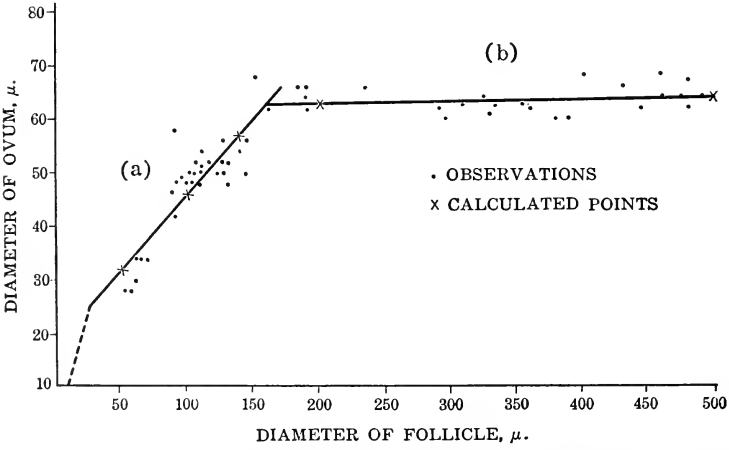

FIG. 8. Same as Fig. 7. Data on the rat. (From the *Proceedings of the Royal Society.*)

preparations are not comparable phenomena. Furthermore, dwarf mice pituitaries can stimulate ovarian growth in immature recipients (Smith and MacDowell, 1931) yet their

follicles do develop to the stage of antrum formation. The absence of eosinophile cells in the pituitaries of dwarf mice may, however, indicate the absence of a necessary link in the chain of steps involved in the hypophysis-gonad relationship.

Whatever the effect of ovarian maturation upon the pituitary may be, it is plain that no follicular response to pituitary hormones occurs until the time when full sized ova are present. Does this mean that the maturation of the follicle

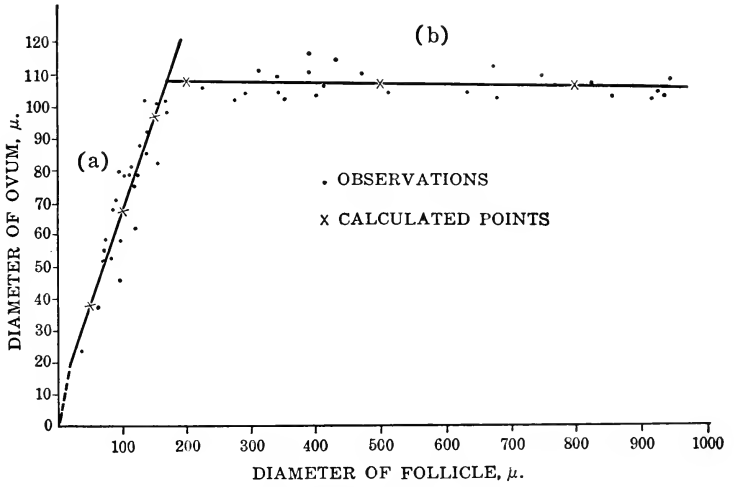

FIG. 9. Same as Fig. 8. Data on the ferret. (From the *Proceedings of the Royal Society.*)

is dependent initially upon some influence of the ovum, or is the simultaneous development of the ovum to full size and follicular growth to stimulable size a coincidence only? The ovum may grow to full size without an investiture of follicle cells as attested by the frequent presence of such ova in the ovaries of dwarf mice (Pincus, unpublished data). On the other hand, anovular follicles do occur in mammalian ovaries (League and Hartman, 1925) though those of large size represent follicles with completely resorbed ova (Engle, 1927*b*). It is interesting to note also that frequent production of anovular follicles from the germinal epithelium takes place in senile rats (Hargitt, 1930).

That the growth of the follicle beyond the antrum stage is independent of the growth of the ovum is amply evident from the data presented by Brambell (1928), Parkes (1931)

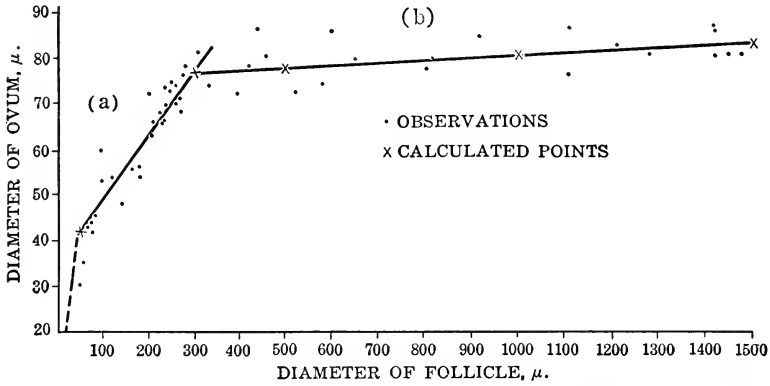

FIG. 10. Same as Fig. 7. Data on the pig. (From the *Proceedings of the Royal Society.*)

and Pincus and Enzmann (1936*b*). In Table III are presented the data collected by Parkes on the relation of ovum size to body weight and follicle size in seven species of mammals.

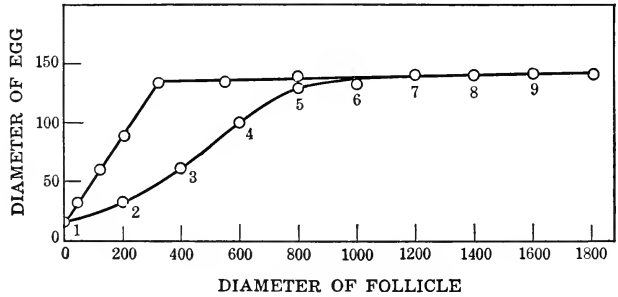

FIG. 11. Same as Fig. 7. Data on the rabbit. The lower curve represents ovum diameter plotted against follicle diameter for the nine types of follicles (see Plate III) distinguished by Pincus and Enzmann, 1936*b*.

Figures 7 to 10 relate the various diameters of ova to the diameters of the enclosing follicles. In the rabbit, Pincus and Enzmann (1936*b*) have identified 9 types of follicles each distinguished by characteristic features of the developing ovum, granulosa and theca (see Plate III). When the

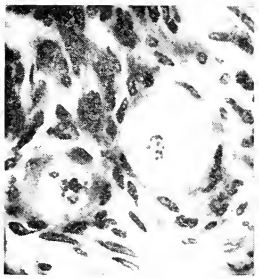

FIG. 1

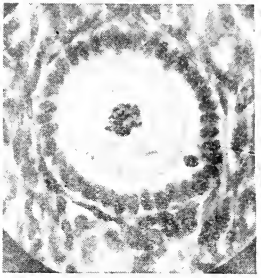

FIG. 2

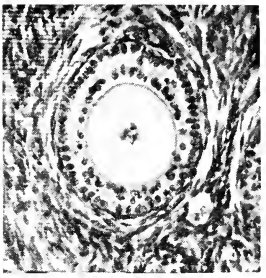

FIG. 3

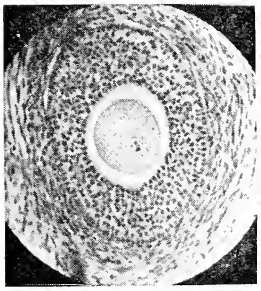

FIG. 4

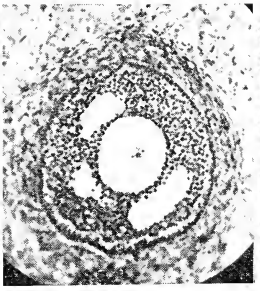

FIG. 5

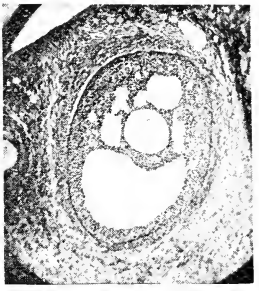

FIG. 6

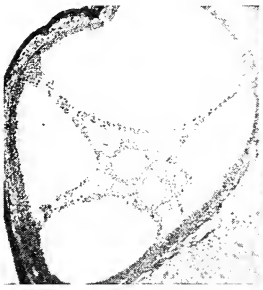

FIG. 7

FIG. 8

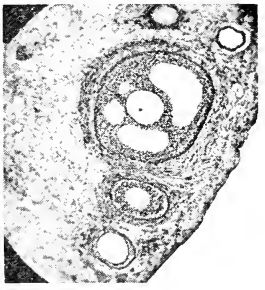

FIG. 9

PLATE III. The development of the follicle and ovum in mature rabbit does.

Fig. 1, Type 1 follicle to the left, type 2 follicle to the right. Nuclei in late condensation of prophase. Fig. 2, Follicle type 3. One row of follicle cells. Fig. 3, Follicle type 4. Two rows of follicle cells. Fig. 4, Follicle type 5. Many rows of follicle cells. Nucleus migrating to periphery. Fig. 5, Follicle type 6. Antra forming. Fig. 6, Follicle type 7. Numerous antra. Fig. 7, Follicle type 8. Ovum suspended in "spider web" of follicle cells. Corona formed. Fig. 8, Follicle type 9. Last preovulatory stage. Fig. 9, Showing position of various follicle types beneath the germinal epithelium.

mean ovum diameters are plotted against the mean follicle diameters (see Figure 11) the resulting curves essentially resemble those illustrated in previous figures, the full ovum size being attained in follicles of type 5 which just precede antrum formation.

TABLE III

SIZE OF THE GRAAFIAN FOLLICLE AT VARIOUS STAGES OF ITS LIFE-HISTORY
(From Parkes, 1931)

SPECIES	1 APPROXIMATE WEIGHT OF YOUNG ADULT FEMALE	2 DIAMETER OF OVUM	3 DIAMETER OF FOLLICLE WHEN OVUM IS FULLY GROWN	4 DIAMETER OF FOLLICLE WHEN ANTRUM APPEARS	5 DIAMETER OF FOLLICLE AT OVULATION
	gm.	μ	μ	μ	mm.
Mouse	2×10	70	125	200	0.55
Rat	1.2×10^2	63	160	200	0.90
Ferret	5×10^2	108	170	230	1.4
Rabbit	2×10^3	84	145	250	1.8
Baboon	1.2×10^4	83	180	310	6.0
Pig	5×10^4	76	300	400	8.0
Cow	4×10^5	—	—	—	15.0

The data plotted in this manner give no indication of the absolute rate of growth of ova though the relative growth rates may be deduced from the rising segment of the curves drawn to these data. These first segments are plotted in Figure 12, wherein it might be deduced that the ferret ovum grows at the most rapid rate, the pig ovum at the slowest rate, if comparable rates of follicular growth occur in the various species.

If it be assumed that the various types of follicles described by Pincus and Enzmann represent developments occurring at equal time intervals then the lower curve of Figure 11 may be taken as a representation of the growth curve of the ovum. The sigmoid shape of this curve is in fact reminiscent of general growth curves. It cannot be taken as a true growth curve, however, until the time necessary for the development of each type of follicle is accurately known. Such information might very well be obtained from ovaries subjected to x-irradiation and examined at various intervals after exposure.

The data of Arai (1920*a*) give a slight indication of the rate of growth of ova since his tables show no ova above 20 μ in diameter in 1 day rats, the first appearance of 20 to 40 μ in ova in 3 day rats, the first appearance of 40 to 60 μ ova in 10 day rats, and ova over 60 μ in rats over 15 days of age. Thus it may be inferred that growth to full size is attained in a little over two weeks. Whether this time is taken also in adult animals is not known exactly, but the minimum period is at least ten days since irradiated mice produce fertile eggs up to ten days after irradiation (Parkes, 1926–27). This implies that there is a sensitive period to x-irradiation in young ova. Marshak (1935) has shown that the pachytene stage of meiosis is especially sensitive to x-rays, and young ova enter into a modified pachytene shortly after leaving the germinal epithelium.

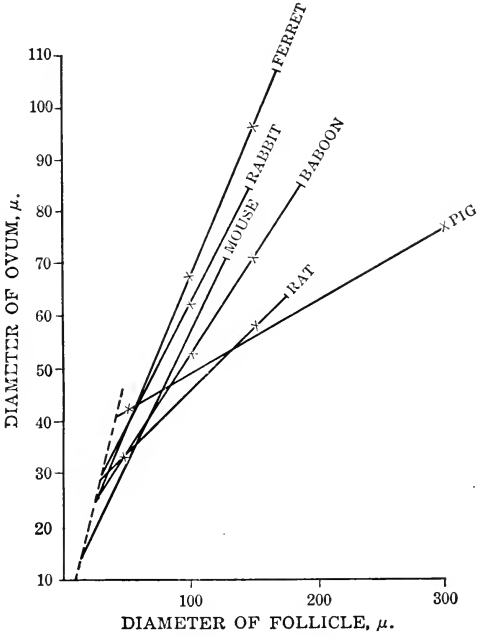

FIG. 12. Same as Fig. 7, showing comparative growth of the ovum in the various species of mammals. (From the *Proceedings of the Royal Society*.)

Not all ova grow to mature size. This is evident at once from Arai's data which show that an average of 0.8 per cent of the ova under 20 μ in diameter attain a diameter greater than 60 μ, and only 2.7 per cent reach 20 to 40 μ in diameter. The factors concerned in the atresia of young ova are as unknown as those determining their growth.

The absolute size attained by mature ova varies from species to species, but the limits are rather narrow (espe-

TABLE IV

ESTIMATES OF THE DIAMETER OF FULL-GROWN MAMMALIAN OVA
(From Hartman, 1929)

ANIMAL	MOST PROBABLE SIZE OF EGG IN MICRA
Monotremata	
Platypus	2.5 mm.
Echidna	3.0 mm.
Marsupialia	
Dasyurus	240
Didelphis	140–160
Edentata	
Armadillo	80
Cetacea	
Whales	140
Insectivora	
Mole (Talpa)	125
Hedgehog (Erinaceus)	100
Rodentia	
Mouse	70– 75
Rat	70– 75
Guinea pig	75– 85
Lagomorpha	
Rabbit	120–130
Carnivora	
Dog	135–145
Cat	120–130
Ferret	120
Ungulata	
Horse	135
Sheep	120
Goat	140
Pig	120–140
Cheiroptera	
Bat	95–105
Lemurs	
Tarsius	90
Primates	
Gibbon	110–120
M. rhesus	110–120
Gorilla	130–140
Man	130–140

cially in the placental mammals) when comparison is made with other vertebrates or inveterbrates. Hartman (1929) has extensively reviewed the available data on fixed and living material and has estimated the average size of the living ovum for a number of species making allowance for the degree of shrinkage in fixed preparations. His estimates

are given in Table IV. Subsequent measurements on living ova have proved these estimates to be on the whole remarkably exact. An excellent brief account of the comparative morphology of living mammalian ova in several species is given by Streeter (1931).

CHAPTER IV

THE DEVELOPMENT AND ATRESIA OF FULL-GROWN OVA AND THE PROBLEM OF OVARIAN PARTHENOGENESIS

Even when the ova have attained maximum size a majority of them are destined to degenerate. We have already mentioned that Allen, Kountz and Francis (1925) estimated that only 14 *per cent* of the medium sized follicles of the pig ovary attain maturity. Engle (1927*b*) finds that in the mouse the percentage of atresia among follicles with antra varies with the stage of the oestrus cycle, the maximum percentage of 86 *per cent* being recorded at the cornified cell stage. While the *percentage* of atretic follicles with mature ova was highest at the oestrus stage the maximum *number* was observed at the beginning of the dioestrus. This is obvious from the data of Table V and Figure 13 which summarize the data on 50 ovaries from non-pregnant mice taken at four stages of the cycle. These data include small atretic follicles as well as antrum-containing follicles, but the fact that the data for pseudomaturation spindles (which occur only in full sized ova) parallel those

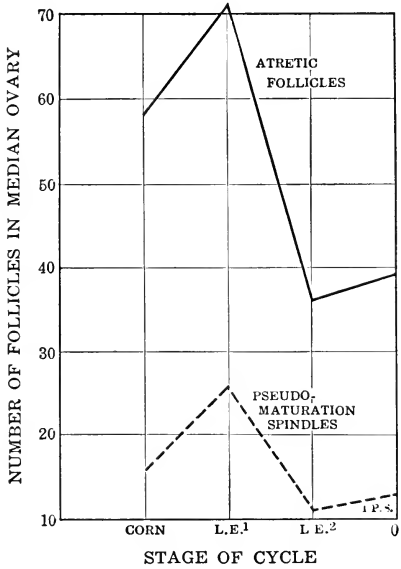

FIG. 13. Showing the number of atretic follicles and pseudomaturation spindles in the median ovary at four stages of the oestrus cycle in the mouse. (From the *American Journal of Anatomy.*)

for follicles indicates that the total number of atretic mature ova reach their maximum in early dioestrus shortly after ovulation. This is doubtless due to the continued formation

TABLE V

THE DEGREE OF ATRESIA OF OVARIAN FOLLICLES OF THE MOUSE AT FOUR STAGES OF THE OESTRUS CYCLE. (From Engle, 1927*b*)

STAGE OF CYCLE	MEDIAN OF SPINDLES	AVERAGE OF SPINDLES	RANGE OF ATRESIA	MEDIAN OF TOTAL ATRESIA	AVERAGE OF TOTAL ATRESIA	RANGE OF TOTAL ATRESIA
Corn	16	18.5	6–40	59	60	36– 85
LE[1]	26	28.4	7–51	71	77.9	50–129
LE[2]	11	11.2	5–17	36	42.1	29– 63
O	13	12	0–26	39	39.8	15– 70

of antrum-containing follicles at a fairly high rate for a short time after ovulation.

We have seen that ovogenesis continues during pregnancy. Engle's data demonstrate that the formation and atresia of full-grown ova also occurs during pregnancy, for he observed an appreciable number of pseudomaturation spindles in ovaries taken during the first 4½ days of pregnancy. These data are summarized in Table VI and Figure 14. It is notable that both the total amount of atresia and the atresia of mature ova is less throughout this period than during the period of least destruction in non-pregnant mice. Unfortunately, Engle does not give the *percentages* of atresia during early pregnancy.

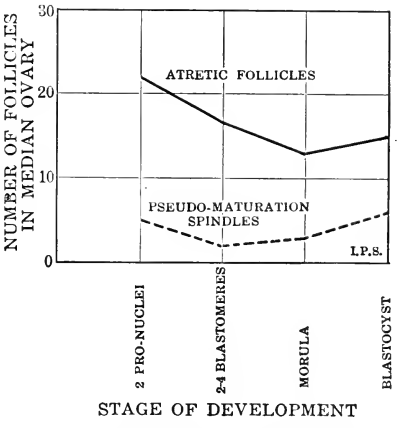

FIG. 14. Showing the number of atretic follicles and pseudomaturation spindles in the median ovary at four stages in early pregnancy in the mouse. (From the *American Journal of Anatomy*.)

The presence of cycles of atresia and growth in animals

other than the mouse has already been noted (Evans and Swezy, 1931). According to Asami (1920) the rabbit exhibits a constant rate of follicular atresia before and after

TABLE VI

THE DEGREE OF ATRESIA OF OVARIAN FOLLICLES OF THE MOUSE AT FOUR STAGES OF EARLY PREGNANCY. (From Engle, 1927*b*)

STAGE OF TUBAL OVA	MEDIAN OF SPINDLES	AVERAGE OF SPINDLES	RANGE OF SPINDLES	MEDIAN OF TOTAL NUMBER ATRESIA	AVERAGE OF TOTAL NUMBER ATRESIA	RANGE OF TOTAL NUMBER ATRESIA
To 2 pronuclei	5	5	0–15	22	23	11–41
2 to 4 blastomeres	2	2.5	0– 7	17	18	10–31
Morula	3	5.6	2–20	13	16.5	7–38
Blastocyst	6	7.1	3–14	15	17.3	14–28

pregnancy. Pincus and Enzmann (1936*b*) found that the younger follicles (types 1, 2 and 3—Plate III) of the rabbit show a much lower percentage of atresia than the larger follicles.

The atresia of mature ova can be prevented by pituitary hormones. This is deduced from the phenomenon of superovulation observed in animals receiving pituitary implants (Smith and Engle, 1927; Smith, 1932). These authors describe, for example, the presence of 49 ova in the tubes of a mature mouse receiving anterior lobe implantations. An adult mouse produces from six to twelve corpora lutea at an ovulation, the absolute number varying with weight of the mouse, the number of previous pregnancies, and certain genetic factors (MacDowell and Lord, 1925; MacDowell, Allen and MacDowell, 1929). In Smith and Engle's mice the largest number of ova ever found in one tube of a normal mouse was seven, and in an immature mouse showing superovulation a maximum of 48 ova was observed in a single tube. Thus the maximum number normally found in one tube is 14.5 *per cent* of the maximum number superovulated. Furthermore, if we assume from MacDowell's data that 9 is roughly the number of ova normally ovulated this is 18 *per cent* of the 49 superovulated in the adult mouse. These percentages agree with the estimations of *per cent*

of antrum-containing follicles maturing. The paucity of antrum-containing follicles and reduction of atresia is directly noted by Smith and Engle. Finally, the ovulated ova are fertilizable although Engle (1931*b*) found evidences of the degeneration of a number of them in the fallopian tubes.

An interpretation of the foregoing data is that normally only a limited amount of pituitary secretion is available to the ovary and consequently only a certain percentage of the ova are able to obtain the amount necessary to prevent their atresia, whereas in animals receiving large amounts of pituitary hormones from implants an abnormal number of ova have available sufficient amounts of atresia-suppressing hormones. It cannot be decided, however, whether the effect on the ova is directly exerted by these hormones, or whether the stimulated follicle tissue produces substances ensuring normal ova, or whether some extraovarian substance released into the circulation by pituitary stimulation reacts upon the ova.

Loeb (1917; see also Meyer, 1913) has indeed suggested that the ovum itself is the controlling factor in follicle development citing the frequent presence of mitoses in follicle cells adjacent to the ovum as well as certain histological evidence that the cumulus oöphorus develops under the influence of the ovum (Walsh, 1917). Allen and his collaborators (1924) also maintained that the ovum is the dynamic center of the follicle apparently on the assumption that the mitosis-inducing action of oestrin upon vaginal and uterine epithelium is reflected in the higher mitosis rate in cells adjacent to the ovum because the ovum either produces oestrin or induces oestrin formation. In the opossum the presence of many atretic ova is correlated with prolongation of the dioestrus interval (Hartman). This supposed oestrinogenic action of the ovum has, however, been largely controverted (1) by the discovery of oestrin in corpora lutea as long as two weeks after ovulation (see Allen, 1932) and (2) by the observation that oestrin is produced in x-rayed

ovaries lacking ova (Parkes, 1926–27). This evidence, however, does not prove that normally oestrin-production may not be under the control of the action of pituitary hormones upon the ovum itself.

In fact, aside from the presumable atresia-inhibiting influence, there seems to be only one other clearly evident influence of pituitary hormones upon the activities of the

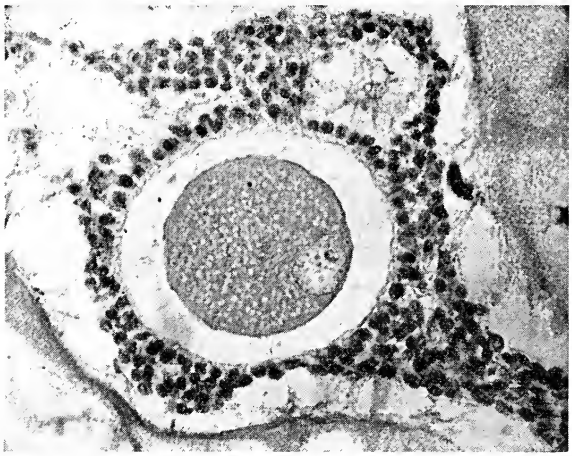

FIG. 15. Ovum removed from a preovulatory follicle of an unmated rabbit showing the vesicular nucleus. (From the *Journal of Experimental Medicine.*)

ovum. That is that the production of the first polar body is dependent upon stimulation by pituitary hormones.

Since this phenomenon is of some consequence to any discussion of the activation of mammalian eggs the writer, in collaboration with Dr. E. V. Enzmann (Pincus and Enzmann, 1935), has undertaken an examination of the mechanism of polar body formation in the rabbit ovary. The rabbit was chosen for these experiments because it ovulates only after copulation and the ova are liberated regularly between 9½ and 10½ hours after copulation (see Heape, 1905; Walton and Hammond, 1932; Pincus, 1930; Pincus and Enzmann, 1932). Furthermore, the mature ova form polar bodies only after copulation. According to Heape (1905)

two polar bodies are formed in the ovary by 9 hours after copulation. Our observations indicate that only the first polar body is given off in the ovary and then the metaphase plate of the second polar spindle is formed. Robinson (1918) observed in the ferret, which also ovulates only after copulation, that only the first polar body is given off in the ovary some time after copulation.

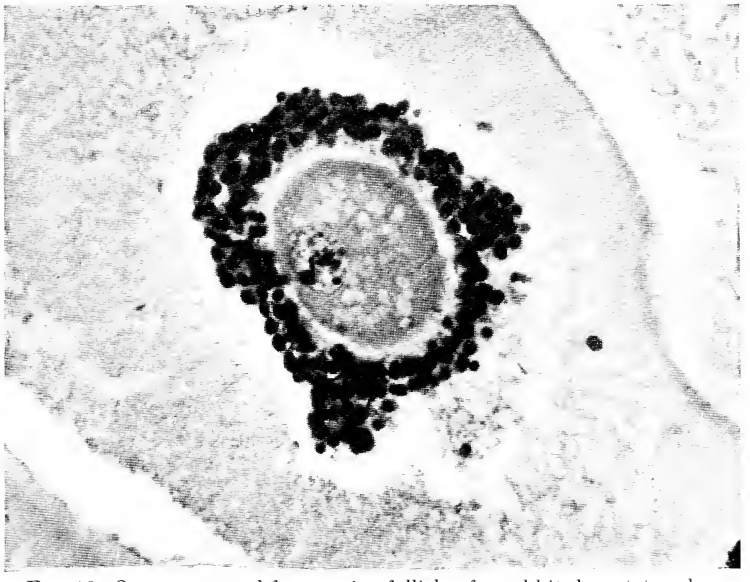

FIG. 16. Ovum removed from a ripe follicle of a rabbit doe at two hours after copulation. Note beginning of chromatin condensation. (From the *Journal of Experimental Medicine.*)

Before copulation occurs the mature ovum contains a single large vesicular nucleus about 30 microns in diameter (Figure 15; see also Plate III, Figs. 4 and 5). At two hours after copulation signs of change are partially evident: some of the ripe ova show the beginnings of tetrad formation in the nucleus but the nuclear membrane is still intact (Figure 16). By four hours after copulation the tetrads of the first polar spindle are formed and the nuclear membrane is ordinarily dissolved (Figure 17). The metaphase plate has a diameter of a little over 10 microns. The first polar

body is given off and the second polar spindle formed at or shortly after 8 hours post coitum (Figure 18). The follicle enlarges during this period also, the first signs of follicular development being evident at two hours after copulation. An exactly similar sequence of events occurs when prolan (pregnancy urine extract) or anterior pituitary extracts are injected.

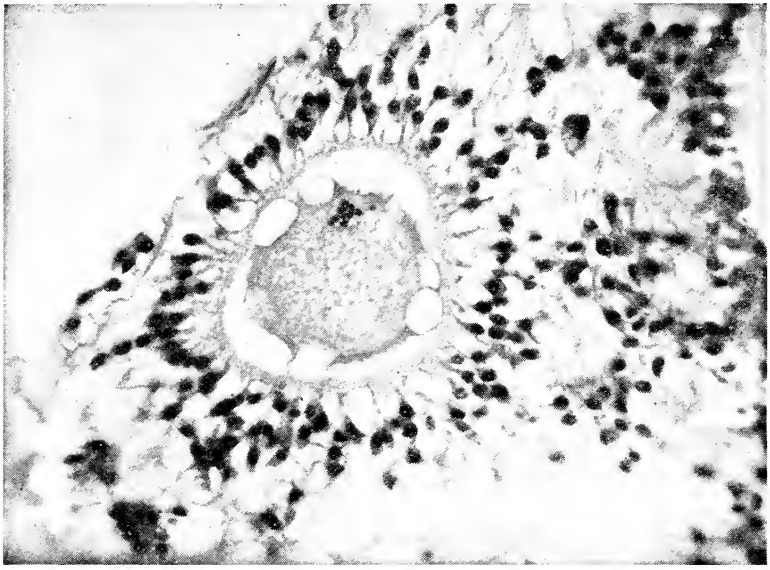

FIG. 17. Ovum from follicle of rabbit doe taken 4 hours after copulation. Formation of metaphase plate and dissolution of nuclear membrane. (From the *Journal of Experimental Medicine.*)

It has been definitely established that prolan and anterior pituitary hormones cause ovulation when injected into the rabbit (Bellerby, 1929; Friedman, 1929). The ovulation occurring after copulation occurs because of the increased level of pituitary hormones secreted into the blood. This level is increased by nervous stimulation of the pituitary consequent on the orgasm. It has been shown by Deansley, Fee and Parkes (1930) that hypophysectomy within one hour of copulation prevents ovulation in the rabbit (see also Smith and White, 1931), and McPhail (1933) has demon-

strated similarly that the critical period of secretion increase in the ferret occurs during the first hour of coitus. It seems evident, therefore, that pituitary secretions are responsible for the activation of the egg resulting in the formation of the first polar body and the second polar spindle. Furthermore, certain observations of Hinsey and Markee (1933) indicate that the threshold for activation is lower than the

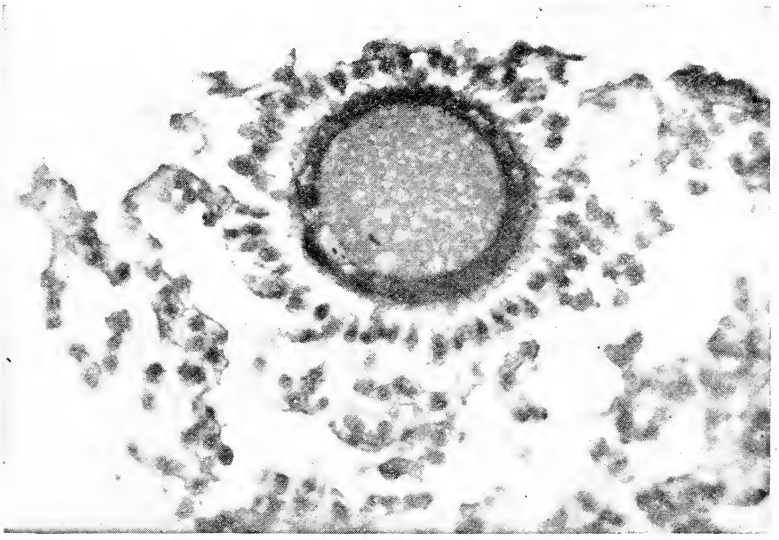

FIG. 18. Ovarian ovum of rabbit doe mated 9 hours previously. First polar body and second polar spindle. (From the *Journal of Experimental Medicine.*)

threshold for ovulation. They observed that ovulation does not occur in large sized (2.6 kilograms and over) hypophysectomized rabbit does if prolan injection is made more than four hours after hypophysectomy. And in small sized hypophysectomized does (less than 2.3 kilograms) prolan ovulation never occurs. Nonetheless in all non-ovulating does polar body formation took place. Friedgood and Pincus (1935) found that stimulation of the cervical sympathetic of the rabbit resulted in maturation phenomena in those preovulatory follicles which failed to liberate ova. The sympathetic nerves presumably stimulated in these cases

the secretion of sub-ovulatory amounts of hormone from the anterior pituitary. Finally, Pincus and Enzmann (1935) found definite ovum maturation with as little as ¼ the minimal ovulating dose of maturity hormone.

In the ovaries of rabbit does which have copulated and then received pituitary injections within six hours after copulation the writer has observed the accelerated ripening of a new set of follicles and the formation of the first polar body. In these rabbits no accessory ovulation occurred though the pituitary extract dosages were at least two to three times greater than those necessary to cause ovulation in unmated does. The absence of ovulation indicates presumably that the expulsion of ova can occur only from full sized follicles, whereas the activation processes may be initiated in ova whenever a sufficiency of pituitary hormones are available. It should be noted, however, that the nuclear activity occurred only in medium sized follicles and never in follicles without antra or with small antra forming. Since the ovum in the rabbit grows to some extent after antrum formation (see Figure 11) it is possible that functional maturity is attained at some time after antrum formation. A new crop of follicles begins to mature in the mated rabbit, and may certainly be stimulated to ovulate by the 4th day of pregnancy as Wislocki and Snyder (1931) have demonstrated by producing superfetation at that time with simultaneous pituitary extract and sperm administration. It is evident, therefore, that any attempt to dissociate *in vivo* the processes involved in polar body formation and those involved in ovulation depends in the mature rabbit upon hormone administration during the very short interval of time following copulation in the hope that active substances reaching the medium sized follicles will differentially affect follicular growth and ovum maturation.

Since the pituitary secretes a thyroid-stimulating as well as a gonadotropic hormone it is possible that maturation (and ovulation) is due directly to thyroid activity and only indirectly to pituitary stimulation. Pincus and Enzmann

(1935) tested this possibility by injecting crystalline thyroxin and thyroprotein into rabbit does on heat. In no instance did ovulation occur but large doses of thyroxin did initiate follicular atresia and a limited degree of ovum maturation. Again we see that atresia-inducing conditions also initiate maturation. The common feature of atretic follicles and preovulatory follicles is an isolation of the ovum from its connections with the follicular epithelium.

It is safe to conclude from the foregoing analysis that the formation of the first polar body in the rabbit ovary (and in the ferret's also) is dependent upon an increase of pituitary hormones in the circulating blood. It happens that in all spontaneously ovulating mammals except the dog the formation of the first polar body occurs in the ovary. Even in the dog (Evans and Cole, 1931) certain signs of nuclear maturation are observable in ovarian eggs. It is natural to infer that in spontaneously ovulating animals the pituitary level reached during oestrus is normally sufficient to induce ovulation as well as polar body formation.

Now it is notable that the atresia of ovarian eggs is often initiated by the formation of a maturation spindle. We have noted that Engle has designated the spindles of ova destined to atrophy as "pseudomaturation" although there is no evidence that they are in fact typically unlike those observed in normally maturing ova. Such spindles are observed only in ova of full size. Measurements of spindle containing ova in mouse ovaries give an average maximum diameter of 70 microns, and mature ova with vesicular nuclei had an average of 69 microns. The writer has also made careful examination of a large number of rabbit ovaries and has never observed typical spindles in immature eggs. What ordinarily occurs is a complex fragmentation of the chromatin (see Figure 19). That the spindles are the indices of impending atresia is indicated by the observation that when they are at a maximum the total follicular atresia is also at a maximum (see Figures 13 and 14). A possible interpretation of their presence may be that they occur as a

result of pituitary hormone action and the subsequent atresia of the ova containing them occurs because these ova are not liberated and fertilized. "Pseudomaturation" spindles have not been reported in hypophysectomized animals although atresia has.

It has long been the contention of certain observers of ovarian atresia that the apparent parthenogenetic develop-

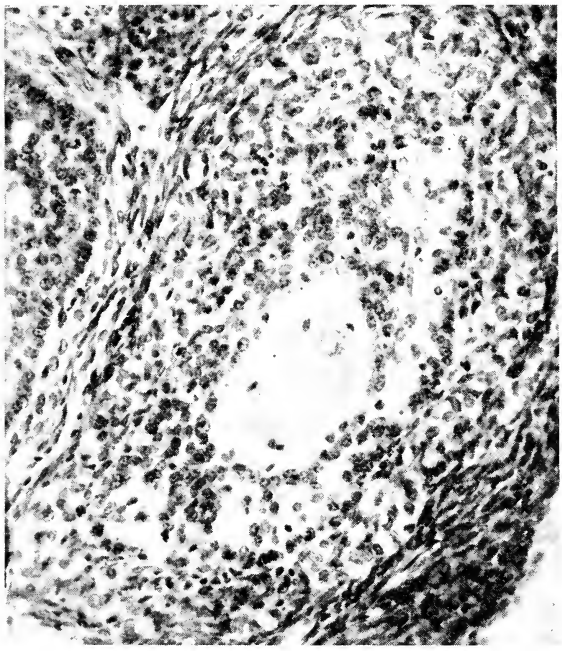

FIG. 19. Atretic ovum from type 3 follicle in the rabbit. Note fragmentation of cytoplasm and chromatin.

ment of ova destined never to be liberated is simply an incident of the process of degeneration and is not in fact true parthenogenesis (Hensen, 1869; Balfour, 1882; Sobotta, 1899; Janosik, 1897; Bonnet, 1899; Rubaschkin, 1906; Athias, 1909; Kingery, 1914; Kirkham, 1916; Stockard and Papanicolou, 1917; Addison, 1917; Long and Evans, 1922; Clark, 1923; Engle, 1927*b;* Kampmeier, 1929). These investigators have observed varied types of fragmentation of

egg nucleus and cytoplasm, most of which cannot be considered the result of true cleavage processes though in some instances a remarkable resemblance to cleaved ova is attained (see Plates IV and V). Another group of investigators generally admit that complex pseudoparthenogenetic fragmentation occurs, but claim that a varying number of ova enter into true parthenogenetic development (Pflüger, 1863; Flemming, 1885; Paladino, 1887; Löwenthal, 1888; Schöttländer, 1891; Henneguy, 1893; Grusdew, 1896; Rabl, 1898; Gurwitsch, 1900; Spuler, 1900; Van der Stricht, 1901; Loeb, 1901, 1905, 1911, *a* and *b*, 1912, 1915, 1923, 1932; Newman, 1912, 1913; Sansom, 1920; Häggström, 1922; Courrier and Oberling, 1923; Courrier, 1923; Branca, 1925; Bosaeus, 1926; Lelievre, Peyron and Corsy, 1927). The resolution of such alternative points of view depends first of all upon a clear definition of what parthenogenesis is and secondly upon the interpretation of the ovarian structures designated as embryonic.

If by parthenogenesis is meant the development of a mature individual from an unfertilized egg then it is at once certain that parthenogenesis does not take place in mammalian ovaries. If, on the other hand, a cleavage of the ovum with an equational division of the chromosomes is the criterion then there is some evidence (Sansom, 1920; Branca, 1925; Engle, 1927*b*) that occasionally parthenogenesis occurs in ovarian eggs (see Plates IV and V). Certainly it is not permissible to consider as parthenogenesis an exact reproduction of events taking place in the fertilized egg, since it is well known, for example, that parthenogenetic individuals arise from ova in which second polar body formation is suppressed.

It seems appropriate, in seeking an understanding of the physiological processes occurring in developing eggs, to distinguish between parthenogenesis and activation. A definite series of physical and chemical events ensue in eggs treated by agents inducing parthenogenesis. An apparently identical set of changes occurs at fertilization. This process

which Needham (1932) has designated "an opening of doors" in the cell initiates the development of the ovum and makes of a static cell one capable of transformation. What happens subsequent to the activation process is often independent of the process itself. The probability of cleavage and the formation of a complete individual depends in part on the nutritional environment and the chromosome constitution of the activated egg.

The activation process in non-mammalian ova has been described in physico-chemical terms (see J. Loeb, 1913; F. Lillie, 1919; Just, 1928; Runnström, 1933; Whitaker, 1933; R. Lillie, 1934). There exists no similar information particularly for the ovarian eggs of mammals. The only established index of an activation of ovarian eggs is the described formation of the first polar body. It is conceivable that this represents the first step in an activation process that would go to completion if conditions were propitious. Perhaps the same pituitary stimulus that induces polar body formation might cause the formation of a cleavage spindle. The first cleavage spindles observed by Branca (1925) may then be considered the result of an activation process carried to completion because adequate pituitary stimulation was available. On this basis the liberation of ova from the ovary results in such a change of environment that the stimulus to completion of activation is ordinarily no longer available. Similarly mature ova retained in the ovary at the time of ovulation ordinarily degenerate either because the proper type of pituitary hormone is not active (*cf.* Hisaw's conception of the alternative action of follicle stimulating and luteinizing hormones) or because of the partition of the active hormone to other tissues (*e.g.*, corpora lutea).

We may consider two further alternative explanations of the activation of ovarian eggs. It is possible that activation occurs in the ova of degenerating follicles because (1) the breakdown of cells near the ovum results in the release of activating substances or (2) the initial stages of atresia in the egg cytoplasm frequently involve structural

changes in the egg cytoplasm which are identical with those changes occurring during normal activation.

According to the first of these two alternatives cell division stimulating substances are released as break-down products (Gutherz, 1925). That such substances are actually formed by mammalian cells has been attested by the study of the growth of tissue cultures (Carrel, 1924; Fischer, 1925) where they have been given the name trephones. Furthermore, signs of atresia in theca and granulosa cells are cytologically evident before signs of ovum breakdown. It has never been conclusively demonstrated, however, that trephones can activate ova (but see Haberlandt, 1922). On the other hand, it is conceivable that regardless of trephone action, the degeneration of follicle cells leads to a stimulating concentration of cytolizing substances (*e.g.*, fatty acids which are known to act as activating agents) or even to a sufficient hypertonicity in the region of the ovum.

The second of these alternatives implies that "the opening of doors" occurring in normal activation is an aspect of degeneration. Atresia certainly involves changes in the colloidal structure of cells, and we have pointed out (*vide supra*) that definite changes in cortical structure mark the activation process. It is interesting, therefore, to note that the cytological appearance of the cytoplasm of retained ova with spindles is markedly similar to that of fertilized eggs. Thus the cytoplasm of unfertilized eggs have upon fixation a rough coarsely reticular appearance (see Figure 15 and Plate III, Fig. 4), whereas retained ova with spindles, like normally activated or fertilized eggs, have a uniformly granular cytoplasm (Figure 18).

Whether stimuli from degenerating follicle cells or endogenous structure changes are involved, it is evident that these factors are in turn conditioned by the supply of available hormone. Insufficient pituitary hormone results in the creation of ovum activating conditions. This is on the face of it, in direct contradiction of the first hypothesis which states that a supraliminal supply of hormone may also

initiate activation. But this contradiction may be resolved if we consider that the same *conditions* may be created by either active pituitary stimulation or absence of it.

It has been shown that pituitary hormones themselves

TABLE VII

THE DEVELOPMENT OF OVARIAN EGGS OF THE RABBIT IN MEDIA CONTAINING VARIOUS HORMONE PREPARATIONS. (From Pincus and Enzmann, 1935)

	Time of Culturing	Number of Cultures	Medium	Results
A	20 min.	14	Ringer-Locke + 1 drop beef pituitary	Vesicular tetrads formed in all cases
	2 hrs.	11	Ringer-Locke + 2 drops beef pituitary	In some cases vesicular tetrads and some free tetrads were formed. Some formed polar bodies
	24 hrs.	9	Ringer-Locke + 1 drop maturity hormone	Vesicular tetrads in all cases except 3 which had free tetrads
	25 hrs.	4	Ringer-Locke + 2 drops maturity hormone	Vesicular tetrads in all cases
	25 hrs.	7	Ringer-Locke + 3 drops maturity hormone	Vesicular tetrads, free tetrads, structures resembling fusion nuclei
	2 hrs.	18	Ringer-Locke	Vesicular tetrads and free tetrads
	4 hrs.	3	Ringer-Locke	Free tetrads
	6 hrs.	3	Ringer-Locke	Rudiment of first polar spindle
	20 hrs.	16	Ringer-Locke	Vesicular tetrads, free tetrads, fusion nuclei
B	24 hrs.	6	Plasma + 1 drop thyroxin	
	22 hrs.	4	Plasma + 3 drops thyroxin	
	22 hrs.	3	Plasma + 4 drops thyroxin	All cultures showed about the same phenomena which included tetrad formation in all cultured eggs. In some of the cultures polar bodies formed, or the vesicular membrane dissolved
	24 hrs.	8	Plasma + 6 drops thyroxin	
	24 hrs.	4	Plasma + 8 drops thyroxin	
	20–24 hrs.	22	Plasma + 2 drops Ringer-Locke sol.	
	20–24 hrs.	8	Plasma + 6 drops Ringer-Locke sol.	

do not act directly upon the ova (Pincus and Enzmann, 1935) by experiments in which ovarian ova with vesicular nuclei were cultured in media containing various pituitary extracts. The data of these experiments are summarized in Table VII–A. They show that in both the extract-containing media and the extract-free media maturation proceeds at about the same rate. Furthermore thyroxin which causes a certain degree of maturation when injected *in vivo* (see page 51 above), causes *in vitro* no further degree of development than thyroxin-free controls (Table VII–B). The isolation of the ova from the normal follicular environment is sufficient to initiate activation. This implies that in preovulatory follicles maturation is caused by either (1) the mechanical separation of the ovum and its corona or (2) the removal of an inhibiting influence. Mechanical separation undoubtedly occurs (*cf.* Plate III, Figs. 8 and 9), but one cannot estimate the exact degree of isolation necessary to initiate maturation, for it is certain (Pincus and Enzmann, 1935) that maturation is initiated in ova still having strands connecting them to the follicular epithelium. In certain forms (*e.g.*, man) the ova remain embedded in the cumulus mass till just before ovulation and the corona forms late. It is notable that Allen, Pratt, Newell and Bland (1930*b*) were able to obtain only one maturation stage in some two hundred ova recovered from 3 to 20 mm. follicles. The writer (unpublished data) has observed one maturation occurring in a primate ovarian ovum, but when primate ovarian ova are cultured *in vitro* considerable nuclear activity occurs. During the first stages of pituitary-induced maturation in the rabbit a secretion of secondary liquor folliculi is observed (Pincus and Enzmann, 1935). This secretion may remove an activation-inhibiting influence. The maturation observed in ova of atretic follicles may be due to a similar sort of secretion rather than to simple isolation of the ovum from its follicular epithelium.

On the basis of the foregoing considerations one might conceivably encounter occasional evidences of activation

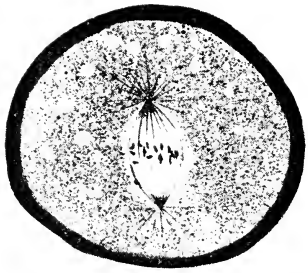

FIG. 1

FIG. 2

FIG. 3

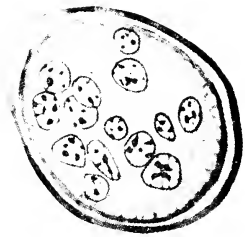

FIG. 4

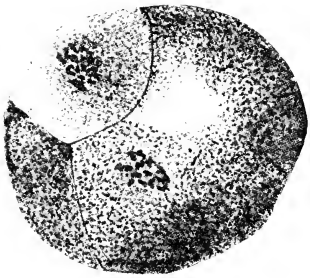

FIG. 5

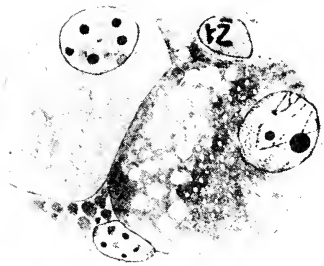

FIG. 6

PLATE IV. Various stages in the development of the mature oöcyte. (From the *Archives de Biologie.*)

Fig. 1, First maturation spindle—guinea pig. Fig. 2, Binucleated ovum, chromosomes oriented for the metaphase of a mitosis—guinea pig. Fig. 3, Binucleated ovum with formed maturation spindles—mouse. Fig. 4, Multinucleate cytoplasm—mouse. Figs. 5 and 6, Typical uninucleate cleaved ovocytes. Fig. 6 shows deutoplasmic extrusions—guinea pig.

where alterations in normal hormone balance occur which are sufficient to cause a preponderating activation stimulus. Such may in fact be the basic cause of certain undoubtedly normal early development in ovarian eggs reported by a number of observers. In Plate IV, Figures 1 to 3 and Figure 1, Plate V, are presented various stages of pre-cleavage development found in ovarian eggs. The multinucleate condition of the egg of Figure 4 may be due to chromatin fragmentation, but the cleavages of the eggs of Figures 5 and 6 of Plate IV and Figures 2 and 3 of plate V are completely normal. It seems clear that at any one of these stages definite atresia of the ovum may set in, preventing further development. Similar arrests of development may occur in parthenogenetically activated invertebrate ova if the activating treatment is not carefully controlled (*cf.* Loeb, 1913; Just, 1928). Entrance into the cleavage process is likewise dependent upon a rather nice balance of developmental events. Furthermore, the processes involved in cleavage may indeed be independent of the activation process. Runnström (1933) has shown that sea-urchin eggs poisoned by monoiodoacetic acid can be fertilized but that segmentation soon ceases and ordinarily just before the dissolution of the nuclear membrane of the first cleavage division.

In later chapters we shall discuss further the problems involved in parthenogenetic activation. Now it is sufficient to indicate that there is a probability of activation of ovarian eggs but that a complete activation is dependent upon a balance of events which must presumably be rarely attained in the ovary. Even if the activation reaction proper occurs and segmentation ensues the probabilities that post-cleavage stages will be entered are made extremely small not merely because of the physical limitations imposed by the structure of the ovary, but because, as we shall demonstrate later (Chapter IX), the growth stage of the embryo is entered into only as the result of a definite hormonal stimulus during the luteal phase, and conversely is definitely inhibited by oestrin. It is therefore surprising that the blastula and

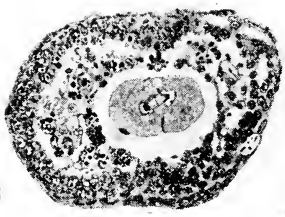

FIG. 1

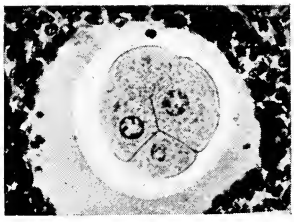

FIG. 2

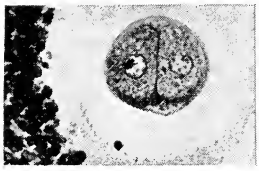

FIG. 3

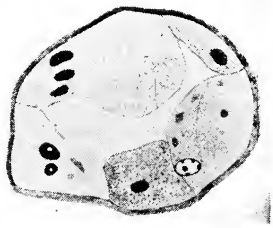

FIG. 4

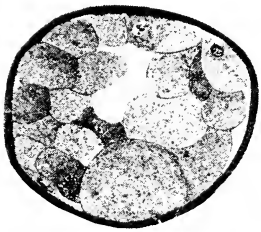

FIG. 5

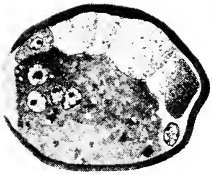

FIG. 6

FIG. 7

PLATE V. (Figs. 1 to 3 from the *Journal of Anatomy;* Figs. 4 to 7 from the *Archives de Biologie.*)

Fig. 1, Line drawing of a section through an atretic follicle of the mouse. First mitotic anaphase. Cytoplasmic division not completed—mouse. Fig. 2, Typical 4-celled ovarian ovum—water vole. Fig. 3, Typical 2-celled ovarian ovum with intact zona—water vole. Fig. 4, Early blastocyst of ovarian ovum—mouse. Fig. 5, Many-celled blastocyst in ovarian ovum—guinea pig. Fig. 6, Multinucleate blastocyst-like ovarian ovum—mouse. Fig. 7, Blastocyst-like ovarian ovum—guinea pig.

neurula-like formations, described by Courrier (1923) (see Figures 4 to 7, Plate V) and Courrier and Oberling (1923) and the atypical ovarian embryos observed by Loeb (1932) should be found. The solution to the controversy concerning their exact nature must await evidence as to the possibility of their formation by experimental means.

It can be seen that the chance of atretic degeneration continually besets the ovarian egg. The evidence indicates this process can be avoided only if sufficient pituitary hormone is available to the ovary. There exists also the possibility that atresia is endogenous in the sense that the ovum as a cell attains a certain maximum degree of development and then inevitably goes down hill. Only the sudden intervention of ovulation and fertilization prevents this process. Such a conception is scarcely amenable to experimental verification chiefly because of the intimate association of the ovum and its follicle. Furthermore, signs of ovum degeneration are preceded by degenerative phenomena in the granulosa cells. If the granulosa and corona cells act as nurse cells to the ovum it is obvious that their behavior must largely condition the behavior of the ovum. Often the ovum becomes detached from the granulosa and corona radiata and floats practically free in the liquor folliculi. We do not know to what extent diffusion of a sufficiency of nutritive substances through the liquor folliculi is possible. The problem of the viability and senescence of the ovum still awaits experimental attack.

CHAPTER V

METHODS EMPLOYED IN THE EXPERIMENTAL MANIPULATION OF MAMMALIAN OVA

The first investigators of living tubal eggs (Barry, Cruikshank, Bischoff, Spee, *et al.*) used rather laborious methods of dissecting the tubes (see Squier, 1932, for an interesting historical discussion). The modern technique of securing eggs from the fallopian tubes of most mammals is a fairly simple one. Nonetheless, certain surprising differences in the behavior of the obtained ova arise when exactly the same methods are applied to two different species. Among the laboratory mammals the rabbit is by far superior, and for one very simple reason, namely, rabbit ova seem to withstand the process of handling better than other ova. Mouse, rat and guinea pig ova, for example, begin to fragment very soon after removal from the tubes (Lewis, 1931; Gilchrist and Pincus, 1932; Squier, 1932; and Defrise, 1933) and to date it has been possible to observe at most one or two cleavages in culture, whereas rabbit ova will go through the whole course of cleavage and blastulation *in vitro*.

The long, fairly straight tubes of most mammals can easily be washed through by a Ringer-Locke or similar balanced salt solution. The writer has found that a Ringer-Locke solution to which has been added an equal amount of homologous blood serum is most useful. It is necessary only to free the tubes of their mesenteric connections, and if the tubes only are to be employed to cut them away from the uterus. It is ordinarily best to cut off the uterus at about one-half inch from the ampulla so that if washing backward toward the fimbria is desired a certain length of uterine lumen will be available for the guidance of the washing pipette. When ova are to be washed downward

from the fimbriated end of the tubes a rather broad bored capillary pipette is used; washing upward from the uterine end requires a very fine pipette. The ova are washed into Syracuse watch glasses and are easily observed under low magnification of a dissecting microscope.

In animals like the rat, mouse and guinea pig with coiled tubes a different procedure is followed. Here the coiled tubes are cut into several fairly straight portions and are squeezed with a pair of fine iris forceps or stroked gently with blunt needles. The contents of the tubal lumen are extruded and the ova are found among the cellular débris.

Ova from the uterus are obtained simply by flushing the uterine lumen with the washing fluid.

Allen, Pratt, Newell and Bland (1930*a*) describe a method for obtaining human tubal ova without removing the tubes or uterus. "The ovaries were examined as soon as possible after the abdominal cavity was opened. In some instances the findings at operation necessitated removal of the most recently ovulating ovary and its tube was not justified, the tube was flushed *in situ* and the corpus luteum alone removed from the ovary. This method consisted of clamping the cervix with a special clamp and injecting isotonic saline solution directly into the uterine cavity from above by hypodermic syringe while first one and then the other uterine tube was gently pinched by the assistant. The injected solution in most cases flowed back freely through the tube and was collected in a series of watch-glasses held beneath the fimbriated end. Apparently the development of valve-like folds of mucosa at the tubo-uterine junction as described for several mammals by Lee (1928) is not appreciable in woman. Usually from 10 to 30 c.c. was flushed through each tube. The most recent corpus was carefully excised from the ovary, and since it is a transitory structure, without sacrificing any considerable amount of ovarian tissue.

"It is believed that this method of flushing the tubes *in situ* is harmless to uterus and tubes and opens up new

possibilities, not only for the recovery of human ova, but also for checking the patency of tubes at operation.

"The tubes which could be removed were washed by direct injection through either the uterine or the fibriated ends after first trimming the tube carefully along the attachment of the mesosalpinx. The trimming seemed advisable, for otherwise when the tube was distended with injected fluid it would often kink badly.

"A search for human tubal ova is sometimes complicated by the follicle cells of the cumulus still surrounding the specimens which make difficult clear observation and certain identification. Although while fresh such specimens are fairly transparent, it is often difficult to observe or measure them accurately. Since it is probable that ova may remain in the tubes for three or more days, degenerative changes may be expected in a certain number of unfertilized tubal ova. Also small masses or balls of cells are often encountered in the tubes. These may originate in the peritoneal cavity, be pinched off from the fimbria of the tube, or (in cases where injected fluid is forced back through the tubes from the uterine cavity) derived from cast-off endometrium. Sometimes such cell balls contain structures which before sectioning can easily be mistaken for ova. For this reason unless an ovum is free from follicle cells or the cells of cumulus are partly dispersed, it would seem necessary that it be sectioned before certain identification is possible. Further check should also be made by histologic study of the most recent corpus luteum."

In obtaining both unfertilized and fertilized ova for culture *in vitro* the use of a warm washing solution is preferable. This is often practically difficult and rabbit ova at least are not materially affected by handling at room temperature over a period of several hours.

The usual methods of tissue culture have been employed in the cultivation of mammalian ova. These include the hanging drop with the ovum held in a plasma clot on a coverslip over a fluid-free cavity; a plasma clot occupying

the total area under a raised coverslip; the Carrel flask; and the watch-glass technique in which the sterile watch glass containing the culture medium is contained in a moist chamber. Blood plasma or serum ordinarily form the basis of the most successful culture media. The longest period of regular development of normally fertilized rabbit ova has been obtained by Lewis and Gregory (1929) who photographed the development of rabbit ova from the initial cleavage stages through late blastocyst stages. They placed the ova in homologous plasma upon glass slides. Pincus (unpublished data) has obtained similar development by this technique and also with ova grown in Carrel flasks. Lewis and Hartman (1933) observed the development of a Macacus rhesus ovum from the 2-cell to the 8-cell stage using the Lewis and Gregory technique. The ova of the rat, mouse, and guinea pig have failed to develop beyond one or two cleavages with the use of a variety of culture media. Thus Defrise (1933) used the following media for culturing rat ova: (1) Ringer's solution, buffered or not with sodium bicarbone; (2) Tyrode's solution, (A) isotonic with NaCl, milimol: (a) 120, (b) 136, (c) 151, (B) $K^+ - Ca^{++} - Mg^{++}$ equilibrium on the basis of the triangular diagram of Löewe, milimol: (a) 2.65 KCL, 1.90 $CaCl_2$, 2.20 $MgCl_2$, (b) 5.63 KCl, 3.60 $CaCl_2$, 0.52 $MgCl_2$: the above solutions were used at pH 6.8, 7.2, 7.6; (3) Tyrode's solution (NaCl: milimol 136 – KCl: 5.6 – $CaCl_2$: 2.16 – $MgCl_2$: 0.52 – $NaHCO_3$: 8.6 – $C_6H_{12}O_6$: 5.5) with the addition of gelatine 0.5 per cent; (4) Tyrode's solution (as above) with the addition of blood serum: (a) 1/1, (b) 3/1; (5) Tyrode's solution (as above) with the addition of plasma (heparin): (a) 1/1, (b) 3/1; (6) pure blood serum of (a) pregnant female, (b) male, (c) newborn; (7) plasma (secured from the heart and mixed with heparin): (a) pregnant female, (b) male; (8) spinal fluid (secured by suboccipital puncture); (9) foetal hystolymph (secured by Martinovitch's technique); (10) uterine fluid (II oestral period): (a) pure, (b) with the addition of blood serum.

In a few cases, in some of the above media, and especially in this: NaCl milimol 130 – KCl 2.65 – $CaCl_2$ 174 – $MgCl_2$ 1.18 – $NaHCO_3$ 8.6 – at pH 7.2 – drops III = blood-serum drops II, one or two mitoses were obtained. The addition of small quantities of embryonic extract, of rat folliculine, of extract of the anterior lobe of the hypophysis to the medium (either solid or liquid, natural or artificial) has not noticeably modified the culture results.

Squier (1932) using a less extensive variety of media was similarly unsuccessful with guinea pig ova (see also Lewis, 1931).

The limitations of the ordinary methods of tissue culture are discussed further in Chapter IX in connection with the investigation of the normal physiological environment of developing ova.

Nicholas and Rudnick (1933) have cultivated rat embryos upon the chorioallantois of the chick, but ovum development has not been studied. The embryos survive and differentiate over a considerable period of time in the foreign environment.

Cinematography of developing ova has been undertaken in a number of recent investigations. Standard motion picture cameras adapted for microphotography are employed.

For a study of the comparative behavior of ova *in vivo* and *in vitro* the writer has transplanted cultured ova into the fallopian tubes of rabbit does (see Pincus and Enzmann, 1934). The operative technique requires the use of a light anaesthesia, *e.g.*, either ether preceded by atropine sulphate injection to inhibit excessive mucous secretion or simple urethane anaesthesia. The exposure of both tubes and ovaries is had by a simple laparotomy. The ova are held in a special pipette with an opening in the tube above the capillary. This type of pipette permits one to take up a minimum amount of fluid with the eggs, and also prevents the ova from being drawn into the wide-bored portion of the pipette. The capillary portion is inserted into the upper $\frac{1}{5}$ of the tubes and the ova expelled by gentle pressure

on the bulb when the opening in the tube is closed over. No amount of pressure on the bulb will expel the ova if the opening is not closed. Extreme care should be taken to expel only the ova and the fluid containing them. If air is also pumped into the tubes it often blows the eggs down too far into the tubes or even into the uterus. Excessive fluid acts in the same way.

The writer (in collaboration with Dr. E. V. Enzmann) has also transplanted mouse ova into the fallopian tubes. Here it is necessary to slit the capsule and expose the tubal opening, which is slightly wider than at the ampulla, but not as wide as the rabbit's fimbriated opening. The tubes are observed under a dissecting microscope and the opening exposed by manipulation with iris or watchmaker's forceps. The delicate mouse ova are best handled in warm Ringer-Locke solution plus serum.

Nicholas (1933*a*) has transplanted rat ova from the fallopian tubes into the uterus. In this case the tubes are excised at the isthmus and the ova expelled from a capillary pipette into the uterine lumen.

CHAPTER VI

THE TUBAL HISTORY OF UNFERTILIZED EGGS

When ovulation occurs without fertilization the liberated ova enter the Fallopian tubes and eventually degenerate. In most polyovular mammals the ova are shed surrounded by an apparently sticky cumulus ovigerus so that a sort of plug is formed due to the adhesion of the various separate cumuli (see Plate VI, Figure 1). This cumulus mass remains more or less intact for some time and then the cumulus cells gradually become detached so that the ova finally float free. The opossum (Hartman, 1925) and sheep (Clark, 1934) appear to be exceptions since very few follicle cells surround the newly shed ova.

The chronology of egg passage in the tubes is best had in the rabbit where ovulation occurs at 9½ to 10½ hours after copulation.

The freshly ovulated ova enter the tubes and become massed together, due to the adherence of the sticky masses of cumulus cells. By 11 hours after copulation (about 1 hour after ovulation) this mass of cumulus cells containing the ova becomes securely lodged in the narrower portion of the tubes just below the broad, fimbriated end. On washing from the uterine end of the tubes this mass (see Figure 1, Plate VI) is first ejected, then the washing fluid. The ova remain thus massed together until about 17 hours after copulation, an occasional ovum separating out of the mass as early as 16 hours after copulation. Figure 2, Plate VI, is the photograph of an ovum still embedded in the mass of follicle cells at 16 hours after copulation. Figure 3 is the photograph of the single one of the 10 ova removed at the same time as that of Figure 2 that had separated out of the mass. Note a number of follicle cells still clinging to the egg.

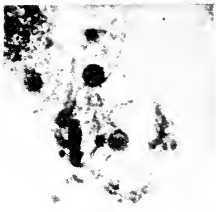

FIG. 1

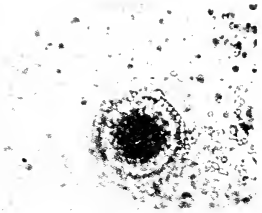

FIG. 2

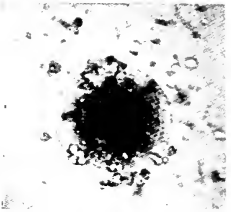

FIG. 3

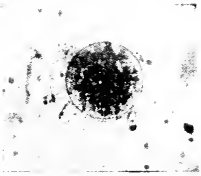

FIG. 4

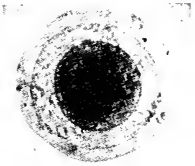

FIG. 5

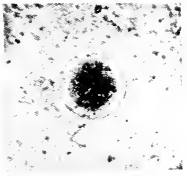

FIG. 6

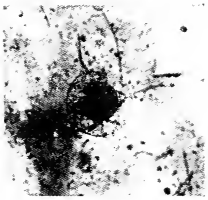

FIG. 7

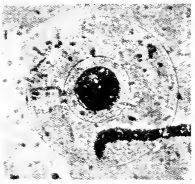

FIG. 8

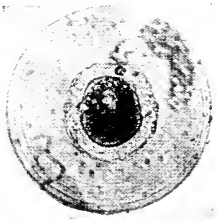

FIG. 9

PLATE VI. (From the Proceedings of the Royal Society.)

Fig. 1, Three ova in the cumulus mass recovered from the fallopian tubes of rabbit doe 12½ hours after a sterile mating. Fig. 2, An unfertilized ovum still in the cumulus mass 16 hours after a sterile mating. Fig. 3, Another 16-hour ovum free of the cumulus mass. Fig. 4, An ovum recovered 19 hours and 5 minutes after a sterile mating with no adherent follicle cells. Fig. 5, An ovum recovered 24½ hours after a sterile mating showing a definite albumin coating. Figs. 6–9, All from sterile matings at the following intervals after sterile copulation: 6. 43 hours, 30 minutes, 7. 73 hours, 40 minutes, 8 and 9. 96 hours, 45 minutes.

As they separate out of the cumulus mass the eggs emerge surrounded more or less by a few adherent follicle cells, and proceed down the tubes where these few adherent cells are lost. At 20 hours after copulation all the adherent cells are gone and a thin layer of albumen is laid down about the zona pellucida. Eggs washed out at this time show very clearly the transparent, shining zona pellucida about the yolky, granular egg cytoplasm, with an extremely thin albumen layer surrounding the zona (see Figure 4). The process involving the separation of the eggs out of the cumulus mass and the clearing off of adherent cells thus involves a period of about 3 hours. When eggs are washed out during this period one observes in a single washing all the stages described, eggs completely clear of adherent cells being preponderant toward the end of the period. One may even find an occasional egg still surrounded by adherent cells as late as 20 hours after copulation.

It is important for reasons that will be obvious later, to note that by 20 hours after copulation all rabbit ova are free of follicle cells and have begun to accumulate a layer of albumen. By 24 hours after copulation this albumen layer is quite appreciable (see Figure 5). Subsequently the ova descend to the uterine end of the tubes acquiring in their passage successive layers of albumen so that the albumen layer may eventually become several times the thickness of the egg itself (see Figures 6 to 9). The zona pellucida no longer presents the clear, shining appearance observed before the deposition of albumen. Most of the ova recovered from the tubes contain at least one polar body, occasionally two or even three. In some cases none have been observed but this may be ascribed to faulty observation as the eggs often come to rest with the polar body hidden.

The eggs enter the uterus between 72 and 96 hours after copulation. No more albumen is added and the eggs undergo rapid disintegration. It is, in fact, very difficult to recover unfertilized ova from the uterus. Pincus (1930) was unable

to obtain the full complement as indicated by the corpora lutea count. They are either rapidly resorbed or washed out into the vagina. The cytoplasm of eggs recovered from the uterus shows distinct evidences of degeneration (Figures 8 and 9).

The persistence of the corona radiata for some time after ovulation occurs regularly not only in the rabbit (*cf.* Yamane, 1930, 1935) but also in the mouse (Long, 1912), the rat (Gilchrist and Pincus, 1932), the dog (Evans and Cole, 1931), and man (Allen, Pratt, Newell and Bland, 1930*a*). It is notable that opossum ova with no surrounding cumulus mass enter the uterine portion of the oviduct in approximately twenty-four hours, whereas all available information indicates that in the higher mammals unfertilized ova entering the uterus do so at approximately 3½ days after ovulation. In the rat (and probably also the mouse) unfertilized ova apparently degenerate in the uterine portion of the tubes (Long and Evans, 1922; Mann, 1924). Albumen deposition about tubal ova occurs in the rabbit and opossum; in most other mammals the ovum traverses the tube surrounded only by the zona pellucida.

The dissolution of the cumulus mass surrounding newly liberated ova seems to involve a definite process in the tubes and is in all probability not due to an autogenous change in the cumulus cells themselves. In guinea pigs the fresh cumulus mass is so tenaciously adherent that it cannot be completely removed by dissection (Squier, 1932). Gilchrist and Pincus (1932) found that rat ova incubated in Ringer's solution did not become free of adherent cells even after many hours. In rabbit ova grown in blood plasma a fibroblast-like outgrowth of the cumulus cells occurs but nonetheless the radial connections to the zona pellucida are not lost (Pincus, 1930). The writer has also observed a similar outgrowth from the cells surrounding cultured human ova, but the extremely tenacious covering of follicle cells is not lost. The likelihood that a slow enzymatic process is involved in the freeing of the adherent cells is substanti-

ated by the great acceleration of this dehiscence in the presence of sperm (see Chapter VII).

The unfertilized ova of most mammals begin to show signs of degeneration when they reach the distal portion of the tubes. In the opossum clear evidences of degeneration are observed by twenty-four hours after ovulation when the

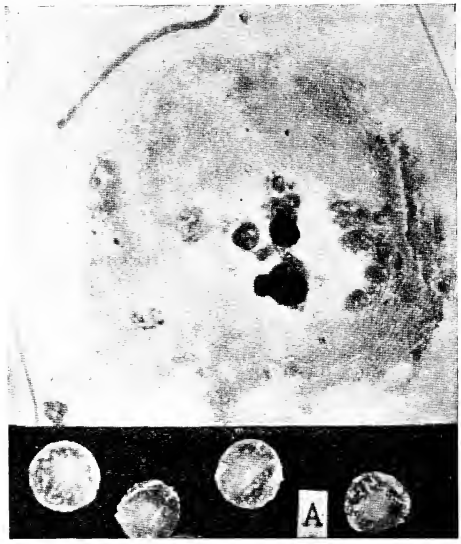

FIG. 20. Fragmenting opossum egg seven days after arriving in the uterus. Section of one of the eggs shown at A, containing three large chromatin masses almost free of cytoplasm. (From the *American Journal of Anatomy.*)

ova enter the uterus (Hartman, 1924). The degenerative changes have been described in detail by Smith (1925). The ovum may remain intact but develop a well vacuolized cytoplasm with clumped or fragmented chromatin. Ordinarily, a definite fragmentation of the whole ovum occurs (Figure 20), and the irregular blastomere-like formations may contain bits of fragmented chromatin or lack chromatin entirely. In some 300 opossum ovum sectioned and examined Smith never observed a true cleavage spindle, and

appropriately concludes that parthenogenetic development never occurs. Her statement that pregnant (or psuedo-pregnant) condition of the animals should favor parthenogenesis is not necessarily correct since activation may require special physiological conditions. In the unmated mouse, however, Charlton (1917) has described identical modes of degeneration in tubal ova with scarcely an approach to normal cleavage, and out of 152 tubal ova in the unmated rat Mann (1924) found only three which appeared to have undergone a belated parthenogenetic development (see Table VIII). In the rabbit (Pincus, 1930) fragmentation occurs rarely; an ovum of the type shown in Figure 21 is occasionally encountered.

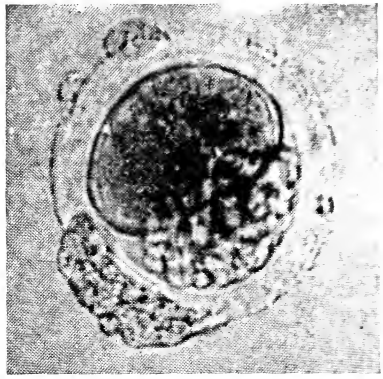

FIG. 21. Rabbit ovum recovered from the tubes 41½ hours after sterile copulation showing polar fragmentation. (From the *Proceedings of the Royal Society.*)

The fragmenting ova found in the tubes of rats and mice

TABLE VIII

THE CONDITIONS OF TUBAL OVA IN VARIOUS PORTIONS OF THE OVIDUCT IN THE RAT. (From Mann, 1924)

Position in Oviduct	Second Maturation Spindle Parallel Surface	Second Maturation Spindle Slightly Rotated	Second Maturation Spindle Perpendicular Surface	Chromosomes Scattered	Unfragmented Multinucleate Cells	Fragmenting Multinucleate Cells	Fragmented Ova	Two Spindles in One Ovum	Central Chromosomes Condensed	2-4 Cell Stages
1–3.5	34	4	2	15	0	0	0	0	2	
4–5	6	0	0	16	2	1	0	1	0	
5–8.5	1	0	0	7	14	13	0	1	0	1
8.5–9	0	0	0	2	0	6	22	0	0	2
Totals	41	4	2	40	16	20	22	2	2	3

finally disappear either through complete disintegration or, what is more likely, by phagocytosis (Figure 22). They usually disappear before the succeeding ovulation, although Hensen (1869) has described the retention in a blocked tube of about 100 rabbit eggs apparently from several ovulations.

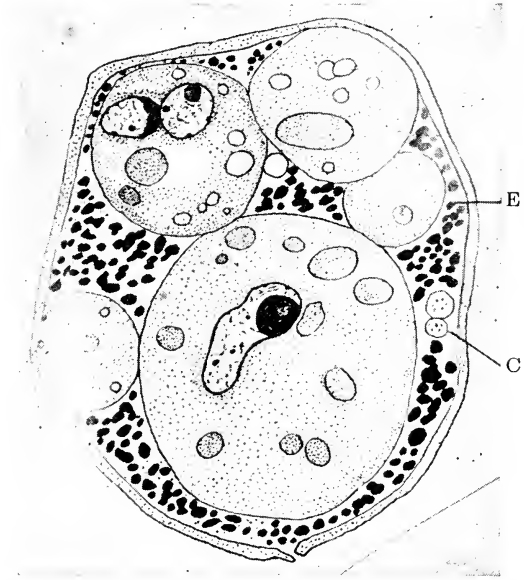

FIG. 22. Section through a fragmented mouse ovum recovered 81 hours after a sterile mating. Phagocytes (C) absorb the degenerated cytoplasmic particles (E). (From the *Biological Bulletin.*)

The rate of passage of ova in the tubes and the method of transport have been the subject of considerable controversy and discussion (see Parker, 1931 and Hartman, 1932*b*). It is generally acknowledged that the passage through the upper portions of the tubes is relatively rapid (Anderson, 1927; Lewis and Wright, 1935) since except shortly after ovulation both unfertilized and fertilized ova are found for the most part in the lower two-thirds of the tube. The method of propulsion of the ova by ciliary and other tubal movements is adequately discussed by both Parker and Hartman and will not be entered into here.

CHAPTER VII

FERTILIZATION AND CLEAVAGE

The events occurring at fertilization in the fallopian tubes have been subject to detailed examination chiefly in polyovular mammals, *e.g.*, the rabbit, rat, mouse, ferret, etc. In all cases the sperm surround the ova embedded in the mass of follicle cells, and penetrate to the ova causing the follicle cells to fall away at the same time. That the sperm swarm present in the tubes is actively responsible for the falling away of the follicle cell mass is abundantly evident from numerous recent observations of fertilization in the rabbit (Pincus, 1930; Yamane, 1930, 1935; Pincus and Enzmann, 1932, 1935). As described previously rabbit ova in does mated to sterile bucks begin to separate out of the follicle cell mass by 16 hours after copulation at the earliest, and the process is normally completed between the 17th and 19th hours. In fertile matings free ova have been observed as early as 11½ hours after coitus, and all ova are invariably free by the 14th hour. Furthermore, when freshly ovulated ova from sterile matings are placed *in vitro* with sperm suspensions there is a rapid dispersion of the surrounding follicle cells which does not occur in control cultures of ova in sperm-free media. Similar phenomena have been observed by Gilchrist and Pincus (1932) in the rat (Figures 23 to 25) and by Pincus (unpublished observations) in the mouse.

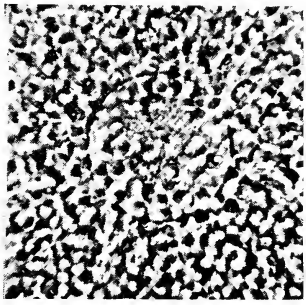

FIG. 23. Rat ovum recovered from the tubes at 16 hours after a sterile mating. Note surrounding follicle cells. (From the *Anatomical Record.*)

Yamane (1930) has ascribed the phenomenon of follicle cell dispersion to the presence of a proteolytic enzyme in the spermatozoa. He was able to secure a similar dispersal of follicle cells from sperm suspensions heated to 60° C. and from preparations of pancreatin containing trypsin. Yamane (1930) believes that this proteolytic enzyme is also responsible for the activation of the egg since he observed "polar" bodies formed in rabbit ova exposed to the suspensions of dead sperm and to the enzyme preparations.

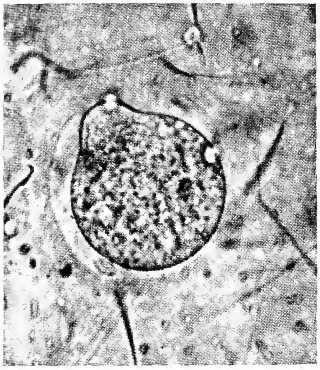

FIG. 24. Rat ovum of Fig. 23 after 2 hours with living sperm. Note absence of follicle cells and protrusion resembling a polar body. (From the *Anatomical Record.*)

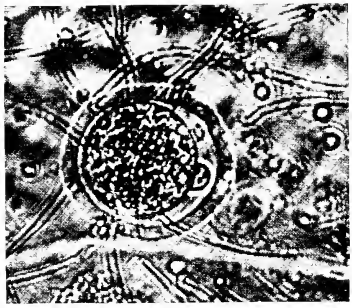

FIG. 25. Rat ovum recovered 11 hours after sterile mating and incubated with living sperm for 2 hours. Note shrunken vitellus and two polar bodies. (From the *Anatomical Record.*)

Pincus and Enzmann (1935) have examined this situation in some detail. Sperm suspensions free of seminal fluid were obtained from the vas deferens of adult rabbit males. Dilutions were made with a buffered Ringer-Locke solution at pH 7.3 – 7.5. The ova were taken at 12½ to 15½ hours after copulation from rabbit does mated to sterile (vasectomized) males; these ova were invariably well embedded in the massed follicle cells. The procedure followed was to place the massed ova in the sperm suspension and incubate for at least two hours. All ova were examined at two hours after semination and in some instances where no obvious signs of fertilization were observed incubated for 12 hours.

In most instances the ova were fixed in Bouin's solution and sectioned in order to determine the nuclear condition. The

TABLE IX

THE EFFECT OF VARIOUS CONCENTRATIONS OF LIVE SPERM UPON FRESHLY OVULATED RABBIT OVA. (From the *Journal of Experimental Zoölogy*)

DATE	CONCENTRATION OF SPERM PER MM.^{3}	EFFECT ON CUMULUS CELL MASS	EFFECT ON EGGS
6/II/34	(undiluted) 185,000	Destroyed in 2 to 3 minutes	2 polar bodies; ova completely dissolved after 24 hours
6/II/34	92,500	Destroyed in several minutes	2 polar bodies
20/I/34	(undiluted) 90,000	Destroyed very rapidly	1 polar body after two hours; polyspermy probable because of very active sperm suspension
10/III/34	80,000	Destroyed	2 polyspermic ova, one polar body; one monospermic with 2 polar bodies
16/I/34	(undiluted) 62,500	"	2 polar bodies; fertilized
20/I/34	55,000	"	1 polar body
20/I/34	40,000	"	1 polar body
26/I/34	38,400	"	2 polar bodies; fertilized
10/III/34	30,000	Destroyed in 20 minutes	1 egg with 3 sperm attached and 2 polar bodies; 2 eggs with single sperm attached and 2 polar bodies; not incubated
6/II/34	32,200	Destroyed	2 polar bodies; fertilized
6/II/34	30,000	"	1 polar body; no sperm entry
26/I/34	25,000	"	2 polar bodies; fertilized
26/I/34	14,300	Partly destroyed	1 polar body; not fertilized
26/I/34	10,700	"	" "
6/II/34	10,100	"	" "
6/II/34	8,000	"	" "
26/I/34	7,200	"	" "
6/II/34	4,000	"	" "
26/I/34	3,600	"	" "
2/II/34	6,000	Destroyed almost at once	1 polar body; no fertilization
2/II/34	3,000	Destroyed in 2 minutes	1 polar body; no fertilization (*rat sperm*)
2/II/34	1,000	Destroyed in 3½ minutes	1 polar body; no fertilization

sectioned ova of the experiments listed in Table IX invariably showed true polar bodies; no achromatic extrusions were observed. Furthermore, all ova with two polar bodies

contained either attached sperm or male pronuclei, whereas all ova with single polar bodies showed no signs of sperm entry with the exception of two heavily polyspermic ova. Polyspermy *may* prevent the second polar division, but probably only when extremely active and dense sperm suspensions are used. The presence of two polar bodies may therefore ordinarily be taken as a sign of activation.

It is evident from the data of Table IX that both the degree and speed of dispersion of the follicle cell mass is roughly proportional to the concentration of the sperm suspensions used and that those sperm concentrations which fail to effect a complete dispersion of the follicle cell mass also fail to cause second polar body formation. But rat sperm as well as rabbit sperm can effect complete dispersal of the follicle cells about rabbit ova and yet no polar body formation occurs. This seems to indicate that the activation of the ovum and follicle cell dispersion involve distinct and separate reactions.

The data of Table X substantiate this conclusion for they show that sperm-free fluid from the vas deferens and sperm suspensions heated to 60° C. for a few minutes cause typical follicle cell dispersion but no polar body formation. That a heat-labile substance is involved in the follicle cell dispersion is evidenced by the data on ova exposed to boiled sperm suspensions. This substance is probably carried by the sperm since similar follicle cell dispersion *in vivo* is brought about by sperm that have travelled the length of the oviducts.

Yamane (1930) found that both rat and horse spermatozoa caused second polar body formation in rabbit ova, and since his pancreatin solutions also caused the same result he concluded that a non-species-specific sperm-borne tryptase was involved. As shown in Table IX above rat sperm were ineffective in causing second polar body formation, but they were more potent than rabbit sperm suspensions in causing follicle cell dispersion. Accordingly Pincus and Enzmann (1936*a*) undertook the experiments with trypsin preparations presented in Table XI.

TABLE X

THE EFFECT OF DEAD SPERM PREPARATIONS AND SPERM-FREE SEMINAL FLUID UPON FRESHLY OVULATED RABBIT OVA. (From the *Journal of Experimental Zoölogy*)

DATE	TREATMENT OF SPERM SUSPENSIONS	EFFECT ON CUMULUS MASS	EFFECT ON EGGS	DILUTION OF PREPARATION
10/I/34	Heated to 60° C. all sperm dead	Destroyed in 10 minutes	1 polar body; no fertilization	Undiluted
30/III/34	Completely dessicated at room temperature; all sperm dead	Destroyed in 2 minutes	1 polar body	Made up to original volume
30/III/34	"	Destroyed in 5 minutes	"	Made up to original volume and diluted ½
30/III/34	"	Destroyed in 11 minutes	"	Made up to original volume and diluted ¼
30/III/34	"	Destroyed in 21 minutes	"	Made up to original volume and diluted 1/8
12/I/34	Centrifuged at 3000 R.P.M. for 5 minutes; heated to 60° C.; supernatant fluid used	Destroyed	"	Undiluted
17/III/34	Centrifuged at 3000 R.P.M. for 40 minutes; heated to 60° C.; supernatant fluid used	Destroyed in 3 minutes	"	Diluted 1/40
17/III/34	"	Destroyed in 4½ minutes	"	Diluted 1/80
17/III/34	"	Destroyed in 7½ minutes	"	Diluted 1/120
17/III/34	"	Destroyed in 8 minutes	"	Diluted 1/160
12/I/34	Centrifuged at 3000 R.P.M. for 5 minutes; not heated; supernatant fluid used; a few sperm present	Destroyed	3 eggs out of 9 with second polar body and sperm	Undiluted
17/III/34	Centrifuged at 3000 R.P.M. for 50 minutes; not heated; no sperm present	Destroyed in 1½ minutes	1 polar body	Diluted 1/20
20/IX/35	Boiled for 12 minutes; all sperm dead	Left intact after 1 hour	"	Diluted ¼

TABLE XI

THE EFFECTS OF EXPOSING FRESHLY OVULATED RABBIT OVA TO VARIOUS SOLUTIONS OF TRYPSIN. (From the *Journal of Experimental Zoölogy*)

DATE	TRYPSIN CONCENTRATION (DRY TRYPSIN PER 100 C.C. RINGER-LOCKE SOLUTION)	EFFECT ON CUMULUS CELL MASS	EFFECT ON EGGS
10/II/34	0.50	Destroyed	3 "polar" bodies in 10 minutes
10/II/34	0.25	"	Egg shrunken
10/II/34	0.125	"	"
10/II/34	0.062	Partly destroyed	"
10/II/34	0.032	"	"
6/II/34	25.00	Destroyed almost immediately	6 to 10 "polar" bodies followed by partial digestion of ova
6/II/34	21.00	"	"
17/II/34	1.00	Destroyed in 1 minute	Egg partly digested
17/II/34	0.50	Destroyed in 1½ minutes	1st polar body digested
17/II/34	0.25	Destroyed in 3 minutes	1 polar body, egg shrunken *
17/II/34	0.125	Destroyed in 6 minutes	" " *
17/II/34	0.062	Destroyed in 14 minutes	" " *
17/II/34	0.032	Destroyed in 31 minutes	" " *

* All these ova showed irregular masses of webbed tissue in the perivitelline space.

The data of these experiments show typical follicle cell dispersion and also "polar body" formation (Figure 26). These are, however, not true polar bodies but rounded cytoplasmic masses caused by the action of the enzyme preparation upon the egg surface. Sections of the ova of these experiments showed the "polar bodies" to be chromatin free. The polar bodies observed by Yamane in his pancreatin experiments were probably of this nature. The polar bodies formed in his experiments with rat and horse sperm may have also have been false polar bodies due to the strongly digestive action of the heterologous sperm suspensions, for, as we have seen, rat sperm suspensions are extremely effective as follicle cell dispersing agents even in very low concentrations. Krasovskaja (1935*b*) believes that

actual penetration and pronucleus formation occurred in his attempts to fertilize rabbit eggs with rat sperm. No figures showing actual sperm penetration are given in this paper. The nuclear configurations shown may, in fact, occur in

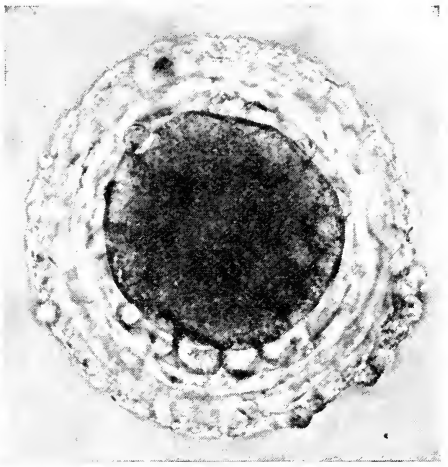

FIG. 26. Rabbit ovum from sterile mating treated with trypsin solution. Note many "polar" bodies. See text.

ova cultured *in vitro* with no sperm added (see Chapter VIII).

The immediate effect of semination (and fertilization) upon mammalian ova is a definite shrinkage of the vitellus (Pincus and Enzmann, 1932). Quantitative estimates of this shrinkage in rat eggs have been made by Gilchrist and Pincus (1932). In Table XII are presented their data on follicular and tubal ova. They show that a 14 per cent reduction in volume occurs in fertilized tubal ova. Furthermore, when unfertilized ova are exposed to sperm suspensions a similar shrinkage occurs (Table XIII). This shrinkage is not due to polar body extrusion since it occurs *in vitro* within 5 to 10 minutes, and polar bodies are normally extruded at 45 minutes to 1 hour after semination *in vitro* (Long, 1912). The ova apparently increase somewhat in volume after this initial shrinkage. Krasovskaja (1935*a*)

has observed an exactly similar initial shrinkage followed by a return to normal in rabbit ova seminated *in vitro*.

TABLE XII

THE VOLUME OF RAT EGGS IN THREE STAGES OF DEVELOPMENT. (From Gilchrist and Pincus, 1932)

STAGE	AVERAGE VOLUME OF ROUND EGGS, CU. MM.	AVERAGE VOLUME OF ELONGATED EGGS, CU. MM.	AVERAGE VOLUME OF ALL EGGS, CU. MM.
Follicular	0.000333 (1)	0.000339 ± 0.000017	0.000337 ± 0.000010
Tubal, unfertilized	0.000251 ± 0.000023	0.000226 ± 0.000013	0.000234 ± 0.000018
1-cell	0.000202 ± 0.000009	0.000200 ± 0.000010	0.000201 ± 0.000010

TABLE XIII

THE SIZE OF RAT EGGS UNDER VARIOUS CONDITIONS OF CULTURE. (From Gilchrist and Pincus, 1932)

TREATMENT	NUMBER OF EGGS	AVERAGE DIAMETER IMMEDIATELY AFTER PUTTING EGGS ON SLIDE, MICRONS	AVERAGE VOLUME CALCULATED, CU. MM.	AVERAGE DIAMETER SOME TIME AFTER INCUBATION, MICRONS	AVERAGE VOLUME, CALCULATED CU. MM.	SHRINKAGE, PER CENT
Incubated in Ringer's solution alone	7	74.4 ± 1.4	0.000216	76.3 ± 0.4	0.000232	—
Incubated with live sperm	9	77.9 ± 1.4	0.000248	72.7 ± 1.4	0.000205	17
Incubated with dead sperm	10	72.8 ± 1.1	0.000204	69.7 ± 1.3	0.000179	12

Sperm penetration into living ova has been observed only once (Pincus, 1930); a modified fertilization cone appears to form at the point of contact. This cone very quickly subsides as is apparent also from fixed preparations of mammalian ova in the tubes (*e.g.*, Lams and Doorme, 1908; Sobotta and Burkhard, 1911; Lams, 1913; and others).

The length of time that the mammalian ovum remains capable of fertilization has been largely a matter of speculation. Exact experimental inquiry has, however, been undertaken in the rabbit (Hammond and Marshall, 1925; Hammond, 1928 and 1934) and in the ferret (Hammond and Walton, 1934). Taking advantage of the fact that the

TABLE XIV

LITTER SIZE AND FERTILITY IN TIMED MATINGS OF RABBIT DOES. (From Hammond, 1934)

No. of Matings	Matings at: Hours after Sterile Coitus	Matings at: Hours before (+) or after (−) Ovulation	Average Litter Size	Matings Fertile, Per Cent	No. of Young per Mating Made
(a) All strains together (52 different does used)					
323	Normal	+10	6.4	79.6	5.3
6	5	+ 5	6.4	82.3	5.3
65	6	+ 4	4.7	64.6	3.0
55	7	+ 3	4.4	58.2	2.5
81	8	+ 2	4.2	42.0	1.8
85	9	+ 1	3.6	37.6	1.4
68	10	0	4.5	22.1	1.0
57	11	− 1	3.4	12.3	0.4
63	12	− 2	3.2	6.3	0.2
(b) C strain (17 different does used)					
131	Normal	+10	7.4	75.6	5.6
25	6	+ 4	5.4	52.0	2.8
18	7	+ 3	3.7	55.6	2.1
22	8	+ 2	2.8	27.3	0.8
21	9	+ 1	4.3	28.6	1.2
20	10	0	4.5	10.0	0.4
18	11	− 1	0	0	0
19	12	− 2	0	0	0
(c) E strain (21 different does used)					
90	Normal	+10	8.1	80.0	6.5
3	5	+ 5	7.0	100.0	7.0
19	6	+ 4	5.8	63.2	3.7
23	7	+ 3	5.6	65.2	3.8
37	8	+ 2	4.9	48.6	2.4
48	9	+ 1	3.6	41.7	1.5
25	10	0	5.4	36.0	2.0
21	11	− 1	4.2	23.8	1.0
21	12	− 2	4.0	9.5	0.4
(d) F strain (14 different does used)					
102	Normal	+10	4.0	84.3	3.4
3	5	+ 5	5.5	66.6	3.7
21	6	+ 4	3.4	81.0	2.7
14	7	+ 3	2.7	50.0	1.4
22	8	+ 2	3.8	5.5	1.7
16	9	+ 1	2.7	47.5	1.0
23	10	0	2.2	37.4	0.4
18	11	− 1	1.5	11.1	0.2
23	12	− 2	2.5	18.7	0.2

rabbit ovulates at 10 hours after copulation and the ferret at about 30 hours, Hammond and his coworkers undertook a series of matings using an initial sterile mating to initiate the ovulation stimulus and then fertile mating to permit sperm access to ova at successively later intervals. In the

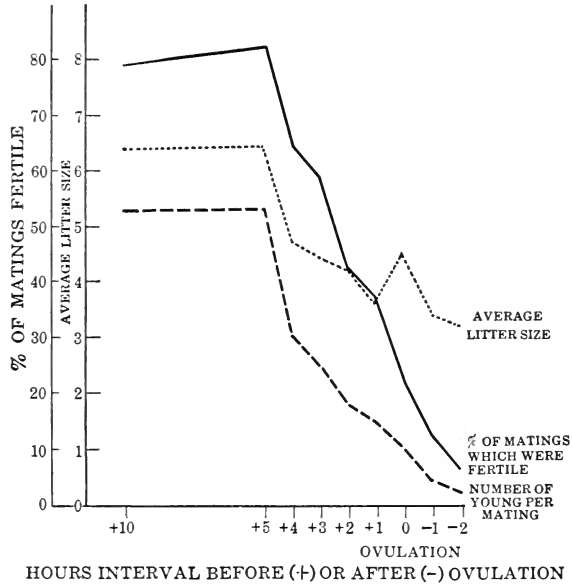

FIG. 27. Fertility of matings made at different intervals of time before or after mating (all strains). (From the *Journal of Experimental Biology.*)

most extensive series of rabbit matings (Hammond, 1934) employed three inbred strains of rabbits in order that homogeneous conditions of fertility might exist in his experiments. The data of his experiments are given in Table XIV, and a graphical representation in Figure 27.

It is at once obvious from these data that matings to fertile bucks made after the 5th hour following a sterile mating show a decline both in absolute (per cent of fertile matings) and relative fertility (number of young produced). When matings are made to fertile bucks at twelve hours after the sterile copulation, *i.e.*, at two hours after ovulation minimum fertility is attained.

In order to make quite certain that the cause of the smaller litters produced after the experimental matings made late in relation to ovulation was due to the ova not being fertilized and not to any interference with the process of ovulation or other causes, a few does so mated were killed during pregnancy and the number of corpora lutea (*i.e.*, ova shed) compared with the number of foetuses present. The results are given in Table XV, and demonstrate that there is a decrease in the number of ova fertilized in the later matings. This implies that the sperm reach the portion of the tubes containing the ova at a time when these ova are for some reason no longer fertilizable.

TABLE XV

THE PERCENTAGES OF RABBIT OVA FERTILIZED IN MATINGS MADE AT VARIOUS TIMES BEFORE AND AFTER OVULATION. (From Hammond, 1934)

MATINGS AT		DOES		NUMBER OF				OVA NOT FERTILIZED, PER CENT
Hours after Sterile Coitus	Hours before (+) or after (−) Ovulation	Number	Strains	Ova Shed	Normal Foetuses	Atrophic Foetuses	Ova Not Fertilized	
6	+4	2	E	25	15	2	8	32
7	+3	2	E, F	20	10	3	7	35
8	+2	3	E	41	18	5	18	44
9	+1	2	E	25	6	0	19	76
11	−1	2	E, F	19	4	0	15	79

On the basis of Heape's (1905) observations that rabbit sperm reach the tops of the tubes in about 4 hours after coitus, Hammond concludes that rabbit ova can remain fertilizable for at most 6 hours after ovulation, by allowing a 2-hour postovulatory interval in the matings made at 12 hours after the ovulation-inducing mating. This period coincides approximately with the time (*i.e.*, 7 hours) that it takes for the ova of sterile matings to begin to separate from the follicle cell mass and start their free travel down the tubes. Hammond concludes therefore that the presence of the plug of massed ova is necessary for fertilization. He reasons as follows:

"The plug, of liquor folliculi and detritus, containing the ova dams up the top of the Fallopian tube and remains there

for some 4 (in fertile matings) to 7 (in infertile matings) hours, during which time the ascending sperms are collecting in its lower layers (see Figure 28). The accumulation of sperms so effected ensures that sufficient shall be available to fertilise the ova as they emerge from the plug. As the sperms are put in progressively later than normal in relation to the time of ovulation, the accumulation of sperms becomes progressively less and the chances of all the ova

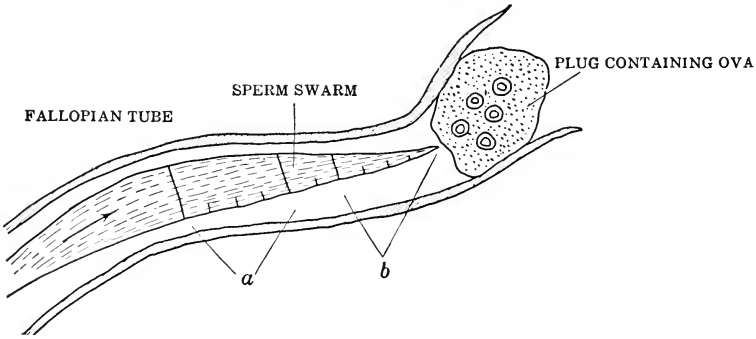

FIG. 28. Diagram illustrating how the chances of the ova becoming fertilized are reduced as the interval between mating and ovulation is reduced. a = amount of sperm swarm which would accumulate if mating were made at the ordinary time—10 hours before ovulation. b = amount of sperm swarm which would accumulate if mating were made 4 hours before ovulation. (From the *Journal of Experimental Biology.*)

becoming fertilised are reduced in proportion to the time the fertile mating is delayed with reference to the time of ovulation.

"The ascent of the sperms can be represented as a curve (see Figure 28 and Hammond and Asdell, 1926) or as a swarm (in the statistical sense). The apex of the sperm swarm (shown, in order to assist visualisation of the problem, very diagrammatically in Figure 28) reaches the top of the tube just at the time the plug is formed, *i.e.*, at ovulation, and so during the time that the plug exists (about 4 hours) it dams up but few sperms as compared with a normal mating made 10 hours before ovulation when the sperm swarm has ascended further (to the point *a* in Figure 28)."

While Hammond's deductions are entirely reasonable, it is possible that the 6 hours of fertilizable life allotted to rabbit ova is possibly too short since in normal matings 1½ to 3 hours are required by the sperm to reach the ova. This would make the critical period some 7½ to 9 hours long. Furthermore it is not the arrival of the first sperm that is effective, since as we have previously seen (pages 77 to 78) a definite minimal sperm concentration is necessary for both follicle cell dispersion and fertilization. If the critical period were thereby further lengthened by 1 to 2 hours it would coincide almost exactly with the time when the ova separating out of the tubal plug begin to acquire a coating of albumen. This coating is impervious to sperm (Pincus, 1930).

Similar experiments of Hammond and Walton (1934) with the ferret show that fertile matings made as late as 30 hours after ovulation result in the production of young. The reasons for the maintenance of the fertilizing capacity of ferret ova for as long as 30 hours are not deducible in detail since the exact tubal history of ferret ova is not known. Hammond and Walton attribute the greater length of fertilizable life in this case to the longer time it takes for the ova to traverse the oviduct, *e.g.*, 5 to 6 days in the ferret compared with 3½ days in the rabbit and the presumably correlated slower dissolution of the plug of massed ova.

In the spontaneously ovulating mammals the fertilizable life of the ova is also of short duration, but exact data are not available since it is ordinarily difficult to ascertain the specific time of ovulation. Hartman (1924) has shown that opossum ova traverse the tubal portion of the oviduct in 24 hours and that upon entry into the uterus unfertilized ova are definitely degenerated. Charlton (1917) found clear signs of degeneration in unfertilized tubal mouse ova by two days after parturition. Since post-partum ovulation occurs in the mouse at about 14 hours after parturition (Long and Mark, 1911) mouse ova may be said to retain cytological normality for about 35 hours. In the rat ova present

in the first third of the oviduct appear cytologically normal (Mann, 1924). According to the data of Long and Evans (1922) the ova remain in this portion of the oviduct for about 33 hours. Hartman's (1932*a*) data on timed matings in Macacus show that fertile matings occur only between the 9th and 18th days of the menstrual cycle with maximum between days 11 and 16. This, of course, does not imply that the ova are fertilizable for several days, but presumably that ovulation may occur at any time during the critical 9 day period. Matings time in relation to the onset of oestrus in the sheep (Quinlan, Maré and Roux, 1932) and the pig (Lewis, 1911) indicate a maximum period of fertility of 48 hours.

It is unnecessary in this monograph to discuss the cytological details of fertilization and cleavage in mammalian ova, since these are now textbook commonplaces. Our interest is primarily in the physiological mechanisms underlying these events and their relation to the dynamics of growth and development. We shall again discuss certain aspects of the fertilization process in the chapter dealing with the activation of unfertilized eggs. Now we shall turn our attention to the relatively scant data that deal with the mechanism of cleavage in tubal ova.

Until fairly recently no very accurate data on the rate of cleavage in tubal ova have been available. This has been due in part to the difficulty of timing ovulation. Even now it is possible to construct only approximate growth curves for a limited number of species. These curves are presented in Figure 29. It will be noted that rabbit ova cleave much more rapidly than those of the other species (see Plate VII). It is a matter of some interest to ascertain whether this difference in the cleavage rate is the result of an especially stimulating tubal environment in rabbits, or whether the cleavage rate is an inherent property of the ova. The data on the monkey were, in fact, deduced from Lewis and Hartman's (1933) observations of cleavage *in vitro*, and may be taken to indicate that segregation from the tubes

results in no great acceleration of cleavage since the growth rate remains at about the level of the other slow-cleaving species. The writer has transplanted mouse ova into the

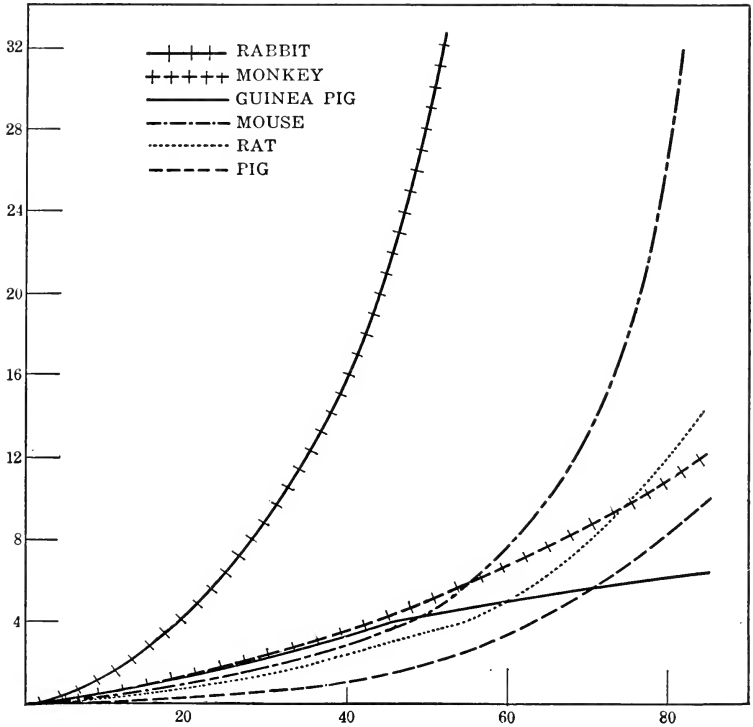

FIG. 29. Showing the cleavage rates of tubal ova in various species of mammals. Abscissa: time in hours after copulation. Ordinate: number of cells. The rabbit = data of Gregory, 1930, and Pincus, 1930. The monkey = data of Lewis and Hartman, 1933. The guinea pig = data of Squier, 1932. The mouse = data of Lewis and Wright, 1935. The rat = data of Gilchrist and Pincus, 1932. The pig = data of Heuser and Streeter, 1929.

fallopian tubes of the rabbit and has noted no increase in the cleavage rate over a period of 72 hours.

Castle and Gregory (1929; also Gregory and Castle, 1931) have, in fact, found certain definite congenital differences in cleavage rate between different races of rabbits. A résumé of their data is given in Table XVI. The animals of their large race (A) attain an average adult weight of about 5500 grams in females and 5400 grams in males. The cor-

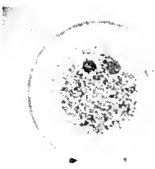

FIG. 4

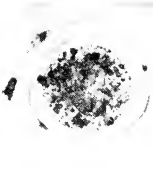

FIG. 5

FIG. 6

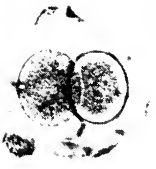

FIG. 7

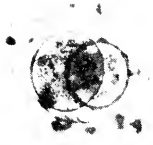

FIG. 8

FIG. 9

FIG. 10

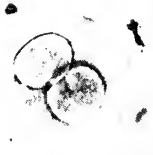

FIG. 11

FIG. 12

FIG. 13

FIG. 14

FIG. 15

FIG. 16

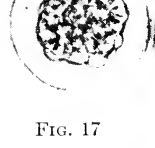

FIG. 17

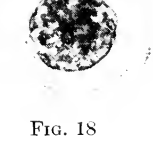

FIG. 18

FIG. 19

FIG. 20

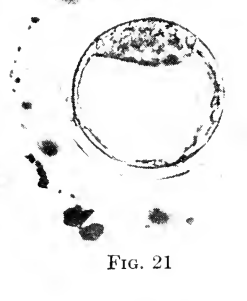

FIG. 21

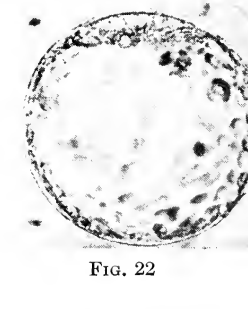

FIG. 22

PLATE VII

(Caption on facing page.)

responding adult weights in the small race (B) are 1500 grams for females and 1400 grams for males. The various hybrid combinations show roughly intermediate adult weights. Their data show clearly that certainly beyond the 32nd hour after copulation the cleavage rate is fastest in the large race animals and the expected sort of intermediate rates occurs in the various hybrid combinations. It is entirely possible that even the earliest cleavages do actually occur sooner in large race animals since large does ovulate later than small does and therefore their ova should be fertilized later. The number of mitoses in cleaving eggs of the large races also exceeds those in the small race, as the data in the columns labelled "prospective" indicate. Since this difference is consistently present in reciprocal hybrids between the races the implication is that the sperm nuclei also participate in the control of the cleavage rate.

In spite of the inherent differences in the speed of segmentation the processes of differentiation occur at the same time in the large and small size rabbits. Thus the blasto-

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PLATE VII. All photographs on this plate were made from the living rabbit eggs in Locke's solution, as soon as possible after removal from oviduct or uterus at an enlargement of 180 diameters (apochromatic objective 16 mm., compensating ocular 8). They are arranged in order of development and show the principal features of cleavage and formation of segmentation cavity. It will be noted that the trophoblast is precocious in its differentiation as compared with the remainder of the egg, and as soon as the trophoblast becomes histologically different one sees fluid begin to accumulate within the egg, thereby forming the segmentation cavity.

Figs. 4 to 9, Litter C 43, 25 hours after coitus. Fig. 4, one-cell stage with two polar bodies; Fig. 5, one cell, with coarse granules, perhaps abnormal; Figs. 6 to 9, showing two primary blastomeres, one tending to be larger than other. Figs. 10 and 11, Litter C 36, 28¾ hours after coitus. Four-cell stage with crossed arrangement of blastomeres. Figs. 12 to 14, Litter C 45, 32 hours after coitus. 5, 6 and 8-cell stages. In Fig. 13 the cell at top is just dividing. Fig. 15, Litter C 35. 16-cell stage. Fig. 16, Litter C 41, 55 hours. Morula of about 32 cells. Fig. 17, Litter C 32, 69¾ hours. Smooth surfaced morula. Fig. 18, Litter C 38, 71½ hours. Differentiated trophoblast cells on surface. Fig. 19, Litter C 33, 76½ hours. Fluid beginning to collect in cleft between trophoblast and inner-cell mass. At this time the albumen coat is at its maximum. Fig. 20, Litter C 33, 76½ hours. Subtrophoblastic lakelets of fluid determining early appearance of segmentation cavity. Fig. 21, Litter C 34, 90 hours. Definite segmentation cavity. Note demarcation between trophoblast and inner-cell mass. Fig. 22, Litter C 42, 92 hours. Zona much stretched and layer of albumen much thinned out. Inner-cell mass flattening into typical germ-disc. From Gregory, 1930.

TABLE XVI

THE MEAN NUMBER OF BLASTOMERES PER OVUM AT VARIOUS TIMES AFTER COPULATION IN LARGE AND SMALL RABBITS AND IN CERTAIN HYBRIDS BETWEEN THEM. (From Castle and Gregory, 1929, and Gregory and Castle, 1931)

HOURS AFTER COPULATION	RACE	NUMBER OF DOES	NUMBER OF EGGS	MEAN NUMBER OF BLASTOMERES	PROBABLE ERROR
32¼	A (actual)	3	31	4.06	—
"	A (prospective)	3	31	4.29	—
"	B (actual and prospective)	3	12	4.41	—
40	A (actual)	4	45	9.94	±0.24
"	A (prospective)	4	45	10.82	—
"	B (actual)	8	27	8.29	±0.19
"	B (prospective)	8	27	8.37	—
"	AB (actual)	1	9	8.44	—
"	AB (prospective)	1	9	8.66	—
41	A (actual)	3	22	11.64	±0.44
"	A (prospective)	3	22	12.68	—
"	B (actual)	6	21	8.62	±0.47
"	B (prospective)	6	21	9.09	—
"	BD (actual)	2	11	8.63	—
"	BD (prospective)	2	11	9.18	—
"	B and BD combined (actual)	8	32	8.62	—
"	AD (actual)	3	20	9.25	—
"	AD (prospective)	3	20	9.55	±0.36
48	F (actual)	4	28	21.75	—
"	F (prospective)	4	28	22.80	—
"	B (actual)	4	15	14.00	—
"	B (prospective)	4	15	14.50	—

A = large race.
B = small race.
AB = F_1 hybrid.
BD = seven-eights small (D = AB × B).
F = three-quarters large (AB × A).
actual = number of blastomeres observed.
prospective = number of blastomeres observed plus the number of mitoses.

dermic vesicle forms at the end of the 3d day (Plate VII, Figs. 18–20), and the embryonic disc by the 168th hour after coitus. Castle and Gregory therefore attribute large size to an inherent mitotic intensity independent of differentiation potentials.

The ova of the rabbit begin their differentiation early in comparison with the eggs of other species. Thus Gregory (1930) detected the beginning of the formation of the inner cell mass just after the 16-cell stage at about 47 hours after

coitus (37 hours after ovulation) and the cavity of the blastodermic vesicle may begin to form while the ova are still in the tubes. Guinea pig (Squier, 1932) ova enter the uterus in the 8-cell stage at the end of the 3d day after copulation and the blastodermic vesicles form only in the uterus at about 4½ days after coitus. In the rat (Huber, 1915) the ova enter the uterus during the 4th day after coitus in about 12 cells and start to form the blastodermic vesicle during the 4th to 5th days post coitum, and in the mouse (Enzmann, Saphir and Pincus, 1932; Lewis and Wright, 1935) blastocyst formation occurs in the uterus during the 4th day after copulation.

The physiological factors governing the cleavage of mammalian ova have been scarcely examined. It has already been stated that the whole course of cleavage of rabbit eggs may proceed normally *in vitro* and in heterologous as well as homologous blood plasma (Pincus, 1930). This would seem to imply that no special environmental factors supervene in the tubes. On the other hand the ova of mice, rats and guinea pigs do not cleave under the ordinary (or a variety of) tissue culture conditions. The reasons for this species difference are not known though the superior vitality of rabbit ova has been attributed to their unique albumen coating; but Lewis and Hartman (1933) have over a period of approximately 24 hours, observed the regular cleavage *in vitro* of a monkey ovum which lacks an albumen coating.

In the case of those ova which have not undergone cleavage *in vitro* one can only deduce that some limiting factor obtaining *in vivo* has not been duplicated. Since it is known that the secretory activity of the tubal epithelium is under hormonal control of the ovary (*cf.* Snyder, 1923) it is possible that a special contribution to the economy of cleaving ova is made by a hormonally induced secretion. The cleaving ova of all mammals journey through the tubes during the early life of the corpus luteum. The secretory activity of the tubal epithelium changes markedly during the transi-

tion from the oestral to the luteal phase. Furthermore, it is possible that the ovarian hormones themselves may directly affect the cleavage process. Oestrin, for example, definitely stimulates the mitotic activity of the vaginal epithelium, progestin inhibits uterine mitoses, etc.

Accordingly Burdick and Pincus (1935; also Pincus and Kirsch, 1936) have investigated the effect of ovarian hormones upon the development of rabbit and mouse ova. They found that the injection of large amounts of oestrin in no way affected the cleavage process although ova in the early uterine stages degenerate and die when only moderate amounts of this hormone are injected (see Tables XXIII to XXV, pages 118–120, 122). That the hormone injected definitely affected the tubal tissue was evidenced by the fact that in both mice and rabbits an effective closure of the tubo-uterine junction was attained, and in rabbits both the contractile activity and the histological appearance of the tubal tissue were definitely altered to the oestrus type. In addition (Pincus and Kirsch, 1936) it was found that rabbit ova grown in cultures containing appreciable amounts of oestrin continued to cleave at the normal rate. Finally fertilized rabbit ova in 1- and 2-cell stages were injected into the fallopian tubes of does on heat (and therefore lacking corpora lutea), and these were found to develop normally up to the early blastocyst stage. Corner (1928) had already shown that in bilaterally ovariectomized rabbit does egg development stops at the early blastocyst stage. The segmentation processes appear, therefore, to be independent of the secretory activity of the ovaries, and of any effect that the ovarian condition may have upon tubal secretion. Rabbit ova will, indeed, go through the morula stage in a carefully balanced buffered Ringer-Locke solution, indicating a fairly complete lack of dependence upon any special organic nutrition. It has, of course, been repeatedly noted by observers of living material (*e.g.*, van Beneden, 1875; Gregory, 1930; Gilchrist and Pincus, 1932; Squier, 1932) and by those who have examined fixed speci-

mens (Sobotta, 1895; Huber, 1915; and others) that mammalian ova show no appreciable increase in size until the blastocyst stage.

The most convenient approach to the study of the physiological processes underlying segmentation has involved the study of the respiratory processes (Warburg, 1908–14; J. Loeb and Wasteneys, 1912–15; J. Loeb, 1913; Runnström, 1930; Whitaker, 1933; and others). Mammalian ova are available in such small numbers that exact quantitative measurements of respiratory activity are difficult to make and have not been made. Nonetheless some indication of the nature of the underlying processes may be had by the use of specific poisons known to combine with and inhibit the reactions of definite components of the chain of reactions involved in respiration. Thus HCN is known to combine with iron-containing enzyme phaeohemin which is the initial activator in the aërobic phaeohemin-cytochrome chain (Warburg, 1932) and so to inhibit the respiration involving phaeohemin activity. Cyanide also inhibits the cleavage of ova of non-mammalian forms (Lyon, 1902; J. Loeb, 1906; see Needham, 1932), as does an oxygen-free medium (J. Loeb, 1895). Runnström (1935) has demonstrated that the mitotic process at segmentation in sea-urchin eggs is not dependent upon the level of respiration since the addition of pyocyanine to cyanide-inhibited egg suspensions restored oxygen consumption to normal levels but no division ensued.

Rabbit ova presumably develop in a medium relatively low in oxygen, since the oxygen tension of the abdominal cavity, and by inference that of the tubes (which have free access to abdominal fluids), is 40 mm. Hg (Campbell, 1924) as compared with 150 mm. Hg, the oxygen tension of the air. It is of interest to inquire whether the segmentation of rabbit ova is linked with the aërobic phaeohemin system. Pincus and Enzmann (1936*b*) have added KCN in appropriate concentration to cultures of cleaving rabbit eggs and the segmentation has ceased. Cinematographs of these ova indicated that the eggs were not "killed" by the poison

since they exhibited the cyclosis (cytoplasmic movements) typical of living ova. Similar experiments with iodoacetamide added to the cultures showed normal cytoplasmic activity of the ova but a limited amount of cleavage. Iodoacetamide presumably combines with the coenzyme concerned in the reduction of pyruvic to lactic acid (Meyerhof and Kiesling, 1933) so that the inhibition of both the oxygen-activating system and its presumable substrate system results in the arrest of cleavage. While the exact coupling of the respiratory system with the mitotic mechanism has yet to be delineated these data do demonstrate that the fundamental processes are alike in mammalian and non-mammalian ova.

We have seen that rabbit ova may be fertilized and cultured *in vitro*. It is a matter of some importance to determine whether such ova may give rise to normal rabbits. Accordingly the writer (see Pincus and Enzmann, 1934) undertook the transplantation of such ova into the oviducts of pseudopregnant rabbit does and found that ova fertilized *in vitro* and also normally fertilized ova kept in culture during the cleavage period apparently resumed normal development after transplantation as evidenced by the production of normal young at term. It is a matter of some interest to note that one set of ova had failed to cleave during 20 hours in culture but nonetheless young were obtained.

The development of a technique for the transplantation of mammalian ova into the oviducts makes possible the testing of a number of problems of development hitherto inaccessible. As we shall see later (Chapter IX) it is necessary that a progestational uterus be available for ensuring differentiation of uterine stages. Thus Biedl, Peters and Hofstäter (1922) transplanted rabbit ova into non-pregnant uteri in some 70 experiments and in only one doubtful case were young recovered. Nicholas (1933*b*) transplanted the isolated blastomeres of the 2-cell stage in the rat under the kidney capsule and observed varying degrees of development of the three germ layers and their various derivatives. The

writer has transplanted single blastomeres of 2-cell rabbit embryos into the tubes and obtained normally differentiating, but small sized blastodermic vesicles from the pseudopregnant uteri of the recipient does. The physiological processes occurring in such embryos are of extraordinary interest and certainly deserve further investigation.

CHAPTER VIII

THE ACTIVATION OF UNFERTILIZED EGGS

We have seen that the fundamental control of the cleavage mitoses is alike in rabbit and sea-urchin ova. We shall now inquire whether the activation of mammalian eggs is also similar to that of other forms.

With the exception of the three 2-cell rat eggs described by Mann (1924) there are no observations of a possible normal parthenogenetic development of unfertilized tubal eggs *in vivo*. With the exception of a single observation by Champy (1927), the first investigation of the behavior of unfertilized tubal ova placed in tissue culture is that of Pincus (1930). His data are presented in Table XVII.

TABLE XVII

THE DEVELOPMENT OF UNFERTILIZED RABBIT OVA IN CULTURE. (From Pincus, 1930)

	Age of Ova (Hours after Copulation)		Number	Medium	Examined (Hours in Culture)	Description	Number Divided	Number Undivided
(1)	0	0	1	RPRE	44	1—unsegmented	0	1
(2)	0	0	4	RPCE	48	3—unsegmented 1—in 3 regular cells	1	3
(3)	0	0	4	RPCE	48	4—unsegmented	0	4
(4)	11	9	1	CPCE	48	1—several polar bodies (?)	1 (?)	0 (?)
(5)	11	25	5	RPRE	24	3—unsegmented 1—8 regular cells 1—4 regular cells	2	3
(6)	11	40	2	RPRE	48	2—unsegmented	0	2
(7)	12	5	5	RPRE	47	3—unsegmented 2—in 12 to 16 regular cells	2	3
(8)	12	30	6	RCPCE	27	1—in 2 regular cells 1—in 3 regular cells 1—in 4 regular cells 3—in 5 to 6 regular cells	6	0

(C = Chicken. R = Rabbit. P = Plasma. E = Embryo Extract.)

TABLE XVII (*Continued*)

THE DEVELOPMENT OF UNFERTILIZED RABBIT OVA IN CULTURE. (From Pincus, 1930)

	AGE OF OVA (HOURS AFTER COPULATION)		NUMBER	MEDIUM	EXAMINED (HOURS IN CULTURE)	DESCRIPTION	NUMBER DIVIDED	NUMBER UNDIVIDED
(9)	13	15	3	RPCE	47	2—unsegmented 1—16 to 20 cells	1	2
(10)	13	35	11	RPRE	45	1—in 2 cells and 2 to 5 polar bodies 3—with about 5 polar bodies 7—unsegmented	4	7
(11)	13	50	4	RPRE	43	2—unsegmented 1—in 4 regular cells and 2 polar bodies 1—in morula	2	2
(12)	14	1	1	RPRE	25	1—unsegmented	0	1
(13)	14	35	6	RPCE	27	2—in 3 regular cells 2—in 4 regular cells 2—in 36 to 40 regular cells	6	0
(14)	15	0	3	CPCE	30	2—in about 4 cells regular 1—with multiple polar bodies	3	0
(15)	15	15	3	RPRE	27	1—unsegmented 1—in 4 regular cells 1—in about 16 cells	2	1
(16)	16	0	5	RPCE	22	2—in 1 very large cell and 10 to 12 small ones 2—in about 16 cells 1—in 32 to 48 cells	5	0
(17)	17	10	3	RPCE	23	1—in 2 regular cells and 2 polar bodies 1—in 3 to 4 large cells and 10 small cells 1—in 1 large cell and 16 small cells	3	0
(18)	17	33	2	CPCE	48	2—no segmentation	0	2
(19)	17	45	4	RPCE	26	1—2 large, 2 small cells and several polar bodies 1—in 16 very regular cells 2—in 20 to 32 cells	4	0

(C = Chicken. R = Rabbit. P = Plasma. E = Embryo Extract.)

TABLE XVII (*Continued*)

THE DEVELOPMENT OF UNFERTILIZED RABBIT OVA IN CULTURE. (From Pincus, 1930)

	AGE OF OVA (HOURS AFTER COPULATION		NUMBER	MEDIUM	EXAMINED (HOURS IN CULTURE)	DESCRIPTION	NUMBER DIVIDED	NUMBER UNDIVIDED
(20)	18	10	7	RPCE	48	3—unsegmented 1—2 unequal cells and 7 to 8 polar bodies 1—3 cells and several polar bodies 1—4 regular cells 1—10 small cells and 1 large cell	4	3
(21)	18	15	9	RPCE	20	4—unsegmented 1—3 cells regular 2—about 4 regular cells, but shrunken 1—in 12 regular cells 1—in about 8 cells, but shrunken	5	4
(22)	18	20	2	CPCE	47	1—unsegmented 1—3 polar bodies	1 (?)	1
(23)	18	25	10	RPCE	24	2—unsegmented 1—1 large cell and 2 to 3 small cells 1—2 regular cells and 2 polar bodies 1—4 regular cells 1—7 regular cells 4—about 8 cells	8	2
(24)	18	30	3	RPRE	48	2—unsegmented 1—in 3 cells	1	2
(25)	18	50	3	RPRE	47	2—in 8 regular cells 1—in 10 regular cells	3	0
(26)	19	5	10	RPCE	22	2—unsegmented 2—in 2 regular cells 4—in 4 regular cells 1—in 7 cells 1—2 unequal cells and 3 polar bodies	8	2
(27)	19	30	4	RPCE	27	1—unsegmented 1—2 regular cells 2—4 regular cells	3	1
(28)	19	45	8	RPCE	22	1—unsegmented 3—in 2 cells 2—in 4 cells 1—in 6 cells 1—in 8 cells	7	1

(C = Chicken R = Rabbit. P = Plasma. E = Embryo Extract.)

TABLE XVII (*Continued*)

THE DEVELOPMENT OF UNFERTILIZED RABBIT OVA IN CULTURE. (From Pincus, 1930)

	AGE OF OVA (HOURS AFTER COPULATION)		NUMBER	MEDIUM	EXAMINED (HOURS IN CULTURE)	DESCRIPTION	NUMBER DIVIDED	NUMBER UNDIVIDED
(29)	20	0	2	CPCE	22	2—many polar bodies	2	0
(30)	20	10	3	RPRE	49	3—in many cells	3	0
(31)	20	20	4	RPCE	47	4—unsegmented	0	4
(32)	20	20	2	CPCE	48	1—unsegmented 1—1 large and 2 to 3 small cells	1	1
(33)	24	25	5	RPCE	27	1—2 unequal cells 3—2 regular cells 1—3 regular cells	5	0
(34)	24	45	2	RPRE	44	1—1 large cell and several polar bodies 1—16 to 20 regular cells and a few polar bodies	2	0
(35)	27	35	5	CPCE	46	1—unsegmented 2—about 8 cells and many polar bodies 2—one large cell and many polar bodies	4	1
(36)	28	35	9	RPCE	47	4—unsegmented 1—4 regular cells 1—6 unequal cells 3—about 8 cells	5	4
(37)	37	20	2	RCPCE	27	2—in many cells and degenerate	2	0
(38)	40	40	3	CPCE	45	3—in many small cells	3	0
(39)	43	10	6	CPCE	46	1—in 2 cells 1—in 4 cells and many polar bodies 2—in 1 cell and many polar bodies 2—in many small cells	6	0
(40)	47	30	6	RPCE	22	3—unsegmented 1—in 2 unequal cells 2—with many polar bodies	3	3
(41)	48	30	8	RPCE	48	5—unsegmented 3—with many polar bodies	3	5
(42)	48	47	6	RPRE	52	1—about 8 large cells 1—3 unequal cells and many polar bodies	6	0

(C = Chicken. R = Rabbit. P = Plasma. E = Embryo Extract.)

TABLE XVII (*Continued*)

THE DEVELOPMENT OF UNFERTILIZED RABBIT OVA IN CULTURE. (From Pincus, 1930)

	AGE OF OVA (HOURS AFTER COPULATION)		NUMBER	MEDIUM	EXAMINED (HOURS IN CULTURE)	DESCRIPTION	NUMBER DIVIDED	NUMBER UNDIVIDED
						4—with many polar bodies		
(43)	50	30	5	RPRE	45	1—unsegmented 4—in many cells	4	1
(44)	68	33	3	RPRE	45	3—unsegmented and degenerate	0	3
(45)	72	0	4	RPCE	22	2—unsegmented and shrunken 2—about 10 polar bodies and shrunken	2	2
(46)	73	40	7	RPCE	45	4—unsegmented 2—16 regular (?) cells 1—5 cells and 3 polar bodies	3	4
(47)	96	45	2	RPCE	46	2—unsegmented	0	2

(C = Chicken. R = Rabbit. P = Plasma. E = Embryo Extract.)

The primary and surprising fact evident from the data is that a majority of the ova placed in culture underwent a certain degree of development, so that out of 213 eggs cultured, 136 or 63.8 per cent are classified as having "divided," the term "divided" including any degree of observable development beyond the 1-celled state of the ova as recovered from the animals. It was the primary objective of these investigations to ascertain the nature of the various degrees of development undergone *in vitro* and to establish any relationship that might exist between the age of the ova and the nature of the development. Before undertaking any detailed analysis of the data it is deemed advisable to describe the various types of development observed.

The ova observed in the 2-cell stage varied in appearance as shown in Plate VIII, Figs. 1–3. The great majority of them resembled that of Figure 1, and showed usually one, sometimes two or three, polar bodies. The ovum of Figure 3 was photographed after the egg had been in cul-

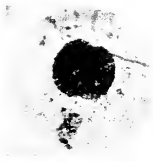

FIG. 1

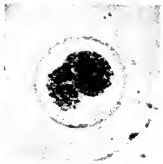

FIG. 2

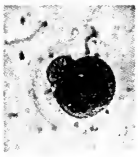

FIG. 3

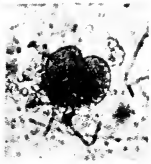

FIG. 4

FIG. 5

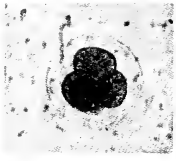

FIG. 6

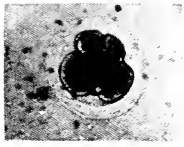

FIG. 7

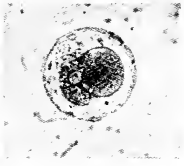

FIG. 8

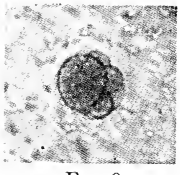

FIG. 9

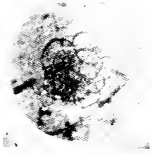

FIG. 10

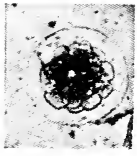

FIG. 11

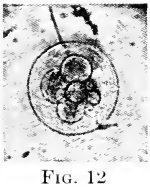

FIG. 12

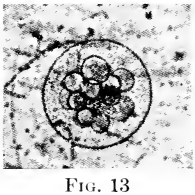

FIG. 13

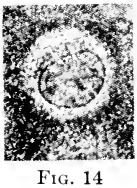

FIG. 14

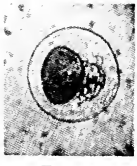

FIG. 15

PLATE VIII. Ova from sterile matings as they appeared after being cultured *in vitro*. (From the Proceedings of the Royal Society.)

Fig. 1, Recovered at 18 hrs. 30 mins. after sterile copulation cultured for 44 hrs. Fig. 2, Recovered at 27 hrs. 30 mins. after sterile copulation cultured for 25 hrs. Fig. 3, Recovered at 19 hrs. 5 mins. after sterile copulation cultured for 22 hrs. Fig. 4, Recovered at 18 hrs. 10 mins. after sterile copulation cultured for 17 hrs. Fig. 5, Recovered at 18 hrs. 15 mins. after sterile copulation cultured for 28 hrs. Fig. 6, Recovered at 19 hrs. 5 mins. after sterile copulation cultured for 22 hrs. Fig. 7, Recovered at 19 hrs. 5 mins. after sterile copulation cultured for 22 hrs. Fig. 8, Recovered at 28 hrs. 35 mins. after sterile copulation cultured for 23 hrs. Fig. 9, Recovered at 17 hrs. 10 mins. after sterile copulation cultured for 23 hrs. Fig. 10, Recovered at 24 hrs. 45 mins. after sterile copulation cultured for 24 hrs. Fig. 11, Recovered at 37 hrs. after sterile copulation cultured for 6 hrs. Fig. 12, Recovered at 73 hrs. 40 mins. after sterile copulation cultured for 45 hrs. Fig. 13, Recovered at 73 hrs. 40 mins. after sterile copulation cultured for 45 hrs. Fig. 14, Recovered from the ovary, and cultured for 28 hrs. Fig. 15, Recovered at 48 hrs. 30 mins. after sterile copulation cultured for 24 hrs.

ture 22 hours. It was subsequently replaced, and when examined 24 hours later had formed eight cells quite regular in appearance. Note is made of this fact because it indicates that ova segmenting irregularly at the first division may eventually assume an appearance characteristic of ova undergoing quite regular division. The ovum of Figure 4 was photographed just as segmentation from two to three cells was being completed. One of the two blastomeres had not quite rounded out at the time of photographing. The segmented ovum of Figure 5 is also in three cells. When first examined after 23 hours of culturing no segmentation had occurred; 5 hours later the ovum had divided as photographed. The ova of Figure 5 were recovered at 18 hours and 15 minutes after copulation and were still surrounded by a number of follicle cells. They were placed *vis-a-vis* in culture and the out-growing follicle cells of each ovum became intermingled and caused the compression of the ova seen in the photograph. Figure 6 is a photograph of a typical 4-celled stage, exactly comparable to the 4-celled stage of fertilized ova (see Plate VII, Figs. 10 and 11). The number of polar bodies in such ova vary from one to three. Again, the great majority of ova observed in four cells presented the regular appearance of the ovum of Figure 6. Figure 7 represents an ovum containing seven cells in which one of the four blastomeres of the 4-celled stage divided twice while the others remained quiescent. Such differential division may begin after the 2-celled stage as illustrated by Figure 8, in which one of the original two cells has remained quiescent while the other divided in two, and one of the two cells formed divided twice to form four small cells. There is also photographed the single polar body of this ovum. Figure 9 represents another case in which one of the early blastomeres has remained quiescent while the others have gone on dividing at a rapid rate. Some such process is responsible for most of the irregular segmentations observed. At the same time segmentation may proceed in a manner comparable to that of normal fertilized ova *in vivo*,

so that one may observe in the same culture the different types described. Figure 10 is a photograph of an ovum segmented to about 20 cells and apparently with a marked degree of regularity. When we come to consider ova segmented into 20 and more cells the interpretation of the course of their development becomes difficult because of a peculiar complication. The ovum of Figure 11 offers a pertinent illustration. It was recovered from the tubes at 37 hours after copulation and was in the 1-cell stage. Six hours later it presented the appearance shown in the photograph. It has apparently segmented into about 36 cells in the course of 6 hours. This means astonishingly rapid segmentation. As a matter of fact what probably occurred was a complex fragmentation of the entire ovum. In the course of filming an ovum recovered at 29 hours and 20 minutes after copulation the course of such fragmentation was observed. After an initial period of quiescence the ovum underwent a period of activity which resulted in the sudden appearance of many small "blastomeres." This was followed by a complete quiescence with the cessation of all cytoplasmic movements. The "cells" of this fragmented ovum, however, were not at all distinct in form or outline. One may observe "many-celled" ova in culture that presented this vagueness of cell outline, but we have also seen well advanced ova in which the component blastomeres were as distinct and clear as in the normal fertilized ovum. Interpretation must, therefore, proceed slowly until the exact mechanics of division *in vitro* is thoroughly investigated. A certain amount of light, however, is shed on the problem by the consideration given below to the relation between the age of the ova and the nature of the development observed. Figures 12 and 13 are photographs of two ova recovered at 73 hours and 40 minutes after copulation. They were photographed after having been 45 hours in the same culture. Note the remarkable regularity of the cells of the ovum of Figure 13. The ova of Figures 14 and 15 represent types ordinarily described as "with many polar bodies." Both

have a very large single cell, beside which lie a number of very small "cells" comparable in appearance to polar bodies. Very often this group of "polar bodies" resembles an irregular indented cytoplasmic mass, and I have actually seen it formed as such a mass budded or divided off from the main body of the cell. This represents the extreme of irregularity observed.

The foregoing account has been given irrespective of the age of the ova figured. It remains for us to ascertain if any relation does exist between the age of the ova cultured and the nature of their development. Before proceeding to a detailed inquiry, however, it must be pointed out that the various types of ova described and figured in the photographs have been observed in ova of all ages so that no absolute correlation exists. Ova have been considered as segmenting regularly only when the cells of the two, four, eight and sixteen cell stages have been of equal size, or when one could obviously trace the regular descent of the cells in ova exhibiting intermediate stages. In the cases of ova exhibiting many cells only those showing clear cell outlines and cells of equal size have been classified as "regular."

TABLE XVIII

Effect of Age of Ova when Removed from Doe on Subsequent Regularity of Division *in Vitro*. (From Pincus, 1930)

Group Number	Age of Ova (Hours after Copulation)	Regular	Irregular	Percentage Regular
(1)	11 to 17	26	8	76.4
(2)	17 to 21	37	16	69.8
(3)	24 to 96	10 (?)	26	27.7
	All ova	73	50	59.3

In Table XVIII the data are collected into three groups as follows: (1) Ova recovered when practically all were in the cumulus mass; (2) ova separating out of cumulus mass and not yet covered with albumen; (3) ova covered with the albumen deposit. It is obvious from these data that the

percentage of ova segmenting with any semblance of regularity decreased perceptibly with the age of the ova. In the group of ova recovered at 24 to 96 hours after copulation 16 of the ova classified as irregular exhibited one large cell and "many polar bodies." In fact, 23 or about half of all the ova called "irregular" are of this type. A number of ova, particularly in the 24 to 96 hour group exhibited "many polar bodies" and a varying number of larger cells. The rest of the ova classified as irregular were either "many-celled" with indistinct cell outlines, or contained cells of unequal size traceable, probably, to the differential division of early blastomeres.

Now this fact that the younger ova tend to segment regularly is presumably related to the state of the egg cytoplasm. The older ova undoubtedly undergo a certain degree of degeneration as they progress down the tubes, and the degree of cytoplasmic degeneration is probably related to the regularity of the subsequent development in culture. The problem is unfortunately complicated by the fact that all ova in culture stop segmenting and degenerate after some time. In these experiments it is probable that practically no development occurs after the ova have been in culture for 36 hours. The time in which the ova may exhibit their potentialities for parthenogenetic development is, under the conditions of these experiments, therefore extremely limited. The surprising fact is that such a large proportion of the ova do exhibit a degree of development that must be classified as parthenogenetic.

The morphology and cytology of parthenogenetic ova have been studied in a number of invertebrate forms where parthenogenetic development has been induced by various methods of treatment. In almost all cases a very large proportion of the parthenogenetic ova exhibit marked irregularities in development (*e.g.*, Wilson, 1901; Scott, 1906; Morris, 1917). In fact all the irregular types described here have been observed in artificially parthenogenetic invertebrate ova. The proportion of regular divisions observed

in these ova compares favorably with those observed in invertebrate ova, with the possible exception of the sea-urchin eggs, a very large proportion of which (as much as 100 per cent) may develop regularly into swimming larvae (Hindle, 1910; Loeb, 1913).

It was not possible to make any extensive cytological study of the ova described. The few sectioned and stained eggs obtained, indicate that in ova segmenting regularly the nuclei and cytoplasm are normal in appearance. In ova segmenting irregularly the situation is apparently rather complicated. There are obvious evidences of degeneration. Some cells contain nuclei, others do not, and the cytoplasm is often quite degenerate. One observes ova with several nuclei and no distinct cell divisions. In the case of one fairly regular ovum there were at least 37 chromosomes in an incomplete metaphase plate.

Upon consideration of the various factors involved in the technique of explanting the ova it seemed most likely that those young ova which underwent a normal parthenogenetic cleavage were stimulated by a gradually developed hypertonicity of the culture medium. For in these experiments the ova were cultured in watch glasses in a moist chamber, where the evaporation of a small amount of water from the plasma culture was possible. If this conclusion is true then at least one of the many types of parthenogenetic stimuli known to be effective with non-mammalian ova is similarly stimulating to mammalian eggs.

In order to examine this question further the writer and Dr. E. V. Enzmann (Pincus and Enzmann, 1936*a*) have studied the effect of known methods of parthenogenetic stimulation upon rabbit ova. We took as our criterion of activation the production of the second polar body, which, as we have seen in the experiments with semination *in vitro*, is entirely adequate.

The data of these experiments are given in Table XIX. They demonstrate that short treatment with solutions of relatively low hypertonicity are certainly effective in in-

TABLE XIX

THE EFFECT OF VARIOUS TREATMENTS UPON THE ACTIVATION OF RABBIT OVA *IN VITRO*. (From the *Journal of Experimental Zoölogy*)

DATE	TREATMENT	RESULT
18/I/34	3 minutes in 2.8 c.c. H/10 butyric acid + 50 c.c. Ringer-Locke followed by 3 mins. in 8 c.c. 2.5% NaCl + 50 c.c. Ringer-Locke followed by plasma culture	Cumulus partly dispersed; one ovum with 2 polar bodies; 7 with 1 polar body; much shrinkage
24/I/34	3 minutes in 5 c.c. N/10 butyric acid + 100 c.c. Ringer-Locke followed by hypertonic solution as above	Cumulus partly dispersed; 2 ova with 2 polar bodies; with 1 polar body; much shrinkage
24/I/34	3 minutes in 7.5 c.c. N/10 butyric acid + 100 c.c. Ringer-Locke followed by hypertonic solution as above	Plasmolysis of ova
24/I/34	3 minutes in 10 c.c. N/10 butyric acid + 100 c.c. Ringer-Locke followed by hypertonic solution as above	Plasmolysis of ova
20/IX/35	10 minutes in 1.8% Ringer-Locke	Cumulus intact; only 1st polar body
20/IX/35	5 minutes in 1.8% Ringer-Locke	Cumulus intact; only 1st polar body
21/IX/35	8 minutes in 1.8% Ringer-Locke	3 polar bodies in 5 hours
21/IX/35	8 minutes in 1.6% Ringer-Locke	1 egg with 2 polar bodies; 1 egg with 3 polar bodies
21/IX/35	8 minutes in 2.0% Ringer-Locke	2 polar bodies in 3 hours
20/IX/35	2 minutes exposure to 45.5° C.	⅓ with 2 polar bodies
20/IX/35	3 minutes exposure to 45.5° C.	2 or 3 polar bodies per egg
21/IX/35	2¼ minutes exposure to 45.5° C.	2 polar bodies formed
21/IX/35	3 minutes exposure to 45.5° C.	2 or 3 polar bodies per egg
18/IX/35	2 minutes exposure to 60° C.	No polar body formation

ducing activation, and that more drastic treatment (*e.g.*, longer treatment, or Loeb's treatment) is only occasionally effective. This indicates that the optimum conditions for the activation of rabbit ova are different from those employed with sea-urchin eggs. The data on the experiments with ova heated to 45° to 47° show that this heat treatment is most effectively activating.

We may conclude therefore that certain of the methods ordinarily employed in the artificial activation of non-mammalian ova are also effective in activating mammalian eggs. In a preliminary group of experiments (unpublished

data) the writer has transplanted ova so activated into the fallopian tubes of pseudopregnant rabbit does and has later recovered the transplanted ova. A number had undergone normal but obviously belated cleavage. A few cleaved at the normal rate and about 10% of the total attained the blastula stage.

In order to obviate any undetected effects of the manipulation of ova *in vitro* Pincus and Enzmann (1936*a*) undertook the activation of ova *in vivo* by injecting into the tops of rabbit fallopian tubes sperm suspensions previously irradiated with ultraviolet light of 2357 A° wavelength. The does used in these experiments had been mated to sterile bucks 12 to 13 hours previously so that their ovulated ova were embedded in the follicle cell plug. Into one oviduct the rayed sperm were injected, into the other an identical sample of unrayed sperm. It was found that ova from the tubes receiving unrayed sperm suspensions were for the most part normally fertilized and cleaved at the normal rate. Ova seminated with rayed sperm showed varying proportions of normally cleavage stages depending upon the time of exposure of the sperm to the ultraviolet light. Long exposures resulted in a preponderance of irregularly cleaved ova. But even the regularly cleaved ova resulting from seminations of sperm given short exposures were markedly retarded when compared with the control ova in the other tube.

The ultraviolet treatment with the particular wavelength used results presumably in the inactivation of the sperm chromatin (see Swann and del Rosario, 1932), and depending on the time of exposure (*e.g.*, intensity of radiation) leaves the non-chromatic portions of the sperm relatively unaffected. Dalq and Simon (1931) have shown that sperm treated with ultraviolet light penetrate into the egg cytoplasm but pronucleus formation does not occur and the chromatin disintegrates. If the sperm centrosome apparatus is not inactivated normal cleavage occurs, otherwise irregular development ensues.

The data of Pincus (1930) indicate that parthenogenetic

cleavages occur later than normal cleavages (although the time taken for the segmentation process itself is the same in fertilized and unfertilized eggs). It thus appears that the retarded cleavages observed *in vivo* as the result of semination with irradiated sperm are parthenogenetic in the sense that the sperm chromatin did not participate in the mitoses.

Novak and Eisinger (1923) attempted to activate rabbit eggs by tying off the tubes at the isthmus to prevent entry of the ova into the uterus. The ova that they recovered were either irregularly cleaved or fragmented with perhaps one or two normal cleavages. Their data thus resemble those of Mann (1924) on rat ova (see Table VIII) which do not descend into the uterus in unmated animals. Grusdew (1896) who injected sperm into the tops of rabbit tubes together with ova from punctured follicles also tied the tubes off at the isthmus and in a number of ova which gave no evidence of sperm penetration he observed ordinarily irregular but occasionally regular development. It would seem then that parthenogenetic development may be induced *in vivo* but that extensive embryonic differentiation has not been demonstrated.

It is obvious, of course, that a mere beginning has been made in the investigation of the parthenogenetic potencies of tubal ova. Presumably normal embryos might develop if a diploid cleavage nucleus could be induced to form. Pincus and Enzmann (1935) have, in fact, found indications that such a process may occur in activated rabbit eggs noting, again, after a rather long latent period, two fusion nuclei in unfertilized ova. The writer has observed an initial nuclear division without cytoplasmic cleavage in a primate ovarian ovum cultured *in vitro*. For full development *in vivo* it seems necessary that parthenogenetic ova should duplicate with some exactitude not only the normal morphological changes but also the rate of these processes. For the differentiating embryo is dependent upon an uterine environment the optimum development of which involves a fairly definite time schedule.

CHAPTER IX

THE GROWTH AND IMPLANTATION OF THE BLASTODERMIC VESICLE

In the cinematographs of Lewis and Gregory (1929) the regular cleavage of rabbit ova *in vitro* is shown to occur at approximately the same rate as *in vivo* and the formation of the blastocyst is initiated. The rapid expansion of the blastocyst into the typical large blastodermic vesicle (see Plate VII, Fig. 21) does not, however, occur. The attempted expansion is apparently barred by the presence of the relatively rigid zona pellucida and albumen coating so that the blastocyst alternately expands and collapses over a period of many hours until degeneration finally ensues. Brachet (1912, 1913) had previously shown that ova recovered from the uterus of the rabbit at 5 to 6 days after coitus will develop normally for 24 hours to 48 hours, passing from the tridermic stage to the stage of the primitive streak, with normal development of the ectoplacenta. Rabbit ova enter the uterus between 72 and 75 hours after copulation (Cruikshank, 1797; Assheton, 1894; Gregory, 1930) in the early blastocyst stage and still surrounded by the zona pellucida and the albumen coat. There is a rapid expansion of the ovum at this time due to the infiltration of fluid into the vesicle cavity so that by 96 hours after copulation the blastocyst is easily three times the diameter of the tubal egg. Very soon after the entry of the ovum into the uterus the viscosity of the stretched albumen layer appears to decrease so that its persistence about the large pre-primitive streak vesicle of the 6th day must be due to a marked softening. By the end of the 6th day to the 7th day it disappears completely due probably to its digestion by uterine fluids since it does not disappear in culture-grown ova. The growth

in culture of whole vesicles during the period when the albumen and zona coverings still remain is extremely difficult for the ova soon degenerate and often collapse (Waterman, 1932, 1934). As soon as the early primitive streak stage is reached, explantation results in a moderate degree of development. Waddington and Waterman (1933) explanted the

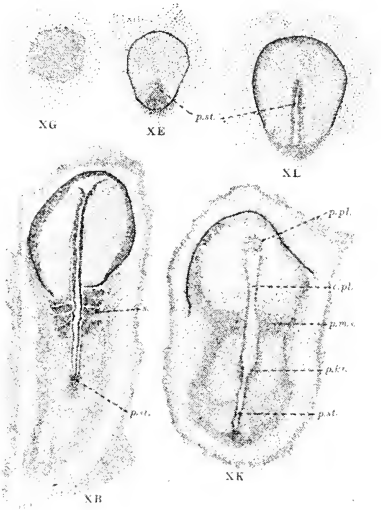

FIG. 30. Camera lucida drawings of embryonic areas of the rabbit at the stages of explantation. XG, late pre-primitive streak. XE, stage of posterior thickening. XL, medium primitive streak. XK, pre-somite. XB, three somite. p.st., primitive streak; c.pl., chorda plate; p.kt., primitive knot; p.pl., prochordal plate; p.m.s., pre-mesodermal somite; s., somite. (From the *Journal of Experimental Biology.*)

embryonic portion of the blastodermic vesicles upon a medium of chicken plasma plus chicken embryo extract and found that the older and more differentiated the embryo at the time of explantation the greater the degree of differentiation in culture. Using the five stages illustrated in Figure 30, the development observed was as follows:

(a) The stage of late pre-primitive streak gives no appar-

ent differentiation as seen in whole mount preparations. Localized thickenings only occur.

(b) The stage of posterior thickening and initial elongation of the embryonic disc develops one or two beating hearts, and localized thickenings after 4–5 days' growth *in vitro*.

(c) The stage of short primitive streak undergoes marked elongation of the primitive streak and embryonic disc on the 2nd day; two, and in one case three, beating hearts appeared after 2–3 days of culture.

(d) The stage of medium primitive streak gives results comparable to (c). In several instances brain, hearts, neural tube and somites appear.

(e) The stage of long primitive streak gave rise to embryos with as many as six pairs of somites after 1 day of culture, and the pre-somite and two-somite stages give only slightly, if at all, better development.

Nicholas and Rudnick (1934) similarly were unable to obtain any adequate development of rat blastocysts in stages earlier than the pre-somite or 5–7 somite. But vesicles in the latter stages developed markedly in a medium consisting of equal parts of rat plasma and 14–15 day rat embryo extract. They report that growth occurs during the first twenty-four hours *in vitro* gradually slowing and ceasing by the 36th hour. "At 48 hours or earlier, differentiation in the embryo has reached a maximum, at which it may be maintained for another 24 hours.

"During this period the embryos in the best cases have differentiated from 2 to 16 somites. The allantoic bud has grown from a small lump of tissue at the angle between the amnion and the posterior part of the embryo to join with the superior surface of the ectoplacental cone. The heart, unformed at the time of implantation, has differentiated a two chambered structure and has initiated its beat, the blood islands have developed in the yolk sac epithelium, and circulation has commenced, both in the yolk sac and in the embryo. The nervous system has differentiated considerably; eyes and ears have differentiated and the embryo as

a whole has gone through a primary torsion, separating it from the embryonic membranes in the region of the intestinal portal and contributing to its apparent reversal of posture.

"The total growth attained in the 48 hour period is less than half that attained by the normal embryo during the same period. The maximum differentiation is nearly three-quarters of that undergone by the normal. The factors limiting growth are affected earlier than those limiting differentiation.

"Apparently respiratory interchange is the most important functional necessity at this stage. The efficiency of this mechanism is not only lowered by the total absence of maternal circulation but even further prevented by the growth of a new enveloping membrane in the nature of a decidua from the marginal cells of the ectoplacental cone. The accumulation of break-down products due to metabolic activity is another checking factor. A few preliminary experiments have shown that these can be removed by washing the entire culture in sterile Ringer's solution and adding fresh embryonic extract. By using this method embryos have been kept alive for 96 hours although growth and differentiation occur only at a low rate during the last 24 hours."

Nicholas (1934) has also observed a few cases of the development of rat embryos from ova dropped into the uterine cavity, and extra-uterine pregnancies in man are of course well known. In the rat the removal of the entire gestation sac from the uterus into the peritoneal cavity may be performed without hindering fairly advanced embryo development in the extra-uterine environment (Selye, Collip and Thomson, 1935*b*). It therefore appears that some somatic influence carries the ova through the critical early blastocyst stages and that this influence does not operate in the ordinary tissue culture media.

It will be recalled that this critical stage occurs at the time of the disappearance of the egg envelopes and Hall (1935) has recently presented data offering a possible clue

to the critical events. He found that the zona pellucida of rat and mouse ova placed in fluids of low acidity quickly disappeared (at pH 3.7 or below). In a few cases the zona pellucida was dissolved in Ringer's solution with a pH as high as 5.4. Deciduomata of the rat have shown pH values as low as 5.7, which are, however, not below the critical levels of the *in vitro* experiments. Pincus and Enzmann (unpublished data) have taken a number of measurements of the pH of pseudopregnant and pregnant endometria and

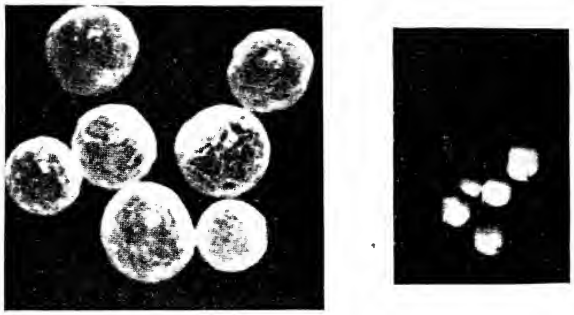

FIG. 31. Left, normal rabbit blastocysts of the 5th day of pregnancy. Right, blastocysts of the 5th day of pregnancy from rabbit doe ovariectomized 18 hours after mating. (From the *American Journal of Physiology.*)

have never observed pH values below 6.5. Nonetheless it is possible that in the small decidual crypts into which the ova fall the critical acidity may be attained.

Burdick and Pincus (1935) and Pincus and Kirsch (1936) have examined this critical stage of development from a somewhat different angle. Corner (1928) had noted that in rabbit does in which both ovaries or all the corpora lutea were removed shortly after fertilization the uterine ova remained in the early blastocyst stage (see Figure 31 and Tables XX to XXII), whereas in control rabbits with corpora lutea normal development occurred. The degenerating blastocysts were associated with an oestrus type of endometrium, and normal growth of a progestational endometrium with implantation of embryos occurred when corpus

luteum extracts were injected daily after ovariectomy (Allen and Corner, 1929). Burdick and Pincus (1935) observed that the daily injection of oestrone begun one or two days after copulation in unoperated rabbits (100–150 rat units per day) and mice (5 rat units per day) resulted in the degeneration of rabbit ova in the early blastocyst stages and of mouse ova in late morula stages, *i.e.*, at the stages during which uterine entry occurs. Pincus and Kirsch (1936) extended these observations in order to fix the critical time of action of the hormone. Injections of oestrone were made at various periods both before and after ovulation, and in the case of the post-ovulatory injections the uteri were examined at the 10th to 12th days to determine the extent of implantation.

Their data presented in Tables XXIII and XXIV indicate clearly that the minimum sterilizing dosage can be given on days 3 to 4 post coitum. These days cover the period of early blastocyst development. The

TABLE XX

GROUP I BOTH OVARIES REMOVED AT 14-18 HRS.			
NO.	AUTOPSIED	STATE OF EMBRYOS	PROLIF.
1	4½ d	DEGENERATED 0.2 MM. DIAM.	0
18	4½ d	" 0.2 " "	0
34	4¾ d	" 0.15-0.2 " "	0
3	5½ d	" 0.2 " "	0
2	7½ d	" 0.4 " "	0
4	7¾ d	" 0.3 " "	0
38	5¾ d	" 0.45 " "	0

TABLE XXI

GROUP II CONTROL OPERATIONS AT 15-18½ HRS.				
NO.	OP.	AUTOPSIED	STATE OF EMBRYOS	PROLIF.
33	 R. L.	4¾ d	7 NORMAL 0.5 MM.	+
24		4¾ d	7 NORMAL 0.6 MM. 4 DEGEN.	+
27		5¾ d	1 ABNORMAL 1 MM.	+
37		5¾ d	7 NORMAL 2 MM.	+
23		6¾ d	3 NORMAL, SHIELD STAGE	+
5		7¾ d	5 NORMAL, 8½ SOMITES	+
21		8¾ d	1 NORMAL, SOMITE STAGE	+

TABLE XXII

GROUP III ALL CORPORA LUTEA EXCISED AT 15-20 HRS.				
NO.	OP.	AUTOPSIED	STATE OF EMBRYOS	PROLIF.
16	 R. L.	4½ d	4 EARLY DEGEN. 0.4 MM.	0
19		4¾ d	4 UNSEG. OVA IN TUBE	0
30		5¾ d	NO EMB. (OVULATION +)	0
31		5¾ d	" "	0
32		5¾ d	" "	0
10		7½ d	4 DEG. BLASTOCYSTS 0.2 MM. IN TUBE	0

TABLE XXIII

THE EFFECT OF OESTRONE INJECTIONS UPON EARLY OVA. (From Pincus and Kirsch, 1936)

RABBIT NUMBER	DAYS INJECTED	RAT UNITS PER DAY	TOTAL RAT UNITS	HOURS AFTER COITUS KILLED	NUMBER OF CORPORA LUTEA	NUMBER OF EGGS RECOVERED	FOUND IN TUBES (T) OR UTERUS (U)	CONDITION OF EGGS
5	3 before mating	150	450	64½	8	7	T	All normal blastulae
14	3 " "	200	600	86	10	10	T	6 degenerating, 4 almost normal blastocysts
31	3 " "	900	2700	24	5	5	T	Not cleaved; sperm in zona
50	3 " "	900	2700	24	5	5	T	All normal in 2-cell stage
6	4 " "	150	600	67½	8	7	T	All normal blastulae
7	5 " "	300	1500	25	5	5	T	Normal in 2–6 cells
10	10 " "	300	3000	25	9	9	T	4 in 1-cell; 3 in 2-cell; 2 in 3-cell
9	15 " "	50	750	25	9	9	T	All degenerating blastulae
15	20 " "	100	2000	86	11	10	T	All degenerating blastulae
35	2 " "	200 *	800	70	8	8	T	Normal blastulae
	2 after mating	200 *	8					

* Oestrone in aqueous solution.

TABLE XXIII (*Continued*)

THE EFFECT OF OESTRONE INJECTIONS UPON EARLY OVA. (From Pincus and Kirsch, 1936)

RABBIT NUMBER	DAYS INJECTED	RAT UNITS PER DAY	TOTAL RAT UNITS	HOURS AFTER COITUS KILLED	NUMBER OF CORPORA LUTEA	NUMBER OF EGGS RECOVERED	FOUND IN TUBES (T) OR UTERUS (U)	CONDITION OF EGGS
1	1 after mating	150 *	150	38	10	10	T	Normal in 8–13 cells
2	2 " "	150 *	300	62	8	8	T	7 normal morulas (32 to 42 cells)
3	3 " "	150 *	450	86	10	4	T	All degenerating blastocysts
						1	U	
4	3 " "	150 *	450	86	9	1	T	1 normal blastocyst in uterus; others all degenerating
						3	U	
12	3 " "	200 *	600	86	11	1	T	Degenerating blastocyst
8	3 " "	3 c.c. .005% NaOH	9 c.c.	86½	7	7	U	All normal blastocysts
11	3 " "	3 c.c. .005% NaOH	9 c.c.	86	9	9	U	All normal blastocysts

* Oestrone in aqueous solution.

TABLE XXIV

THE EFFECT OF VARIOUS TYPES OF OESTRONE INJECTIONS DURING THE PREIMPLANTATION PERIOD UPON THE IMPLANTATION RATIO. (From Pincus and Kirsch, 1936)

ANIMAL NUMBER	DAYS AFTER MATING INJECTED	RAT UNITS INJECTED DAILY	TOTAL NUMBER OF RAT UNITS	NUMBER OF CORPORA LUTEA	NUMBER OF IMPLANTATIONS	REMARKS	
16	1	200 *	200	9	9	Implantations normal	
17	1–2	200 *	400	9	2	" "	
18	1–3	200 *	600	7	1	" "	
20	1–3	200 *	600	5	2	" "	
19	1–4	200 *	800	7	1	" "	
25	1–5	200 *	1000	10	0	———	
24	4	200 *	200	10	8	Implantations normal	
38	4	400	400	7	1	" "	
21	4–5	200	400	7	0	———	
41	4–5	100	200	9	8	3 dying; 5 normal	
26	4–6	200	600	8	0	———	
44	5–6	200	400	12	7	Implantations subnormal in size	
48	5–6	200	400	To term		No litter	
47	3–4	100	200	"	"	Litter of four	
37	3–4	200	400	12	0	———	
45	3–4	150	300	10	0	———	100% dead
71	3–4	150 †	300	11	0	———	100% dead
69	3–4	150 §	300	8	6	Implantations normal	
40	3–4	100	200	14	5	Implantations normal	60.3% dead
52	3–4	100	200	13	6	2 embryos subnormal	60.3% dead
60	3–4	75	150	8	1	Implantations normal	87.5% dead
56	3–4	37½	75	11	1	Implantations normal	
61	3–4	37½	75	6	5	Implantations normal	72.7% dead
66	3–4	37½	75	5	0	———	72.7% dead
58	3–4	30	60	10	3	Implantations normal	
65	3–4	30	60	11	11	Average diameter of egg chambers 1.43 × 1.07	33.3% dead
59	3–4	25	50	9	7	Implantations normal	
62	3–4	25	50	10	10	Implantations normal	11.1% dead
64	3–4	25	50	8	7	Implantations normal	11.1% dead
13	1–5	3c.c. .005% NaOH	15c.c.	6	5	Implantations normal	
54	No injections			10	10	1 subnormal in size	9.8% dead
55a	"	"		5	5	Implantations normal	9.8% dead
55b	"	"		11	8	" "	9.8% dead
63	"	"		9	9	" "	9.8% dead

* Oestrone in aqueous solution (Parke-Davis Theelin).
† Crystalline oestrone in oily solution. § Crystalline oestrone in aqueous solution.

minimum daily sterilizing dosage for days 3 and 4 is 150 rat units of oestrone-in-oil. When lesser dosages are injected a partially sterilizing effect is observed. This partially sterilizing effect is measured by observing the ratio between the number of corpora lutea and the number of implantations. The relation of the implantation ratio to the hormone dosage is given in Figure 32. It will be seen that even relatively low hormone dosages have a lethal effect upon a number of the embryos. This effect may be due either to prevention of implantation of vesicles developing normally till implantation time or to a

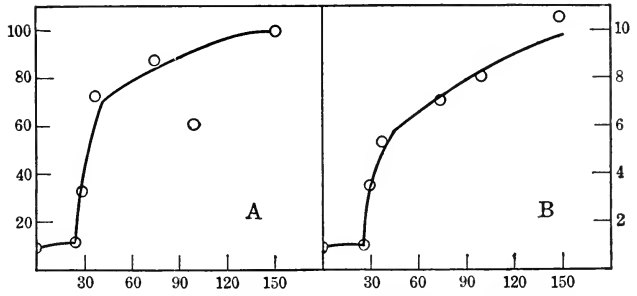

FIG. 32. Abscissa: oestrone dosage in R.U. per day. Ordinates: A, per cent of embryos unimplanted; B, number of unimplanted embryos per female. (From the *American Journal of Physiology.*)

degeneration before implantation. The latter alternative seems most likely when one observes the degenerated condition of the preimplantation blastocysts. In addition practically all the embryos that do become implanted are normal in appearance, and, in fact, give rise to normal young at term (rabbit no. 47).

When eggs in the blastocyst stage are placed in culture they will develop normally for 24 to 36 hours (Brachet, 1913; Pincus, 1930). Cleaving ova will, as we have seen, develop for several days and collapse when the blastocyst stage is reached and presomite stages continue development for 3 to 9 days. This implies that the explanted blastocyst either carries with it from the uterine environment a limited supply of necessary nutrition or that it rapidly exhausts the necessary materials from the ordinary culture medium.

If oestrone in some way directly interferes with the assimilation or metabolism of this critical nutrition then blastocysts cultured with this hormone should show inhibited development compared to that of controls in a normal medium. Pincus and Kirsch (1936) cultured early blastocysts taken from the uterus of rabbit does with varying amounts of oestriol (12.5 to 25.2 γ per culture) and found that control blastocysts developed at the same time as those in the oestriol-containing media. Oestriol was used instead of oestrone because the former is much more soluble in aqueous media and it also has a lethal effect upon developing blastocysts when injected *in vivo* (see Table XXV). These experiments show that the lethal effect of the hormone is not due to the direct action of the hormone upon the developing blastocyst. The sterilizing effects of oestriol and dihydrooestrone (Table XXV) indicate that the lethal effect is not oestrone specific, and point again to the disturbance of a needed nutritive condition.

TABLE XXV

THE EFFECT OF VARIOUS INJECTIONS OF OESTRIOL AND DIHYDROOESTRONE UPON THE IMPLANTATION RATIO. (From Pincus and Kirsch, 1936)

ANIMAL NUMBER	DAYS AFTER MATING INJECTED	AMOUNT INJECTED DAILY (IN GAMMA)	TOTAL AMOUNT (IN GAMMA)	NUMBER OF CORPORA LUTEA	NUMBER OF IMPLANTATIONS	REMARKS
46	3–4	16.7*	33.3	9	8	Implantations normal
70	3–4	16.7†	33.3	9	6	" "
78	3–4	18.0†	36.0	8	0	———
51	3–4	22.2*	44.4	12	0	———
73	3–4	22.2†	44.4	10	0	———
42	4–5	11.1*	22.2	16	16	Implantations subnormal in size
43	4–5	5.5*	11.0	10	10	Implantations normal
49	4–5	11.1*	22.2	To term		No litter
68	3–4	66.0§	132.0	6	6	Implantations normal
74	3–4	100.0§	200.0	7	3	Average diameters of egg chambers 1.90 × 1.43 cm.
76	3–4	150.0§	300.0	9	2	Egg chambers = .8 × 1.0 and .9 × 1.1
77	3–4	225.0§	450.0	8	0	———

* Dihydrooestrone in aqueous solution.
† Dihydrooestrone in oily solution.
§ Oestriol in oily solution.

Just what special conditions are needed for carrying the blastodermic vesicle over this critical stage cannot be explicitly stated. It is obvious that corpus luteum activity is necessary for the establishment of these conditions, and the oestrone effect is due to an inhibition of this activity. Thus it is possible to overcome the partially sterilizing effect of low oestrone dosages by the simultaneous injection of a corpus luteum hormone preparation (Pincus and Kirsch, unpublished data). Other substances (*e.g.*, vitamins A and C) are ineffective as inhibitors of complete sterilization. There seem to be two alternatives: either (1) progesterone or some corpus luteum product act directly upon the blastocysts or (2) corpus luteum secretions induce a special uterine environment through their action upon the endometrium. Pincus and Enzmann (unpublished data) have made crude extracts of the endometrium of pseudopregnant rabbit does, and have cultured blastocysts in media containing these extracts. No marked effect was obtained with the particular preparations employed, but further investigation may disclose the presence of an active substance. It is certain that blastocyst death due to oestrone action occurs in a uterus the endometrium of which still shows at least partial pseudopregnant proliferation. The minimum sterilizing dosage employed by Pincus and Kirsch is insufficient to abolish pseudopregnant growth completely (Leonard, Hisaw and Fevold, 1931; Courrier and Raynaud, 1933). Courrier and Raynaud (1934) have also found that dosages sufficient to prevent implantation are below the level necessary for the abolition of pseudopregnant growth. The data presented here on sub-sterilizing dosages demonstrate explicitly that a certain number of vesicles fail to develop in a uterus in which others proceed normally. We may consider therefore that there is necessary at least a threshold amount of a necessary active substance, or an optimum-hydrogen ion concentration alterations of which differentially affect the various blastocysts, or a rate of uterine contraction which causes the proper lodging of the blastocysts in the endo-

metrium thus preventing their injury. The fact that blastocysts in culture also show unusual sensitivity leaves the first two of these alternatives.

The behavior and differentiation of the blastodermic vesicle at the time of implantation have been the object of extensive investigation by mammalian embryologists since the publication of Bischoff's (1852) classical memoir on the subject. These investigations have been concerned chiefly with presenting exact descriptions of the mode of implantation in various classes of mammals (see Robinson, 1904; Grosser, 1909; Bonnet, 1903; Spee, 1915; Wilson, 1928; Sansom and Hill, 1930) and the accompanying differentiation of the vesicle. The physiological processes underlying these phenomena have been scarcely investigated.

The writer has been interested in the phenomenon of the delayed pregnancy which seems to offer an opportunity to exploit the processes occurring at implantation. Delayed pregnancy, or late parturition, occurs notably in the lactating mouse or rat which is carrying a set of fertilized eggs during lactation. This is a result of the fact that mice and rats have an oestrus period within 48 hours of parturition in which normal mating and fertilization take place. Enzmann, Saphir and Pincus (1932) have analyzed all the available data in the literature and found that in mice and rats each suckling young on the average prolonged pregnancy by about 21 hours (see Figure 33), though this time of prolongation seemed to vary somewhat from strain to strain. An examination of mated mice in a series of timed matings disclosed the fact that the preimplantation vesicle in suckling females failed to implant at the normal time but some time later depending upon the number of young suckling (see Kirkham, 1916, 1918). Once implantation occurs the growth of the embryo proceeds at the rate characteristic of normal embryos (Enzmann, 1935). Obviously the lactation process results in the establishment of conditions *in utero* which inhibit implantation, and the rather exact relationship between the degree of delay of pregnancy and the number of

young suckling suggests that definite quantities of necessary substances are withdrawn from the uterus as the result of mammary gland activity.

Teel (1926) found that the daily injection of a NaOH extract of the anterior hypophysis delayed implantation in rats when injections were begun on the day of mating. Injections on days 1 to 6 caused delayed implantation with parturition occurring in normal fashion but several days

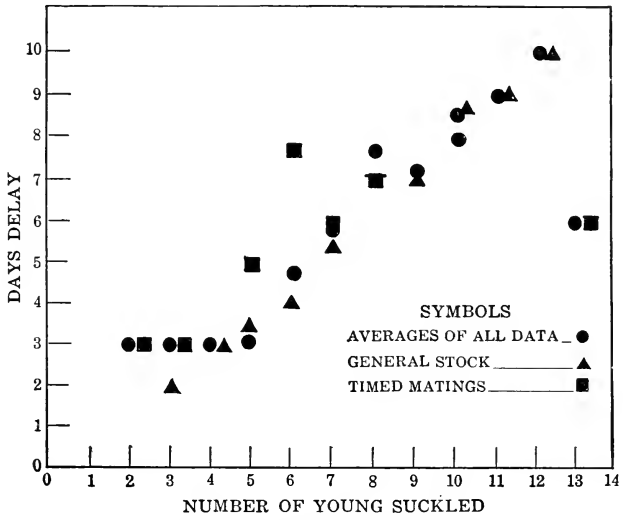

FIG. 33. Showing the relationship between the degree of delay of pregnancy and the number of suckling young. (From the *Anatomical Record.*)

after term; injections on days 1 to 12 also caused delayed implantation but a definite interference in the birth mechanism so that only one of a series of females produced normal living young in a late parturition; injections over a longer period resulted not only in delayed implantation but also in stillbirths 5 to 7 days after normal term. The impairment of the birth mechanism can therefore be avoided by early injection and is presumably a phenomenon distinct from that of delayed implantation. The inhibition of parturition can be caused not only by alkaline pituitary extracts (Evans and Simpson, 1929*b;* Snyder, 1934) but also by corpus

luteum extracts (Nelson, Pfiffner and Haterius, 1930). Since the pituitary extracts employed by Teel caused marked luteinization of the ovaries of injected animals it is possible that the delay in implantation may be due to excessive corpus luteum secretion. Selye, Collip and Thomson (1935*b*) have ingeniously demonstrated that the rat ovary during lactation presumably produces little or no oestrin, so that the hormone-producing tissue of the ovary during lactation is predominantly the luteal tissue. One need not postulate hypersecretion by the corpus luteum during lactation but merely an unbalance in which corpus luteum hormone predominates (thus Selye, Collip and Thomson actually obtain larger corpora lutea in lactating mice when oestrin is injected).

Wislocki and Goodman (1934) injected a preparation of progestin (after Allen, 1930), for 8 days after mating into two rabbits but no delay of pregnancy ensued. Antuitrin-S and antuitrin-G injected in fairly large amounts during early pregnancy were also ineffective although these preparations induced a fresh ovulation and new corpus luteum formation. The ineffectiveness of progestin in the two experiments of Wislocki and Goodman may have been due to an insufficient dosage. On the other hand it is possible that delayed pregnancy is due to an insufficiency of corpus luteum secretion, so that the immediate effect of Teel's extract may be considered the stimulation of oestrin production with inhibition of luteal secretion followed by corpus luteum activity which induced or completed the implantation process. Hamlett (1935) is in fact of the opinion that delayed implantation is due to hyposecretion of the corpus luteum. He has found (1932) that copulation and cleavage occur in the nine-banded armadillo of Texas during July, and the unimplanted vesicle lies free in the uterine lumen until early November when implantation takes place. Correlated with the quiescent period is a large corpus luteum the cells of which contain few or no secretory droplets or granules. Shortly before implantation vacuolization and lipoidal secre-

tion occurs in the luteal cell cytoplasm, and the removal of such corpora lutea leads to abortion whereas removal during the free vesicle period has no discernible effect upon the uterus or ovum. Hamlett (1935) quotes a number of instances of naturally-occurring delayed implantation of a presumably similar nature.

This possibility has been tested by injecting oestrone-free corpus luteum extracts into lactating pregnant mice during the early part of pregnancy (unpublished data). Injections of approximately 1/20th of a Corner-Allen rabbit unit were made over a 5 to 8 day period. A number of the mice failed to produce any young but seven females gave birth to normal litters. These were born not at term but much later; in fact, the average date of birth was 4 days later than would occur in delayed pregnancy if the expected delay is calculated on the basis of 21 hours per suckling young.

The implication is clear that excessive corpus luteum secretion caused a delay of pregnancy in mice. Since Teel (1926) found that deciduomata formation could be readily induced in the uteri of unmated females treated with his extracts corpus luteum activity undoubtedly occurred as a result of luteinizing hormone injection. The act of suckling then, by prolonging corpus luteum activity (which it does—see Parkes, 1929; Turner, 1932), results in a delay of implantation. Selye and McKeown (1934*a*) have in fact shown that suckling in rats prolongs pseudopregnancy and that the effects of suckling do not occur in the absence of the ovary (Selye and McKeown, 1934*b*).

The fact that Teel obtained definite deciduomata in animals subjected to a treatment that produces delayed pregnancy indicates either: (1) that mechanical irritation is more effective than ovum contact and that therefore the corpus luteum effect is really subnormal or (2) that excessive corpus luteum activity in some way inhibits the actual process of implantation of the blastocysts. The problem is an interesting one and is receiving further investigation.

CHAPTER X

SUMMARY AND RECAPITULATION

For the purposes of this monograph an ovum is considered as such from the moment of its functional differentiation in the ovary until its implantation in the uterine endometrium. An examination has been made of the experimental investigations of the growth and development of the mammalian ovum during the various stages of its life history in the ovary and oviducts.

The problem of the origin of the definitive ova has received much attention, but it cannot be said to have been completely resolved. If we are to judge by evidence from non-mammalian forms the large amoeboid primordial germ cells must enter the embryonic gonad if it is to differentiate as a functional organ. A functional ovary develops only from embryonic gonads in which the secondary sex cords proliferate to form a true ovarian cortex associated with the germinal epithelium.

The ovaries of young mammals contain large numbers of primitive oöcytes. The conception that these oöcytes are the only precursors of the definitive ova is controverted by a large body of recent evidence which indicates that new ova are proliferated from the germinal epithelium and that the rate of this proliferation varies with the various stages of the oestrus and pregnancy cycles. Ovogenesis in the adult seems to be partially inhibited by certain secretions of the anterior pituitary, the gonad-stimulating hormones affecting follicle growth primarily. The exact relation of the gonad-stimulating hormones to the ovogenetic processes is not at all obvious. It seems certain that the prophase stages of the oöcyte nuclei occur independent of pituitary hormone activity.

Pituitary hormones are definitely concerned in the final stage of ovum maturation, the first polar division which normally occurs in the ovary of most mammals. The pituitary secretions do not affect the eggs directly but initiate changes in the follicles which make for maturation in the ova. Similar changes occur in atretic follicles with a resulting "pseudomaturation" in the ova of such follicles. The initiation of ovum activation represented by the first maturation division occurs *in vitro* simply upon the explantation of ovarian eggs. Maturation *in vivo* and *in vitro* can be explained as the result of a functional isolation of the ovum from the follicular epithelium. It is held probable therefore that the parthenogenetic development of ova observed in mammalian ovaries occurs as the result of the establishment in the follicle of special activating conditions.

Parthenogenetic development of unfertilized tubal ova rarely if ever occurs *in vivo*. In most eutherian mammals the eggs are shed surrounded by follicle cells. If sperm are not present the surrounding cells slowly fall away, and the naked ova descend into the lower portion of the tubes where they degenerate and are eventually either resorbed or washed out into the uterus. When sperm are present there is a rapid dissolution of the surrounding follicle cells due to the action of a heat labile substance carried by the sperm. It has been claimed that this same substance activates the ova into forming the second polar body, but the available evidence is contradictory. Tubal eggs remain fertilizable for a few hours in the rabbit, and for thirty hours in the ferret.

Mammalian ova may be fertilized *in vitro* and normal cleavage ensues. This is most readily demonstrated with rabbit ova, for the ova of most of the other forms examined do not cleave or develop appreciably under the ordinary conditions of tissue culture. Segmentation *in vivo* occurs at fairly characteristic rates in the various species of mammals. The cleavage rate in rabbits is definitely correlated with the adult size of the strain employed. The cleavage process

itself is under the control of a cyanide-labile system. The process of cleavage is apparently independent of the activity of the primary sex hormones, oestrin and progestin.

Tubal rabbit ova readily exhibit parthenogenetic cleavages under certain conditions of explantation *in vitro*. Parthenogenetic activation can be initiated experimentally by treatment with cytolytic agents, by exposure to hypertonic solutions, and by heat treatment.

The development of the blastodermic vesicle *in vivo* is conditioned by the activity of corpus luteum secretions. In the absence of the corpus luteum development does not occur beyond that stage in which ova just entering the uterus are found. The evidence indicates that the corpus luteum secretions either stimulate the eggs directly or provide through stimulation of the uterine endometrium a suitable environment for the developing blastocysts. Oestrin and allied compounds prevent blastocyst growth by inhibiting the corpus luteum effect, the ova being most sensitive to this inhibition during the early blastocyst stages.

The implantation process itself is also under hormonal control. In the rat and mouse ovum implantation is delayed during lactation. This delay appears to be due to excessive corpus luteum secretion.

The development of various techniques for the explantation of ova both *in vivo* and *in vitro* makes available a variety of experimental investigations of the mammalian ovum. The ova of certain forms are particularly adapted to experimental manipulation. Mammalian ova normally develop in a homeostatic environment. Certain components of this homeostasis sharply limit the extent and nature of ovum development at certain stages. During other phases of its growth the ovum appears to be a relatively independent organism. Careful investigation of the physiological processes occurring in the ovum itself and in its homeostatic environment is made possible by the various explantation and transplantation techniques.

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