George D. Snell, Editor

BIOLOGY OF THE LABORATORY MOUSE

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OF THE

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by

THE STAFF

of

THE ROSCOE B. JACKSON MEMORIAL LABORATORY

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J. J. BITTNER
A. M. CLOUDMAN
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G. W. WOOLLEY

With a Chapter on
INFECTIOUS DISEASES OF MICE

by

J. H. DINGLE
Harvard Medical School

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Whose generous grant
Made its preparation possible
This book is dedicated

PREFACE

Of all the laboratory mammals, probably none has contributed more to the advancement of knowledge than the common mouse. Certainly among all the mammals it is the most widely used, for not less than one million mice are raised each year in this country for research in bacteriology, cancer and genetics.

A result of this extensive use of the mouse is that a large body of information has grown up concerning it. This, however, is so widely scattered through the literature that it is often a major undertaking for the research worker who wishes to use it to locate and gather the particular facts that he needs. Much of this information is assembled in this book. In a number of cases, where there are important gaps in the literature, these have been filled in by special research projects. In general, controversial material has been avoided or given only brief mention. The emphasis is placed on established facts useful to the research worker.

Certain fields, for example anatomy and endocrinology, have of necessity been largely omitted. In most cases material omitted is adequately covered in other recent books.

Because it deals with the mouse alone, this book presents a vertical cross-section of biological knowledge rather than the more usual horizontal cross-section. It contains information about one animal drawn from various branches of zoology, rather than information about one branch of zoology drawn from observation of a variety of animals. There is, I believe, one notable virtue in this vertical method of presentation, namely, that it makes the synthesis of biological knowledge somewhat easier. There is a widespread feeling among biologists that progress will depend increasingly on the synthesis of the specialized techniques which have been developed within the individual cubby-holes into which science is somewhat arbitrarily divided. The departmentalization of biology is a convenience not to say an absolute necessity, but within the organism the tissues, the genes, the endocrines, the diseases and the processes of development are all intimately related, and the biologist frequently finds that research in his own specialty is leading him straight into another field of

knowledge. At the present time there are, for example, increasingly well beaten paths between genetics and embryology, between endocrinology and cancer research, between cancer research and bacteriology, between bacteriology and genetics. It is a major purpose of this book, by gathering together the fundamental knowledge about the mouse from several fields of study, to make it easier for the research worker using mice as his experimental material to traverse these interconnecting paths of science.

The preparation of the book has been financed by a grant from the John and Mary R. Markle Foundation. This generous support has made possible the conduct of several pertinent research projects and the preparation of many original photographs and drawings. The embryological studies described in Chapter 1 have also been aided by a grant from the Alexander Dallas Bache Fund of the National Academy of Sciences. In the preparation of their material the authors have been ably assisted by the following persons: Miss Olive Bartholomew, preparation of embryological and histological sections; Miss Bernette Bohen, drawings; Mr. Joshua Burnett, tabulation of linkage data; Dr. Elizabeth Chase, histological sections; Dr. Katrina P. Hummel, photography; Mr. Arthur Lieberman, bibliography; Mr. John Mowat, photography and construction of apparatus; Mr. William Payne, photography; Miss Ella Rowe, preparation of sections; Miss Elizabeth Keucher, assistance in preparation of the index. Prof. C. H. Danforth has made valuable suggestions in regard to several parts of the text.

In conclusion, the editor would like to express his appreciation to the other members of the Laboratory Staff for their continued cooperation and for many valuable suggestions, and to Dr. C. C. Little for his hearty support and, in a broader sense, for the wise direction in a large measure responsible for the friendly atmosphere so essential for successful collaboration.

GEORGE D. SNELL, *Editor*
Roscoe B. Jackson Memorial Laboratory
Bar Harbor Maine

CONTENTS

Chapter 1

THE EARLY EMBRYOLOGY OF THE MOUSE

By GEORGE D. SNELL, *Roscoe B. Jackson Memorial Laboratory.*

The early embryology of the mouse and rat has been the subject of numerous studies during the past 50 or 60 years. Because the results of these studies are published in several languages and in many different journals, some of them not accessible in most libraries, because errors were inevitably present in the earlier articles, and because many of the published figures are not adequate for conveying a quick and clear understanding of the subject, the author has undertaken, and here presents the results of, a complete reinvestigation of nearly the whole field. The material used in the study consists of sections of embryos spaced at six hour intervals from 4 days to 9 days. In some cases ten or more embryos of a single stage have been sectioned. The sections were prepared by Olive Bartholomew, Elizabeth Fekete and the author. The technique used has been described elsewhere (14). To this description need only be added that, because in most cases the females used as mothers were hybrids between two strains, and because the fathers were from a third strain, thus giving both embryos and mothers a maximum of hybrid vigor, the stages as here described are usually earlier, often by as much as a day or more, than comparable stages described by other authors. While this procedure gave embryos which developed rapidly and were normal in a high proportion of all cases, it did not eliminate variability. No attempt has been made to describe the varia-

tions that have been noted in the rate of development of embryos or in the rate of development or form of separate parts. It should be emphasized, however, that the range of variation in these respects is considerable.

Wherever it is applicable to the mouse we have in general followed the terminology employed by Patten in the "Embryology of the Pig."

Contentious material is described in footnotes rather than in the text. Some readers will wish to skip these altogether. A complete bibliography is given at the end of the chapter, including a number of articles not referred to anywhere in the text.

Fertilization.—By fertilization is meant the entrance of a sperm into the egg. Fertilization in the mouse occurs in the upper end of each oviduct where the eggs are found, usually gathered into clumps, after their discharge from the ovaries. The sperm thus have to traverse the length of the uterus and oviduct to reach the eggs, a process accomplished partly through their own motility but for the most part through a churning action of the female duct. Since the beginning of heat in the female commonly occurs about two hours before ovulation, sperm may already be present in the oviduct when ovulation occurs.

The egg consists of a sphere of living protoplasm, the vitellus, surrounded by a transparent, non-living membrane, the zona pellucida (Fig. 1A). The zona pellucida in turn is surrounded by follicular cells which, however, are dispersed soon after fertilization. Within the vitellus is the egg nucleus, not clearly visible in living eggs such as the one shown in Fig. 1A, but easily seen in fixed and stained material.

Mature eggs within the ovary average about 95 μ in diameter (outside diameter of the zona). Following fertilization the zona pellucida expands until its outer diameter becomes about 113 μ (= .0044 inches). This is just within the limits of visibility for the unaided eye (35).

Usually only one sperm enters each egg. Almost immediately after entry, which may occur through any part of the egg's surface, the vitellus shrinks slightly in size and the zona pellucida expands, so that a space forms between them (35, 50). This is the perivitelline space. At this time only the first polar body has been formed. Within the next few hours the second maturation division occurs and the second polar body is budded off from the surface of the vitellus (Fig. 1B).

Not only the sperm head but also the middle piece and sometimes the whole tail enters the vitellus. The sperm head carries in one complete set of chromosomes from the male parent, while the middle piece contributes mitochondria from this parent. These latter are soon distributed through-

out the vitellus, and at the first cleavage division are divided more or less equally, along with the mitochondria already present in the egg, to the two

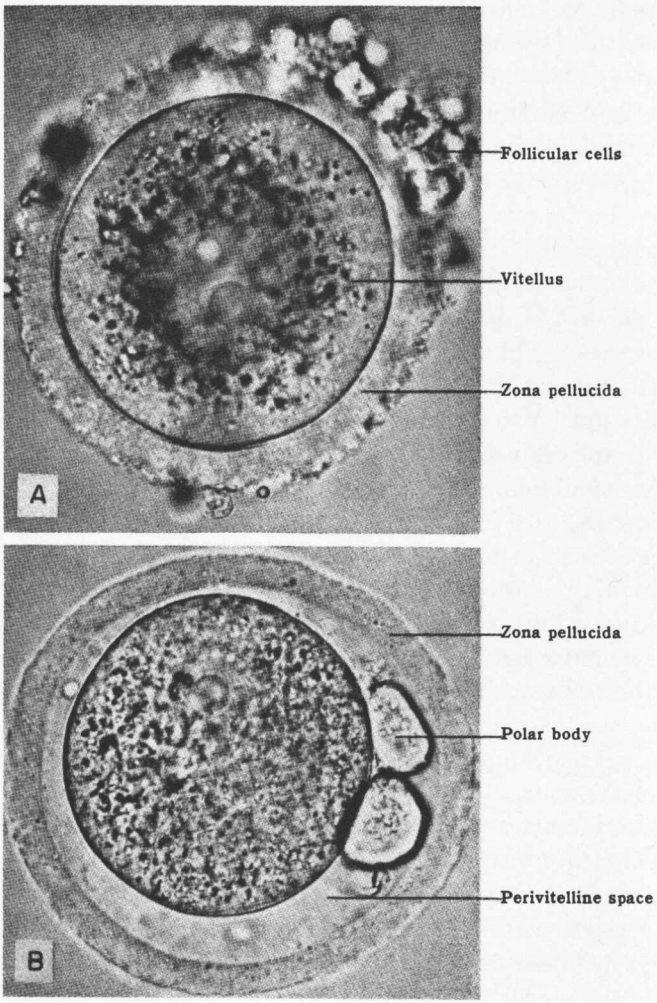

FIG. 1.—Photographs of mouse eggs (×600). A. Egg removed from ovary. B. Fertilized egg from oviduct 20 hours after copulation. Two polar bodies and sperm in perivitelline space. (*From Lewis and Wright.*)

daughter cells. There is some evidence that Golgi material is also carried by the sperm into the egg (19, 22, 34).

The sperm and egg nuclei, now both within the vitellus, are known as the male and female pronuclei. They move towards each other until they lie

side by side, each appearing at this stage as a typical resting nucleus, though the male element is a little the smaller of the two. At the first cleavage division the nuclear walls break down, the chromosomes split longitudinally, and one-half of each split chromosome is carried to each daughter cell. Hence at this division, as at all future somatic divisions, each cell receives a full complement of chromosomes from each parent.

Cleavage.—Cleavage in the mouse occurs while the eggs are still in the oviduct. The first cleavage occurs about 24 hours after copulation and

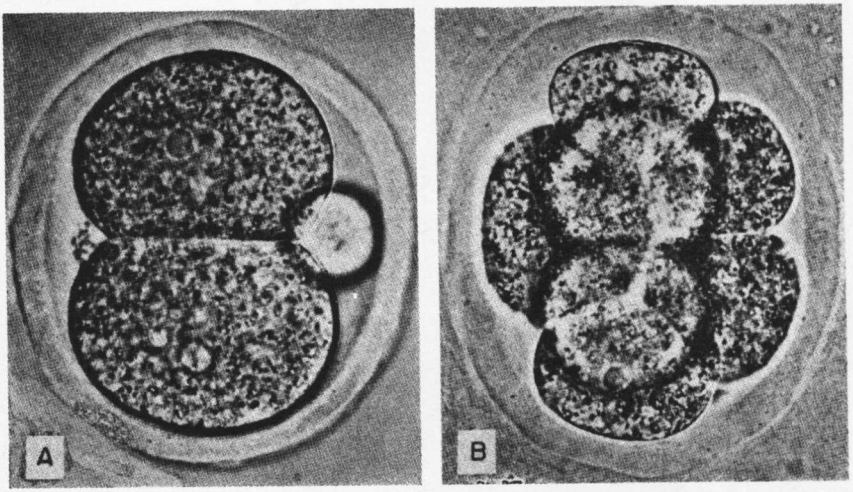

FIG. 2.—Photographs of mouse eggs (×600). A. Two-cell egg from oviduct 24 hours after copulation. Large second polar body and disintegrating first (on opposite side). B. Seven-cell egg from oviduct 48 hours after copulation. Note one cell on left larger than the rest. Division of this cell would give the eight-cell stage. (*From Lewis and Wright.*)

results in two cells not quite equal in size (Fig. 2A). Following divisions occur somewhat more rapidly, giving rise to 4-cell, 8-cell stages, etc., and are usually nearly synchronous in the different cells. Occasionally, however, eggs are found with some divisions completed, others still incomplete, and hence showing an odd number of cells (Fig. 2B). The actual act of division requires only 5 or 10 minutes; the interval between divisions lasts about 12 hours. Eggs of 16 cells or more, but in which no cavity has appeared, are called morulae. Eggs usually reach this stage about 60 hours after fertilization, and pass from the oviduct, through which they have been gradually moving, into the uterus, some 6 to 12 hours later (35). This is subject to

considerable variation, however, and in one study passage into the uterus at 4 days was found to be the rule (7).

The blastula.—Shortly after entering the uterus, and usually sometime after the egg has reached the 32-cell stage, an eccentrically located, fluid filled cavity appears among the cells of the morula. This enlarges rapidly

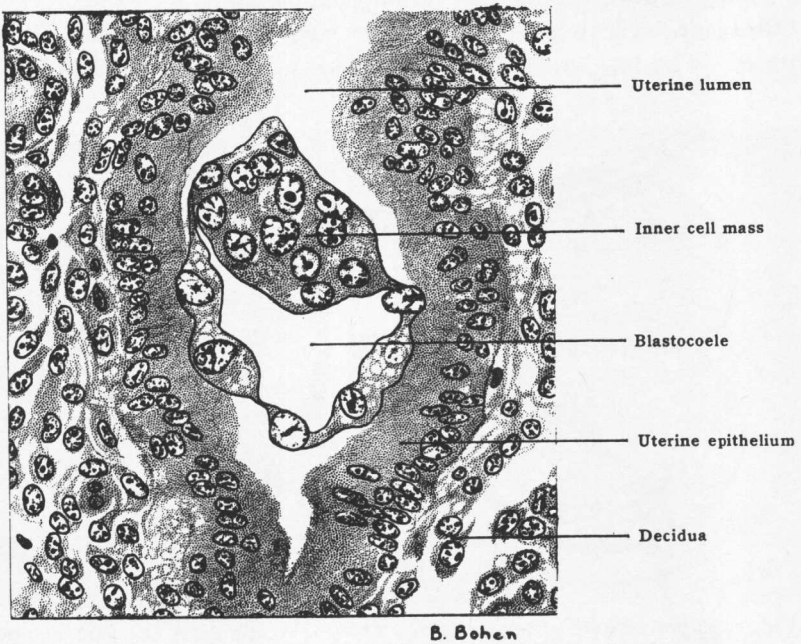

FIG. 3.—Blastula in uterine crypt 4 days after copulation. Projection drawing (×600).

to produce the segmentation cavity or blastocoele (Fig. 3). The cavity is bounded by only a single layer of cells except on one side where most of the cells are grouped to form a structure called the inner cell mass. Eggs in this stage are known as blastulae.

Implantation and early growth.—The uterus in the mouse is duplex, consisting of two horns which unite just anterior to their junction with the vagina, and each of which is attached to the dorsal body wall by a mesentery, the mesometrium (Fig. 4). There are two layers of muscle in each horn, an outer longitudinal layer and an inner circular layer. The uterine lumen is lined with epithelium. Between the epithelium and the muscle layers is the mucosa, a tissue which forms the bulk of the uterine wall. The epithelium is indented by numerous small crypts.

Very shortly after entering the uterus the eggs become spaced more or less evenly throughout its length, and each egg finds its way into a uterine

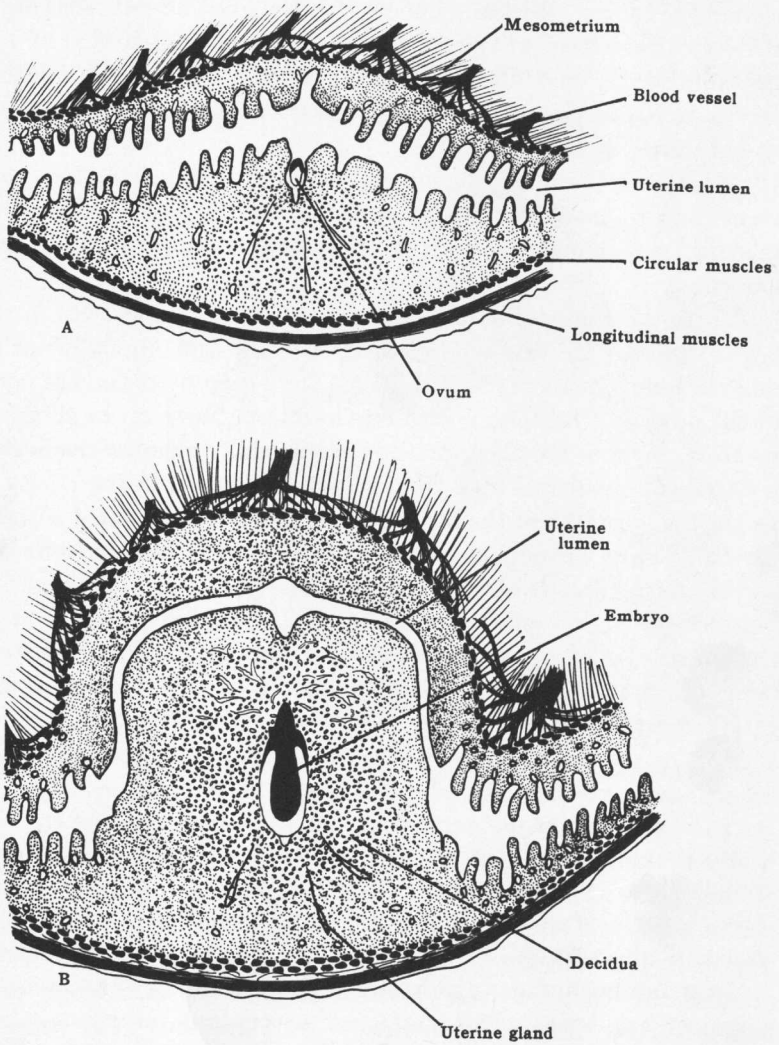

FIG. 4.—Diagrams showing implantation (×45). A. Longitudinal section through horn of uterus about 5 days after mating. An ovum has recently become implanted in one of the uterine crypts. B. Longitudinal section through implantation site about 7 days after mating. (*After Burckhard.*)

crypt on the ventral or antimesometrial side of the lumen, thereby coming into close contact with the uterine epithelium (Fig. 3). The presence of the

blastula quickly sets up changes at the implantation site. Within a few hours the epithelium begins to loosen, and its nuclei to show degenerative changes (Fig. 5). Within 15 hours it is sloughed off entirely (Fig. 6). At the same time active growth commences in the mucosa, so that by 1 day after implantation (5 days after mating) there is an appreciable swelling in the uterus at the implantation site. The swollen mucosa at the implantation site is known as decidua.

Meanwhile the zona pellucida has been lost from around the egg, perhaps through a process of digestion by means of an enzyme secreted by the uterine mucosa (11), though neither the exact time nor mechanism is thoroughly known.

Up to the time of implantation there has been no growth in size in the egg. Cleavage has resulted in a division of the egg, originally one large cell, into numerous smaller cells, but little if any new protoplasm has been formed in the process. Beginning with implantation, however, rapid growth commences. At first the blastocoele enlarges, while the inner cell mass assumes a flattened cup-shape with the concave face towards the cavity (Fig. 5). In the living condition the blastocoele is probably distended with fluid, and its walls tightly pressed against the uterine epithelium, but in fixed material at this stage there is always some collapse. This initial expansion of the blastocoele requires only a few hours and is quickly followed by a growth of the inner cell mass down into the enlarged cavity (Fig. 6). Blastocoele and inner cell mass both are known thereafter by new names; namely, yolk cavity for the former and egg cylinder for the latter. A comparison of Figs. 7, 8, 10 and 12 will show the rapid growth of the egg cylinder that occurs during the next two and one-half or three days.

The formation of the entoderm.—At the same time that the blastocoele begins to enlarge, the inner cell mass can be seen to be composed of two types of cells (Fig. 5). Adjacent to the blastocoele is a single layer of darkly staining cells. This is the entoderm, one of the three primary germ layers. The rest of the blastocyst is composed of ectoderm, divided into the ectoderm of the inner cell mass, and the trophectoderm, a single celled layer bounding the blastocoele ventrally and laterally. The trophectoderm (troph from the Greek word for nourishment) derives its name from the fact that it probably plays a rôle in the nourishment of the young embryo. The mesoderm has not yet appeared.

Very shortly after the first appearance of the entoderm, single cells or strands of cells grow out from its margin down along the inner surface of the trophectoderm. At first these cells are few and widely separated (Figs. 7

and 8), but by 6½ days they lie evenly spaced and quite close together over the trophectoderm's entire inner surface (Fig. 10). The layer of cells thus formed is known as the distal entoderm. Meantime the inner cell mass has grown down into the yolk cavity to form the egg cylinder. This is composed of an inner mass of ectoderm cells and an outer layer of entoderm cells (Fig. 8). This layer of entoderm cells bounding the egg cylinder is known as the proximal entoderm. The entoderm is thus divided into two distinct parts, distal and proximal, lining the distal and proximal walls of the yolk cavity.

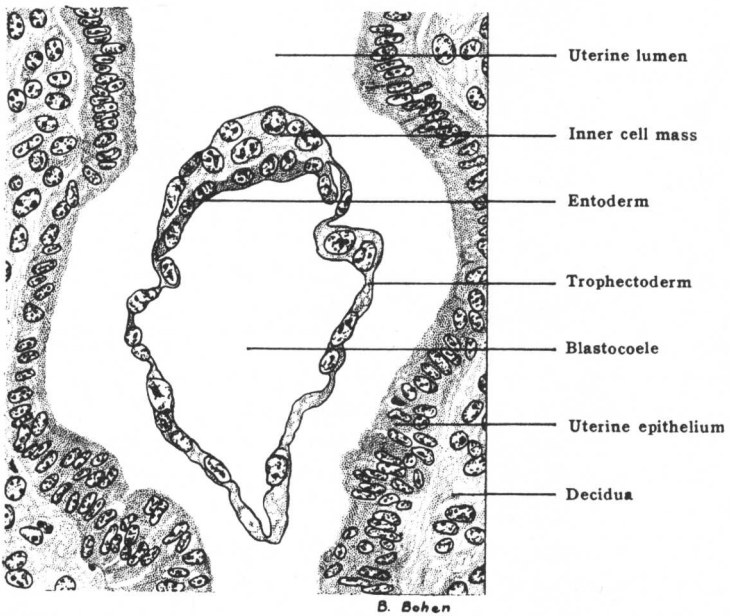

FIG. 5.—Section of implanting blastula 4 days 5 hours after mating. Projection drawing (×400).

Embryonic and extra-embryonic ectoderm.—At about 4½ days, when the egg cylinder first begins to form, it can be seen that the egg cylinder ectoderm is divided into two parts, a dorsal,* more darkly staining† region with

* Most authors have used the terms mesometrial and antimesometrial to distinguish the two poles of the egg, the former being toward, and the latter away from, the mesometrium or supporting mesentery of the uterus. However, as the dorso-ventral axis of the embryo coincides with the dorso-ventral axis of the mother for at least the first 8 days of development, the usage dorsal and ventral would seem to be perfectly clear in most cases besides having the advantage of simplicity. The dorsal side is up in the drawings.

† When counterstained with congo red.

elongated nuclei, and a ventral, more lightly staining portion with round nuclei* (Fig. 6). The former gives rise to various extra-embryonic struc-

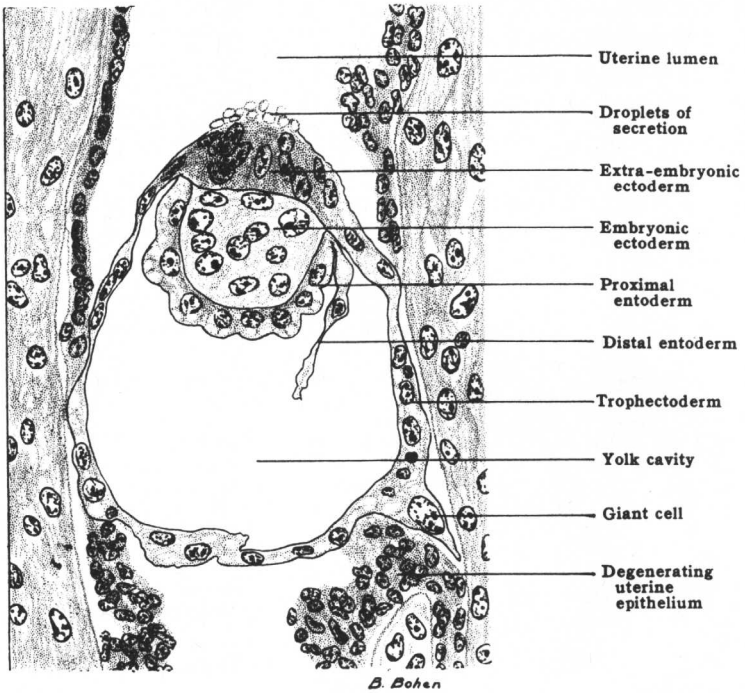

FIG. 6.—Longitudinal section of early egg cylinder stage at 4 days 15 hours after mating. Projection drawing (×400).

tures and is, therefore, called the extra-embryonic ectoderm; the latter gives rise to the ectoderm of the embryo proper and is, therefore, called the embryonic ectoderm. While the difference in staining reaction and in the shape of the nuclei has disappeared by 5½ days, the division between the

* It is possible that the division between embryonic and extra-embryonic ectoderm can be traced back to stages earlier than 4½ days. One author (41) contends that the ectoderm of the inner cell mass at a stage corresponding to that shown in Fig. 5 is divided into two regions, a lighter staining outer layer continuous with the trophectoderm and a darker staining area between this and the entoderm, but the existence of such a division has also been denied (22, 61). In our preparations at the 4¼ day stage we find occasional flattened, dark-staining nuclei on the outer surface of the inner cell mass and in some cases these appear to form a layer continuous with the trophectoderm. It seems probable that these represent an early stage of the extra-embryonic ectoderm. Phylogenetically the extra-embryonic ectoderm is probably derived from the trophectoderm, so that a similarity of structure is not surprising.

two regions is still quite distinct (Fig. 8). Strictly speaking the trophectoderm is also extra-embryonic ectoderm, but as a matter of convenience the term will be used only for the extra-embryonic ectoderm of the egg cylinder.

At about 5 days a cleft or cavity, the proamniotic cavity, appears in the embryonic ectoderm (Fig. 7). This is followed very shortly by the appearance of a similar cleft in the extra-embryonic ectoderm, and by the fusion of these two, so that by 5½ days the egg cylinder contains a narrow lumen (Fig. 8).

The ectoplacental cone.—Beginning at 5 or 5½ days, active growth at the dorsal end of the extra-embryonic ectoderm gives rise to a new structure,

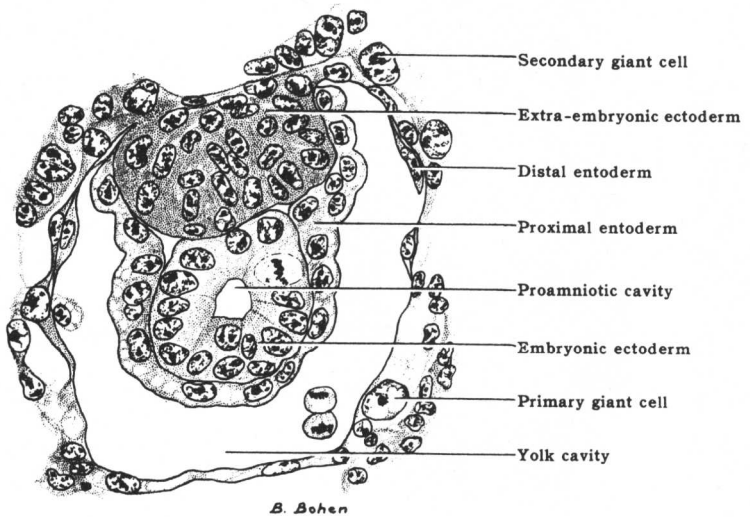

FIG. 7.—Longitudinal section of early egg cylinder. Age unknown, but probably about 5 or 6 days. Projection drawing (×400).

the ectoplacental cone, which joins the egg cylinder ventrally, and extends dorsally towards the lumen of the uterus (Fig. 8). This develops rapidly, its cells showing numerous mitoses, and by 6½ days it composes almost one-half of the total length of the embryo. Its structure, particularly at the upper extremity, is porous, and the interstices between the strands of cells that compose it soon become infiltrated with maternal blood (Fig. 10). In later stages it becomes part of the placenta.

The inversion of the germ layers.—At 5½ days (Fig. 8) the egg cylinder is a structure consisting of a double wall enclosing a narrow lumen. The inner layer of the double wall is composed of ectoderm, the outer of entoderm. This relation of ectoderm and entoderm, found in the mouse, rat,