THE EMBRYOLOGY OF FLEAS

(WITH 12 PLATES)

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INTRODUCTION

Aside from its general scientific aspects, the study of flea embryology is of interest from two particular standpoints. In the first place, such an investigation has been needed to extend our knowledge of the pulicine life cycle. Hitherto the embryological phase of this cycle has been neglected in spite of the fact that its other aspects have received considerable attention. A careful search of the literature reveals only five contributions to the subject of flea embryology, and they are all brief and fragmentary. In some cases the claims made therein have been found to be erroneous in the light of the present study. These discrepancies are doubtless due to the difficulties involved in the technique of preparing the eggs for successful observation.

Weismann (1863) was the first to undertake an investigation of the development of the flea in the egg stage, but he dismisses the subject with a single page of his lengthy paper which is otherwise concerned with the embryology of *Chironomus* and *Musca*. He chose the dog flea, *Ctenocephalides canis* (Curtis), as the species for his consideration and reports that in the egg of this form "although the chorion is not exactly opaque it does not allow observation of the finer details, which, to be ascertained accurately, require a well related series of observations. Yet it mocks at every attempt to remove it without injury to the yolk membrane." Weismann concludes, therefore, that the egg of the flea is not favorable for embryological study.

The second worker to publish on the subject of flea embryology was Packard (1872). He used the eggs of the cat flea, *Ctenocephalides felis* (Bouché), although, believing like other workers of his day that cat and dog fleas constituted a single species, he titled his discussion "The Development of *Pulex canis*." Like Weismann, he was forced to restrict his studies to superficial observations inasmuch as the section method of investigation was not well developed.

Balbiani (1875) was the third to attempt to work out the early ontogenetic development of fleas. He followed Packard in the use

of cat flea eggs and observed that in them the chorion is more transparent than in the ova of other siphonapteran species. He recognized the inadequacy of the study of whole eggs, for he says in this paper, "The delicate question of embryology can only be studied with profit by the sectioning method, but the egg of the flea is too small to bear this mode of investigation." Balbiani can scarcely be blamed for reaching this conclusion when the small size of cat flea eggs is considered together with the fact that at that time no insect eggs had been sectioned with any degree of success. Considering the absence of efficient microtomes and biological stains, necessitating the limited methods of research employed by Balbiani and his two predecessors in the investigation of flea embryology, the results which these pioneers obtained must be admired. Although obviously superficial, as well as inaccurate in some instances, the papers of these first students of flea development are, nevertheless, definitely superior to those of the two writers on this subject who followed them and who had many of the technical advantages of modern equipment.

The first one to utilize the section method in studying flea eggs was Tikhomirowa (1890). This paper is not available to the writer, but a review of the main points given therein is presented by its author in another of her papers (1892) which deals with the development of *Chrysopa*. For the most part her conclusions regarding flea development are inaccurate.

Strindberg (1917) was the second to apply the section method to the investigation of pulicine embryology. His brief paper is concerned only with the intermediate developmental phases of *Archeopsylla erinacei* (Bouché). Concerning the other periods he says: "The early embryonic stages scarcely present worthwhile observations." "The later embryonic stages do not show anything worthy of note and therefore are not taken into account." A critical examination of this author's statements necessitates the conclusion that his material was very inadequate.

The second respect in which the study of flea embryology is of particular interest is that which is concerned with its application to the phylogenetic position of the Siphonaptera. A study of the literature suggests that there has been more speculation on the subject of the affinities of the fleas than of any other insect order. This interest in the natural relationships of fleas seems to have been the chief stimulus which has prompted previous investigators to undertake studies of their development, for their papers include discussions of the racial origin of these insects. While accurate comparative embryological observations are invaluable in the solution of phylogenetic problems,

it must be recognized that much of the past work in the field of insect embryology is inaccurate. This is evidenced by the distinctly opposite interpretations which frequently have been given by workers on the same species. In view of this fact it is inadvisable for the writer to attempt a phylogenetic application of the present observations until he has personally investigated the embryology of those forms which are suspected of being most closely related to the Siphonaptera. Only then can it be certain that the same methods and interpretations have been applied to the different subjects and that the comparisons are worthwhile.

SPECIES

The observations made during this study indicate that the embryological development of all fleas is essentially the same. Except for such superficial characteristics as size, nature of the chorion, and the number of micropyles, the corresponding stages of the eggs of different species appear to be identical. This was shown by making careful studies on the development of three species of diverse systematic positions. These included *Ctenocephalides felis* (Bouché), the cat flea; *Nosopsyllus fasciatus* (Bosc.), the common rat flea of temperate regions; and *Hystrichopsylla dippiei* Roths., a giant form which lives in the nests of the wood rat *Neotoma*.

Following the classification of Oudemans (1909), the order Siphonaptera is divided into two suborders on the basis of the presence or absence of a dorsal suture on the head. Fleas which possess such an incrassation are placed in the suborder Fracticipita, while those which lack it constitute the suborder Integricipita. If this division is a natural one based on true relationships, the choice of the three species used in this study is valid. *Ctenocephalides felis* and *Nosopsyllus fasciatus* belong to the Integricipita, while *Hystrichopsylla dippiei* is classed in the Fracticipita.

In contrast to Oudemans' system, many authors, including Ewing (1929), consider that the subdivision of the Siphonaptera into these two groups is artificial and unwarranted owing to the number of intermediate forms in which it is difficult to determine whether or not a dorsal suture is present, and also because of the lack of sufficient correlating characters. These authors prefer to follow the classification of Baker (1905) which regards the Siphonaptera as a compact group and, accordingly, divides it directly into families. Based on this classification, the choice of species for this study is again found to be a satisfactory one inasmuch as the three forms selected belong to three different families. *Ctenocephalides felis* represents the Puli-

cidae, *Nosopsyllus fasciatus* the Dolichopsyllidae, and *Hystrichopsylla dippiei* the Hystrichopsyllidae. Moreover, these three families to-·gether contain over 80 percent of the total number of genera belonging to the order. The three subject species used in this study must be regarded, therefore, as a valid representation for the Siphonaptera as a whole.

EGG SOURCES

Ctenocephalides felis proved to be the most prolific of the three species studied. The eggs of this flea were collected from a cat's sleeping blanket by means of a camel's hair brush.

Nosopsyllus fasciatus eggs were obtained from a pure culture of this species reared on white mice by the methods described by Leeson (1932). Gravid females were confined in voile-capped vials until they had oviposited. It was found that these imprisoned insects lived longer and deposited more eggs if a piece of cotton was placed in the vial with them.

The eggs of *Hystrichopsylla dippiei* were obtained either from the nests of wood rats or from gravid females taken from such nests. Because the eggs of this species are so much larger than those of the several other forms which inhabit *Neotoma* nests, there was no danger of mistaken identity.

AGE DETERMINATION

In order to determine the developmental age of embryos in terms of days, cat flea eggs, newly laid by females imprisoned in vials, were incubated under a constant temperature of 25° C. and a relative humidity of 79 percent. Relatively few eggs were incubated in this manner; these were used as standards and the others studied were interpolated into the series in their proper places. As no attempt was made to obtain *Nosopsyllus fasciatus* and *Hystrichopsylla dippiei* eggs of accurately determined incubation age, the developmental stages of these species were compared to known ones of *Ctenocephalides felis*.

MICROTECHNIQUE

In the preparation of whole mounts of *Ctenocephalides felis* embryos, dechorionation was facilitated by heating the eggs at 90° C. for a few minutes. The heat served to coagulate the yolk so that it drew away from the chorion, thereby providing a working space between the shell and the vitelline membrane. The resulting solidifica-tion of the vitellus was a further advantage because a yolk so hardened

does not rupture easily. The relatively thin chorion of the cat flea egg was an added factor in making dechorionation in this species a simple matter. This membrane collapses as soon as it is punctured and is thereafter quite easily removed under the binocular by dissecting it away with a fine pointed needle.

Because the eggs of *Nosopsyllus fasciatus* and *Hystrichopsylla dippiei* have thicker shells and therefore do not ordinarily collapse when punctured, a different procedure was necessary for the removal of their chorions. After heating, the surfaces of these eggs were dried thoroughly with blotting paper and they were immersed in liquid paraffin on a glass slide. When the paraffin had hardened, a series of needle punctures was made completely around the egg through both the paraffin and the chorion. Next, the adjacent punctures in the paraffin were connected and the paraffin cap, thus formed, was removed. Finally, the punctures through the exposed chorion were connected and the upper surface of this shell was removed in one piece. The shrunken vitellus with its attached embryo was lifted through the resulting opening by means of a minute spatula. Dechorionated eggs were fixed in Bouin's picro-formol-acetic fluid, stained with alcoholic borax-carmine, destained in 70 percent acid ethyl alcohol, cleared in cedar oil, and mounted in xylol-balsam.

Eggs used for sectioning were neither heated nor dechorionated. However, owing to the impermeability of the chorion, this membrane was punctured before fixation. Puncturing was done in the same Bouin's fluid which was to serve as the fixing reagent. Several punctures were made in each egg. Tertiary butyl alcohol, as recommended by Johansen (1935), was used for washing and dehydration. Imbedding was accomplished by a combination technique involving modifications of two previously used methods. These were Boycott's well-known paraffin-celloidin technique and Walls' (1932) adaptation of the hot-celloidin process to animal tissues. From absolute tertiary butyl alcohol, the eggs were placed in a 2 percent solution of celloidin dissolved in ether-alcohol. Infiltration was done in small glass tubes, 3 mm. in diameter. These were cut into short lengths and closed at one end. Such infiltration bottles, containing the eggs in 2 percent celloidin, were placed, uncorked, in a metal cylinder capable of withstanding high pressure. After being sealed, this cylinder was placed in an electric oven with the temperature set at 60° C. where the heat, acting on the ether-alcohol solvent, provided the necessary pressure to force the celloidin into the tissue. After 24 hours the cylinder was removed from the oven, cooled, and opened. At this point the step from Boycott's method was incorporated into the procedure. About

half of the 2 percent ether-alcohol celloidin was removed from each tube, and an equal amount of 3 percent celloidin dissolved in clove oil was added, after which the infiltration under heat in the pressure cylinder was continued for another 24 hours. Following this second period, the celloidin was gradually thickened by suspending a tiny piece of parlodion in the upper part of the solution, after which the pressure infiltration was carried on for another day. Such additions of parlodion were made daily until the solution reached the proper consistency for imbedding.

Hardening of the celloidin blocks was accomplished by immersing the open infiltration tubes in chloroform for several days. It was important, however, that the contents of the tubes be thoroughly cooled before the hardening was begun. After hardening was completed, each tube was tapped with some hard object until its glass wall was cracked in several places. The resulting pieces of glass were carefully picked away from the celloidin, and the block was soaked in chloroform until it ceased to float.

The completely hardened celloidin block was infiltrated in paraffin preparatory to double imbedding. During this process the clove oil in the celloidin matrix was partially replaced by paraffin, thereby eliminating the possibility of the celloidin pulling out from its paraffin coating during sectioning.

Because of the tendency of the celloidin matrix to warp during heating and drying, the following method for mounting the ribbons was used. The surface of the slide was first swabbed with a thick layer of albumen fixative. Good quality cigarette papers were cut in half longitudinally, and each half was laid on a flat surface and saturated with distilled water. The microtomed ribbon was cut into strips of the desired length, and these were laid in reverse order on the paper. More distilled water was then applied to the paper, and the resulting surface tension aided in pulling the ribbons out until they were fairly flat. Thereafter, the paper was lifted up with the sections adhering to it, reversed, and laid ribbon-side down on the albumen-smeared slide. The whole was immediately blotted with several thicknesses of filter paper, a finger being rolled over this paper from one end of the slide to the other, thereby insuring direct contact between the sections and the slide at all points. Sections mounted in this manner were certain to be flat and therefore suitable for photographic purposes.

Again, because of the tendency of the celloidin to warp, no period of drying was allowed following mounting. Instead, as soon as the cigarette paper was pulled away, leaving the ribbon adhering to the

slide, all traces of water were quickly wiped from the under surface of the glass, and the slide was passed directly into xylol. Absolute tertiary butyl alcohol was used following the removal of the paraffin by xylol because it was desired to keep the celloidin matrix intact, and this alcohol does not dissolve celloidin.

In order to make certain that the sections would remain firmly attached to the slide during the staining process, a step was adapted from Galigher (1934) and introduced into the procedure at this point. This consisted of dipping the slide into a 1 percent solution of ether-alcohol celloidin and then passing it into chloroform to harden the film. Thereafter, the slide was transferred to 95 percent tertiary butyl alcohol for the beginning of hydration preparatory to aqueous staining. The lower alcohols used in the series were either tertiary butyl or ethyl solutions, inasmuch as the latter possessed sufficient water to prevent the dissolution of the celloidin matrix and protective film. It was necessary that dehydration following staining be completed with tertiary butyl alcohols.

Sections were stained by various methods, the combination of Delafield's haematoxylin with Eosin Y as a counterstain being used for most of them. Mayer's haemalum with a counterstain of Fast Green FCF, and Heidenhain's iron haematoxylin with a counterstain of Eosin Y were also used to some extent. Heidenhain's iron haematoxylin was found unsatisfactory for the earlier stages because it colored the numerous yolk spheres so darkly that they dominated the entire picture and obscured both the cleavage nuclei and the tropho-nuclei (pl. 1, fig. 13).

Because the eggs used in this study were obtainable in such large numbers, it was possible to infiltrate and imbed them en masse, several hundred to a block. No attempt was made at orientation, and those sections which were cut at undesirable planes were ignored (pl. 4, figs. 39, 40).

CHARACTERISTICS OF THE EGGS

The eggs of fleas are regularly prolate-spheroidal in shape. Consequently, they do not possess any dorsoventral differentiation such as is exhibited by the ova of many other insects. When first deposited, the eggs of all three species of fleas considered in this paper are glistening white. There is a pronounced difference in the degree of their transparency, however. Those of *Ctenocephalides felis* are relatively transparent, those of *Hystrichopsylla dippiei* less so; those of *Nosopsyllus fasciatus* are the most opaque. While the eggs of all fleas

tend to become more opaque as they age, those of the cat flea are by far the most transparent at every stage of development. Color differences also become manifest as development progresses. The eggs of the cat flea remain relatively colorless, those of the other two species assume a cream color within a few hours after being laid. This coloration is retained by the eggs of *Hystrichopsylla dippiei,* but in the case of *Nosopsyllus fasciatus* the eggs often acquire a darker hue which sometimes approaches a reddish brown before hatching. These variations in color and transparency are due primarily to chorionic differences between the species, correlated with differences in egg size. A relatively thick chorion, combined with small egg size, makes an egg more opaque and darker in color.

Of the three species here considered, the eggs of *Hystrichopsylla dippiei* are much the largest, approximately 1.9 mm. in length and 0.9 mm. in greatest diameter. Corresponding measurements for the two other species were 0.8 mm. and 0.4 mm. for *Nosopsyllus fasciatus,* and 0.5 mm. and 0.3 mm. for *Ctenocephalides felis.*

The outer surfaces of most insect eggs are sculptured with a reticulum of slightly raised ridges which enclose polygonal spaces. These markings represent the imprints of the ovarian follicle cells which serve to secrete the chorion. In the eggs of *Nosopsyllus fasciatus* and *Hystrichopsylla dippiei* this sculpturing of the chorion is rather pronounced. However, in the cat flea egg it is scarcely perceptible, even when the egg surface is thoroughly dried after oviposition. The chorionic markings of this species are so indistinct that Balbiani (1875) characterizes the chorion of the cat flea egg as unsculptured. He says, "The rugose appearance, like scales, that the egg presents on its surface, is not inherent in this membrane as Leuchart thought, but is due to the coating that the egg receives at the moment of deposition." Balbiani does not state what procedure he followed in reaching this conclusion, but it may be assumed that he failed to see markings on the chorionic surfaces of eggs dissected directly from the ovarioles. A failure of this type is attributable to the fact that as long as the chorion is wet its sculpturing is difficult to make out. This is true even in the case of *Nosopsyllus fasciatus* or *Hystrichopsylla dippiei* eggs in which the reticulations of the chorion are very evident when this membrane is dry. As cat flea eggs tend to collapse in a short time after they are dissected from the ovariole, it is probable that Balbiani examined the eggs before they had dried sufficiently to show the chorionic sculpturings clearly. The faint reticulations which may be seen on the chorion of the *Ctenocephalides felis* egg are without doubt

homologous to the sculpturings on the egg surfaces of other insects, representing the imprints of the cells of the follicular epithelium.

In section the chorion of the cat flea egg appears as an extremely thin single membrane (pl. 4, figs. 39 to 42). By contrast, the chorions of *Nosopsyllus fasciatus* and *Hystrichopsylla dippiei* are considerably thicker. In the latter of these species this membrane appears as a rigid shell made up of two laminae which are united by countless, extremely slender, transversely arranged, supporting pillars (pl. 1, fig. 2). These two chorionic layers appear to correspond to the exochorion and endochorion which Blochmann (1887b) and Wheeler (1889) have described for certain other insects.

In all of the three flea species studied, a second protective covering lies beneath the chorion. This is the yolk or vitelline membrane (pl. 6, fig. 53; pl. 11, fig. 79). It is thin in all species and, like the chorion, is noncellular. Its relative position in regard to the chorion and the yolk surface varies in different preparations, but as a rule it lies nearer to the latter in the younger stages. The vitelline membrane appears to be merely the attenuated limiting membrane of the developing oocyte which has been secreted by the superficial protoplasm. It remains intact until the time of eclosion.

Spermatozoa traverse the chorion and the vitelline membrane of insect eggs by means of micropylar openings. In flea ova these are arranged in two circular areas, one at each pole (pl. 1, fig. 1). These micropylar apparatuses have been observed previously in the eggs of *Ctenocephalides felis* by Balbiani (1875). The number of openings at the respective poles in the eggs of any one species is variable, but is always considerably greater at the anterior pole in any individual egg. Furthermore, the minimum for the anterior pole number is always somewhat greater than the maximum for the posterior pole number in the same species. Balbiani found that in the case of cat flea eggs the number of anterior micropylar openings varied from 45 to 50, whereas his counts gave only 25 to 30 such pores for the posterior pole. However, the writer's counts show a greater variation in the case of each pole. The micropylar openings at the anterior pole were found to number as few as 35 or as many as 55, whereas the posterior apparatus was found to consist of from 20 to 30 such openings.

It appears that the micropylar apparatuses in the eggs of *Nosopsyllus fasciatus* and *Hystrichopsylla dippiei* have not been described previously. In the former the number of openings at the respective poles corresponds closely to the range given above for *Ctenocephalides felis*. In *Hystrichopsylla dippiei* the pores are fewer in number at both

poles; the anterior apparatus in this species was found to possess 30 to 40 such openings, whereas the posterior one gave counts of from 15 to 25. In all three species studied, the openings at each pole were found to tend toward a spiral arrangement.

In *Hystrichopsylla dippiei* there may be a number of volcanolike raised processes arranged irregularly around the margin of the micropylar area (pl. 1, fig. 2). Their general appearance suggests the so-called micropyles of *Phyrrhocoris apterus* illustrated by Packard (1898, fig. 501), but longitudinal sections through them have failed to demonstrate for certain that the minute canals which pass inward from their craters perforate the chorion. Therefore, their function as supplementary micropyles is doubtful. The eggs of *Nosopsyllus fasciatus* and *Ctenocephalides felis* appear to lack these peculiar structures.

Balbiani (1875) was of the opinion that in cat flea eggs only the anterior group of micropylar openings served as passageways for the entrance of spermatozoa, since he found what he interpreted as spermatic filaments caught only in the anterior openings. None of the preparations made during the present study show any structures lodged in the openings of either pole which may be interpreted with any degree of certainty as spermatic tail pieces. Furthermore, longitudinal sections of the eggs of all three species demonstrate clearly that the lumina of the pores of both the anterior and the posterior micropylar apparatuses pass through the chorion. It is probable, therefore, that in the case of flea eggs the openings of both the anterior and posterior micropylar areas serve as passageways for the entrance of spermatozoa.

The difference in the number of micropylar pores present at the poles is the only external indication of anterior-posterior differentiation in flea eggs. The identification of the poles was made by opening eggs in which the embryos had developed sufficiently to permit positive recognition of the cephalic and caudal ends. This identification made it possible to test the Law of Hallez (1886) as applied to flea eggs. Eggs for this purpose were dissected from the oviduct, and invariably their anterior poles were directed toward the head of the mother. Because flea eggs possess no dorsoventral differentiation until development is well advanced, no attempt was made to test the Law of Hallez in respect to this second axis.

Some authors, such as Huettner (1923), describe a third protective membrane for insect eggs. This they term the plasma membrane. It is described as an extremely thin investment adhering closely to the

superficial protoplasm and difficult, therefore, to demonstrate. Such a covering, if present, would lie inside of the vitelline membrane and would be evident when the germ cells push out at the posterior pole as described in a later section. Such a membrane is not apparent in flea eggs.

The superficial protoplasm of insect eggs is known as the periplasm or cortical ooplasm. The periplasm at the anterior pole is called the anterior polar plasm, and that at the posterior pole is termed the posterior polar plasm. In the eggs of many forms either one or both of the polar plasm regions are widened to form protoplasmic caps which some authors regard as adaptations to facilitate the entrance of spermatozoa. As will be described later in the section on germ cells, most writers on insect embryology believe that the posterior polar plasm plays an important role in the differentiation of the primordial germ cells. In comparison with the eggs of most other insects, those of fleas possess a very narrow periplasm, and none of the sections made during this investigation showed it widened to form an anterior protoplasmic cap. However, some of the sections have this cortical ooplasm perceptibly thickened at the posterior pole (pl. 5, fig. 43). The entire periplasm is uniformly granular and no darker-staining granules are in evidence in this posterior protoplasmic cap.

The periplasm is continuous with an anastomosing reticulum of protoplasmic strands which ramifies throughout the inner portion of the egg. Contained within the meshes of this reticulum are numerous vitelline spheres which make up the deutoplasm or store of yolk for the nourishment of the developing embryo. These vitelline spheres vary considerably in size. Lying within each of them are several small, rounded, and refringent bodies. These are the vitelline bodies. Both vitelline spheres and their vitelline bodies appear in many of the accompanying photomicrographs (pl. 1, figs. 4, 5, 8, etc.). Delafield's haematoxylin was found to leave both the vitelline spheres and vitelline bodies colorless, and was used for the sections from which the above illustrations were made. By contrast, Heidenhain's iron haematoxylin stains both structures black (pl. 1, fig. 13).

Multitudes of granulelike objects, apparently identical with the granules of the periplasm, may be present along the strands of the protoplasmic reticulum (pl. 5, fig. 45). These are by no means uniformly distributed, and their concentration varies in different preparations. They appear to correspond to the Blochmann's corpuscles which have been observed in the eggs of certain other insects. These bodies were first observed by Weismann (1863), and, although he includes a short discussion of the development of the dog flea egg in this

same paper, he fails to mention the presence of such bodies in this species. Blochmann (1884, 1886, 1887a) studied these small corpuscles in the eggs of a number of insects and concluded that they play an important part in embryonic development. Because he observed their multiplication by transverse fission, he concluded them to be bacterial organisms. Mercier (1906) substantiated Blochmann's supposition by successfully culturing them in artificial media. Owing to the great variability of the number of these bodies in flea eggs, it seems improbable that they are of importance in the embryonic development of these insects. No attempt was made to culture these structures.

MATURATION

In unfertilized flea eggs, the position of the female pronucleus is somewhat variable, being dependent upon the maturation processes. Some of the preparations of newly deposited eggs show that they contain only two nuclei and that these are lying close together in the margin of the anterior polar plasm. Such a preparation apparently represents a stage immediately following the first maturation division, one of the two nuclei representing the first polar body and the other the secondary oocyte. Sections from other newly laid eggs show that the second maturation division has already taken place, as two polar body nuclei may be seen lying in the periplasm, while a third or ootid nucleus lies toward the interior of the egg. The polar body nuclei of fleas, like those of other insect forms, are never extruded from the egg but remain within the anterior periplasm until they disintegrate. The first polar body appears to remain undivided. Following the second maturation division the ootid nucleus migrates toward the center of the egg. Oviogenesis, or the differentiation of the ootid nucleus into the female gametic nucleus, takes place during this migration. This maturing process prepares the female nucleus for syngamy with the male gametic nucleus.

SYNGAMY

Figure 12 (pl. 1) shows a longitudinal section through the anterior half of a cat flea egg in a stage just prior to syngamy. Only four nuclei are evident in the entire egg and three of them are present in this section. These are the female gametic nucleus, the male gametic nucleus, and one of the polar body nuclei. The second polar body lies at a different level and appears in another section. The large number of micropylar openings occurring in flea eggs is suggestive of

polyspermy, but if other spermatozoa have entered this particular egg they have apparently disintegrated. The actual process of fertilization, or the fusion of the male and female gametic nuclei, is shown in figure 37 (pl. 4). It occurs in the central region of the egg, as a rule somewhat toward the anterior pole. The zygotic nucleus resulting from syngamy is shown in figure 38, and the complete section from which this enlargement was made is shown in figure 39.

CLEAVAGE

As is typical for the eggs of insects, cleavage in fleas is meroblastic peripheral, owing to the centrolecithal nature of the ovum. By a series of synchronous mitotic divisions, several generations of so-called cleavage cells are produced by the zygotic nucleus. As Blochmann (1887b) first pointed out, these bodies are not independent cells, inasmuch as each is connected by protoplasmic strands to the protoplasmic reticulum which ramifies throughout the vitellus, and by this reticulum, in turn, to the periplasm. In flea eggs, each cleavage nucleus is surrounded by a star-shaped protoplasmic mass, and this is continuous with the protoplasmic reticulum. Such cleavage nuclei, therefore, have the same general appearance as the zygotic nucleus. Like it, they stain rather faintly.

Synchronous cleavage divisions have been reported for a number of insect species. Huettner (1923) found them to occur in *Drosophila,* Auten (1934) in *Phormia,* and Butt (1936) in *Brachyrhinus.* Their presence in flea development, however, has not been observed heretofore.

During the early cleavage divisions the resulting nuclei lie rather close together in the central region of the egg. Not until after the completion of the fifth such division, when the egg is in the 32 nucleus stage, is there an indication of any orderly migration of the cleavage nuclei toward the periphery. Following the next or sixth cleavage division, when 64 segmentation nuclei are present in the egg, the majority of these have definitely begun their migration to the surface. In doing this they collectively assume a hollow spheroidal arrangement which is proportionate in all its dimensions to the outline of the egg. The nuclei which outline this spheroid are all approximately equidistant from the cortical ooplasm which forms the surface of the egg mass. Huettner (1923) says that in *Drosophila* the cleavage nuclei start their migration to the surface after the eighth cleavage division, or when the egg is in the 256 nucleus stage. In flea eggs, therefore, this migration starts somewhat earlier.

It has been stated above that the majority of the cleavage nuclei migrate to the surface of the egg. The relatively few that remain behind in the vitellus are destined to produce the primary trophonuclei.

The migrating cleavage nuclei continue to rise toward the surface of the egg but undergo another cleavage division, the seventh, before they reach the periplasm. In flea eggs it is at the 128 nucleus stage, therefore, that the cortical layer is first supplied with nuclei. Mention should be made of the fact that the mitotic spindles of this seventh division are parallel to the egg's surface and, as a consequence, the daughter nuclei retain the spheroidal arrangement already described for their parent nuclei. Wheeler (1889) for *Blatta*, Heymons (1895) for *Forficula*, and Lécaillon (1897) for *Clytra*, have described a similar orientation for the mitotic spindles of the cleavage division just prior to the arrival of the nuclei in the periplasm.

There is a great deal of variation among insect eggs as to the exact region of the periplasm at which the cleavage nuclei first reach the surface. In *Pieris* (Bobretzky, 1878) and *Chironomus* (Weismann, 1882) the first nuclei arrive in the cortical protoplasm at the anterior pole. In *Formica* (Ganin, 1869) and *Musca* (Graber, 1889) the surface of the egg is attained first at the posterior pole. Auten (1934) says that in *Phormia* the nuclei arrive at the two poles slightly before they reach the other surfaces of the egg. In *Hydrophilus* (Heider, 1889), *Platygaster* (Kulagin, 1897), and *Calendra* (Wray, 1937) the surface of the egg first reached is between the poles in the equatorial region. In certain other insects such as *Blatta* (Wheeler, 1889) and *Apis* (Nelson, 1915) the ventral surface somewhat anterior to the equator is the region first reached. In *Callopteryx* (Brandt, 1869) the nuclei are said to arrive at the surface in groups. In the development of flea eggs the arrival of the nuclei at the surface does not correspond to any of the above cases for, as a rule, in pulicid ova the nuclei arrive at the periplasm simultaneously at all points. This is not surprising in view of the regular symmetry of these eggs and the fact that the nuclei approach the surface arranged in the form of a hollow, single-layered figure, the outline of which conforms very closely to the shape of the egg surface. The only exception to the simultaneous arrival of the nuclei at the surface is that in some eggs the most posterior nuclei arrive at the periphery a little later than the others. This is apparently due to the fact that the zygotic nucleus sometimes begins dividing at a point slightly anterior to the middle of the egg. As these most posterior cells tend to become germ cells, their further history is postponed for later consideration.

The number of cleavage nuclei arriving in the cortical ooplasm of flea eggs is inadequate to constitute at once a continuous epithelium such as is characteristic of the blastula stage. As stated above, it is at the 128 nucleus stage or after the seventh cleavage division that the periplasm is first supplied with nuclei. The 15 or so nuclei which are to be seen at the periphery in a median longitudinal section at this stage are rather regularly distributed but widely separated from each other (pl. 1, fig. 4). This condition contrasts with the situation which occurs in *Calliphora* (Blochmann, 1887b) and *Hydrophilus* (Heider, 1889), in which the nuclei reach the periplasm closely crowded together. In the embryological development of fleas, therefore, there must be a further multiplication of the nuclei in the periplasm before the stage comparable to that in which the nuclei arrive at the periphery in *Calliphora* and *Hydrophilus* is reached. For the sake of convenience, although there is as yet no cessation of nuclear division at any surface region, cleavage may arbitrarily be said to terminate with the arrival of the cleavage nuclei at the egg's surface. The embryonic differentiations which immediately follow this stage and which lead up to the completion of the blastoderm collectively constitute what is herein termed blastulation.

BLASTULATION

At the conclusion of cleavage the egg may be said to be in the blastema stage. This term was first used by Weismann (1863) to designate the nucleated periplasm in the eggs of dipterans before its division into cell territories. Although he was mistaken in assuming that the nuclei in this layer have a spontaneous origin, later workers, such as Patten (1884) and Wheeler (1889), have used the term without objection. When the general definition of blastema, viz, the primitive basis of a structure yet undifferentiated and from which such a structure grows, is considered, it must be admitted that the nucleated periplasm of insect eggs comes within the meaning of the term when its function as the primitive basis for the blastoderm is taken into account. For this reason, the precedent established by Patten and Wheeler is herein followed.

Four substages of the blastema are to be recognized in the eggs of fleas, each determined by the number of nuclei which are present in the periplasm. The first of these has already been described as being synonymous with the 128 nucleus stage. The second, third, and fourth blastema substages correspond to the 256, the 512, and the 1024

nucleus stages respectively. The details of the differentiations of these various stages deserve special consideration.

Mention has been made of the fact that at the last or seventh cleavage division, which occurs during the migration of the pre-blastema nuclei to the periplasm, the mitotic spindles are arranged parallel to the egg's surface, thus insuring that the daughter nuclei will be equally distant from the periplasm when they are formed. All the division planes of the three subsequent nuclear divisions likewise are at right angles to the egg's surface. This phenomenon serves to retain the single-layered arrangement of the nuclei in all the blastema substages.

Immediately following the eighth nuclear division or when the egg is in the 256 nucleus substage of the blastema, the two daughter nuclei of any one parental nucleus lie close together. Soon, however, they draw away from each other until they are approximately equidistant from the adjacent nuclei and from each other (pl. 1, fig. 5; pl. 4, fig. 40). The egg is then ready for the ninth nuclear division. This, and the tenth division, take place in the same manner as the eighth, although the pairs of daughter nuclei are less apparent owing to the greater number and resultant crowding of the nuclei in the periplasm. The third blastema substage, following the ninth nuclear division, is shown in figures 3, 10, and 11 (pl. 1) and in figures 41 and 42 (pl. 4). Figures 8 (pl. 1) and 49 (pl. 6) represent the fourth blastema sub-stage. This follows the tenth nuclear division and corresponds to the 1024 nucleus stage.

In the fourth blastema substage, all of the first true blastodermic nuclei are accounted for. All that remains to transform the blastema into the blastoderm is the separation of the individual nuclei with their adjacent cytoplasm into distinct cell territories. The cells delineated in this manner are the first true cells of the developing egg. Some 120 of them are present in a median longitudinal section of the primary blastodermic stage or the first substage of the blastula (pl. 1, figs. 7, 9; pl. 5, fig. 48). The discussion of the second and third blastula substages is postponed to the section on the formation of the germ band.

The cytoplasm of the blastoderm has two sources. Part of it is derived from the thin periplasmic layer, and the remainder has been carried into this region by the immigrating cleavage nuclei. The first indication of the delimitation of the cell territories of the blastoderm is the appearance of slightly rounded swellings at the surface of the egg. These swellings are opposite the individual peripheral nuclei. Almost immediately the lateral cell walls begin to form. They begin at the surface and gradually extend inward. The nuclei do not lie in

the absolute centers of their forming, respective cell territories. Instead, each is located somewhat toward the outer margin. With the completion of the lateral cell walls, the cell territories still remain incompletely demarcated, as the cytoplasm of their bases is still connected. It is also continuous with the reticular protoplasm of the interior of the egg. Although these phases of blastulation are typical for centrolecithal insect eggs, the final step, consisting of the development of the inner walls of the blastoderm cells, varies as to details in different forms. In the eggs of certain insects such as *Hydrophilus* (Heider, 1889) and *Apis* (Nelson, 1915) an inner peripheral or cortical protoplasmic layer is produced. It is believed to be composed entirely of protoplasm which has come to the surface with the cleavage nuclei. This zone has only a temporary existence owing to the fact that it is ultimately absorbed by the blastoderm cells. A true layer of inner peripheral protoplasm was not found by Marshall and Dernehl (1905) in their study of *Polistes,* although a small amount of cytoplasmic substance was observed just inside the bases of some of the completed blastodermic cells. An inner layer of peripheral protoplasm is said by Carrière and Bürger (1897) to be entirely lacking in *Chalicodoma* and *Anthophora.* As the eggs of these last two genera have a relatively thin periplasmic layer, and as this is also the case in flea eggs, an inner peripheral protoplasmic layer was expected to be lacking also in the developing ova of these insects. A deficiency of cytoplasmic material in the cortical layer would be expected to necessitate the incorporation at once into the blastodermic cells of all the protoplasm which had come to the surface with the cleavage nuclei. However, careful examination of sections of flea eggs representing this stage indicates an agreement with the eggs of *Polistes* rather than with those of *Chalicodoma* and *Anthophora.* Although thin, irregular, and ill-defined, cytoplasmic patches may be seen lying just below the bases of certain of the blastoderm cells. This may be regarded as homologous to the definite inner layer of peripheral protoplasm of certain other insects.

TROPHONUCLEI

As already stated, after the sixth cleavage division when 64 segmentation nuclei are present in the egg, a variable number of these start migrating toward the surface as the preblastema nuclei. Within the spheroidal figure which they outline are the remaining nuclei which, with their progeny, stay in the central portion of the egg. Here they probably serve to liquefy the yolk which surrounds them, making

it available as nourishment for the cells of the developing embryo. Because of this function, they may be termed trophonuclei or vitellophags. It would be incorrect to call them trophocytes because, like the cleavage nuclei, the cytoplasm surrounding them is not marked off into cell territories. In order to emphasize this point, the term trophonucleus is used hereafter. During the preliminary cleavage divisions all of the trophonuclei divide simultaneously with the other nuclei. Later, however, the division of the trophonuclei is irregular. This is demonstrated by the fact that while these nuclei disperse soon after division, some preparations show a few lying close together in pairs. This irregular division of the trophonuclei has been described previously for *Apis* by Nelson (1915).

In some insects, such as *Leptinotarsa* (Wheeler, 1889) and *Clytra* (Lécaillon, 1898), a phenomenon known as secondary yolk cleavage is said to occur. In these forms the yolk is divided into polyhedral masses, each of which contains a trophonucleus. This segmentation serves to divide the protoplasmic reticulum of the vitellus as well, so that in these forms each yolk segment with its trophonucleus and surrounding cytoplasm may properly be regarded as a yolk cell. Only in such cases is the term yolk cell applicable. In agreement with the majority of other insects, such segmentation of the vitellus is not shown in any of the numerous sections of flea eggs made during this study. Therefore, for the vitellophags of the eggs of Siphonaptera the term trophonucleus rather than yolk cell is correct.

The origin of the primary trophonuclei from cleavage nuclei which have remained behind in the yolk has been demonstrated in numerous insects by various authors including Bobretzky (1878) for *Pieris*, Kowalevsky (1886) for *Calliphora*, Wheeler (1889) for *Leptinotarsa*, and Nelson (1915) for *Apis*. On the other hand Patten (1884) for *Neophylax*, Will (1888) for *Aphis*, Wheeler (1889) for *Blatta*, and recently Du Bois (1932) for *Sciara*, state that all the cleavage nuclei migrate to the surface so that none of them are left to produce the primary vitellophags. In the eggs of fleas a few nuclei, as in the majority of insect forms studied, remain behind in the vitellus and these in turn give rise to the primary trophonuclei. Tikhomirowa (1892) has already made this observation for the composite species *Pulex serraticeps*. Strindberg (1917), in his study of the development of *Archaeopsylla erinacei*, failed to find yolk nuclei at any stage.

Not all trophonuclei are of this primary type, however. They must of necessity have some other source in those forms in which all of the nuclei pass to the periplasmic layer. Patten (1884) says that in *Neophylax* the yolk nuclei arise at a later stage by the immigration of

nuclei into the vitellus from both the serosa and the ventral plate. Graber (1871) had previously shown that in forms possessing primary trophonuclei, these are augmented by additional or secondary vitellophags which enter the yolk from the germ band. Numerous other workers, including Korotneff (1885) for *Gryllotalpa,* Noack (1901) for *Calliphora,* Nelson (1915) for *Apis,* Butt (1936) for *Brachyrhinus,* and Lassmann (1936) for *Melophagus,* have substantiated his observations. In flea eggs, such an augmentation of the primary vitellophags by others of a secondary nature occurs, notwithstanding the statement of Tikhomirowa (1892) to the contrary. This augmentation is by immigration from the surface. These secondary trophonuclei may enter the yolk either from the blastoderm or from the mesoderm when this germ layer has differentiated. None of them appear to come from the serosa as Patten (1884) described for *Neophylax.* In spite of their origin from the completely delimited cells of the blastoderm or mesoderm, the secondary vitellophags appear to lose their cellular nature as they enter the yolk. Their cell boundaries break down and they become incorporated into the cytoplasmic syncytium which ramifies through the vitellus. Having lost their demarcations, these vitellophags can no longer be regarded as independent cells. Instead, they must be termed trophonuclei like the primary vitellophags. Such secondary trophonuclei appear indistinguishable from those of the primary group once they have passed deeply into the yolk. When first given off by the blastoderm, they stain rather deeply and correspond in the density of their coloration to the cells of the superficial layers. The subsequent decrease in the staining ability of the secondary trophonuclei appears to be due to the dispersion or dissolution of their substance. A similar phenomenon has been observed by Heymons (1895) in *Gryllotalpa, Gryllus,* and *Forficula,* by Friederichs (1906) in *Meloë,* and more recently by Eastham (1927) in *Pieris.*

ORIGIN OF THE GERM CELLS

The origin of the primordial germ cells in insects was first observed by Robin (1862) in *Chironomus.* He failed, however, to recognize their true nature for he regarded them as polar bodies. Weismann (1863), again in *Chironomus* and also in *Calliphora,* observed not only the separation of these cells from the posterior pole of the egg but also found that they reentered the egg and finally disappeared among the embryonic tissues. He concluded, therefore, that they were not polar bodies and termed them "pole cells" instead. Like Robin, however, he failed to understand their significance. Metchnikoff

(1866) found similar pole cells in *Miastor* and succeeded in tracing them to the larval gonad, thereby indicating their true character. His work was confirmed by later workers, particularly Balbiani (1882) who saw in *Chironomus* not only the constriction of these cells but their reentry into the interior of the egg and incorporation into the gonads as well. All the germ cell work up to this time was done on entire eggs, and with the introduction of modern sectioning and staining techniques, further evidence was accumulated to show that the pole cells are in reality the primordial germ cells. Ritter (1890) especially, again working on *Chironomus,* was able to trace, by means of extensive sections, the development of the germ cells up to the time that they reached their final positions in the gonads of the fully formed larva. Ritter's work was reexamined and confirmed in the main by Hasper (1911). Subsequent to these earlier fundamental studies, germ cells have been discovered in the eggs of a number of other insects. These investigations indicate, however, that the differentiation of germ cells among insects takes place by two general methods. Before comparing these two methods it is necessary to discuss the characteristics of the posterior polar plasm and the role it plays in the origin of the primordial germ cells of hexapods.

Weismann (1863) described what he termed "yolk granules" in the pole cells of *Chironomus*. Ritter (1890), in his study of the germ cells of this same fly by the section method, demonstrated that Weismann's "yolk granules" came from a disk-shaped mass of granular substance located in the periplasm at the posterior pole of the egg. Hasper (1911), in his reinvestigation of Ritter's work, was able to show that this granular substance was incorporated into the cytoplasm of the germ cells. This incorporation of granular plasm by the germ cells has been reported in numerous insects and has led to the belief which is held by some workers that this substance possesses the peculiar power of incorporating itself into the cytoplasm of differentiating cleavage cells and thereby transforming them into germ cells. Hegner, in a series of publications dating from 1908 to 1917, champions this view and terms the granules in question "germ cell determinants."

According to the descriptions of numerous other authors, these posterior polar granules are congregated in a disk-shaped area in the peripheral periplasm at the posterior end of the egg. Gambrell (1933), in discussing this mass of granules in *Simulium,* says that it lies just beneath the surface at the posterior end and appears as a sharply outlined, irregular, and darkly staining body which is completely imbedded in the formative protoplasm. Butt (1934) describes it for *Sciara* as

follows: "At the posterior pole in the periplasm lies a saucer-shaped granular plate, the germinal cytoplasm or germ line determinant." Huettner (1923), in his work on *Drosophila,* used Heidenhain's iron haematoxylin almost exclusively for staining his sections and found that the posterior polar granules stained black like the yolk spheres. Likewise Auten (1934), working on *Phormia,* found that the posterior granular plate stained deeply with Heidenhain's iron haematoxylin. In view of these and other similar observations which appear in the literature, it was expected that a typical disk-shaped area of darkly staining granules would be found at the posterior pole of the flea egg. However, in none of the numerous preparations of pulicid eggs made during this investigation is such an area indicated. The general appearance of the posterior polar plasm of flea eggs has been described in a previous section. In view of the importance of this substance in the differentiation of the germ cells, the following further details are given. At the posterior pole the periplasm is shown to be widened, in a few sections, to form more or less of a cap (pl. 5, fig. 43). This cap is entirely free from yolk spheres. This is its chief distinguishing characteristic, for, in the remaining portions of the periplasm, the yolk structures often appear to extend nearly to the surface. This posterior polar cap is uniformly faintly granular like the rest of the peripheral protoplasm. Furthermore, like the other regions of the egg, it may possess Blochmann's corpuscles. But never in any of the sections prepared for this study does it contain any structures which may be interpreted as corresponding to the darkly staining polar granules of other insect eggs. As previously stated, some of the sections were stained with Heidenhain's haematoxylin and, although the yolk spheres were colored black, there were no similarly stained posterior polar granules present. The remainder of the sections made of the early stages were stained with Delafield's haematoxylin with a counterstain of eosin. Gambrell (1933) used this same combination extensively and found the polar granules clearly indicated thereby. Butt (1934) used Bouin's fluid for fixation, a reagent utilized for the majority of the eggs sectioned in this study, and successfully demonstrated the granular plate in *Sciara.* It may be assumed, therefore, that the fixation and staining techniques employed during the present investigation are favorable to the visibility of posterior polar granules, and their apparent absence in this case may be taken as an indication that such granules do not occur in the eggs of the three flea species under consideration.

This apparent absence of posterior polar granules, however, appears to be not without precedent in the eggs of insects. Nelson (1915), in

his thorough and extensive study of the embryology of the honey bee, makes no mention of the presence of posterior polar granules in the eggs of this insect. He, too, used Bouin's fluid for fixation and iron haematoxylin for staining and found that the yolk spheres stain deeply by this method. As he could hardly have failed to observe a limited posterior polar area of granules had one been present, it may be inferred that the eggs of the honey bee, like those of fleas, lack these particles.

While Hegner's interpretation of the significance of posterior polar granules has been accepted by numerous workers, other embryologists have opposed his view. Huettner (1923), particularly, has taken this position. In 1901 Noack reached the conclusion that these granules are yolk spheres. Huettner, however, demonstrated that this is not the case, and showed furthermore that they are not mitochondria. He also noticed, as Noack had already pointed out, that all of the germ cells do not receive an equal number of these granules. Therefore, in contrast to the germ cell determinant hypothesis, he believes it "probable that the posterior polar granules may be only by-products of the posterior germ plasm and have nothing to do with the determination of the germ cells." In spite of his suggestion that the term be laid aside, some recent workers continue to use it. Du Bois (1932), Gambrell (1933), and Butt (1934), all write of these granules as germ cell determinants. The fact elucidated above, that the eggs of certain insects such as fleas lack posterior polar granules, appears to be conclusive evidence that these structures are not the differentiators of the primordial germ cells.

The two general types of germ cell differentiation in insects are illustrated by *Miastor* and *Drosophila* respectively. In the former, as shown by the studies of Metchnikoff (1866), Kahle (1908), and Hegner (1912 and 1914a), a single primordial germ cell gives rise to all the germ cells of the organism. In *Miastor* the parent nucleus of all the germ cells is differentiated from its fellows in the 8 nucleus stage when it migrates into the posterior polar plasm. Thereafter, this entire polar plasm with its nucleus is cut off from the egg to form the primordial germ cell, which by subsequent divisions produces all the gonial cells of this fly.

In contrast to this single cell origin for all the gonial cells, the majority of insects appear to derive their germ cells by a somewhat different method. In *Drosophila,* for example, as shown by Huettner (1923), a variable number of cleavage cells migrate into the posterior polar plasm and each is constricted off as a primary germ cell. In *Drosophila* this number varies from 6 to 20. In some eggs, the total

number extruded indicates beyond question that they could not all have arisen by the synchronous division of a single primordial germ nucleus. Noack (1901) found the same type of germ cell differentiation occurring in *Calliphora*. In this form 15 to 20 polar nuclei are pushed out posteriorly at one time. Recently, Lassmann (1936) has shown that germ cell differentiation in *Melophagus* is of this type.

In the case of flea eggs, the germ cells develop by a method comparable to that which takes place in *Drosophila, Calliphora,* and *Melophagus*. A variable number of cleavage nuclei pass into the posterior polar plasm and constrict off from the body of the egg, surrounded by some of this differentiating ooplasm, to become the first germ cells. Figures 46 and 47 (pl. 5) show several of these completed germ cells lying just within the vitelline membrane. The enlargement for figure 46, showing three germ cells, was made from the whole egg section shown in figure 42 (pl. 4).

Numerous insect embryologists, including Gatenby (1918) working on *Trichogramma,* have shown that the polar nuclei which are destined to become germ cell nuclei can in no way be distinguished from the other cleavage nuclei except by their location in the posterior polar plasm. In the eggs of fleas, all the nuclei present in the egg at this stage are similarly indistinguishable, and because the posterior polar plasm is not demarcated, as in most other insect eggs, by its unique granular appearance, it is impossible to recognize them with certainty. Until they begin to protrude from the egg's surface, one may only assume that the nuclei located nearest to the longitudinal axis of the egg at its posterior pole are destined to become germ nuclei (pl. 5, fig. 44).

Several germ cells make their appearance in the eggs of fleas simultaneously. The first indication of their constriction is the formation of rounded swellings of the egg surface at the extreme posterior pole. Figure 45 (pl. 5) shows three germ cells bulging out preparatory to constriction. In some eggs, such as the one illustrated, the cytoplasm of the protruding cells may possess a granular appearance. The minute structures which are responsible for this appearance are not to be confused, however, with the described posterior polar granules of other insects. Instead, as already stated, they are to be identified as comparable to Blochmann's corpuscles. Their number is negligible in some preparations and profuse in others. Furthermore, unlike polar granules, they are distributed throughout the entire vitelline and periplasmic regions of the egg.

In the discussion on cleavage it was stated that in the eggs of fleas all nuclei entering the periplasm usually arrive there at the same time,

the only exception being that in some cases the most posterior nuclei arrive at the periphery shortly after the others. Judging from their position, these extreme posterior polar nuclei are to be regarded as future germ cell nuclei. Their delayed arrival at the periplasm appears to be at variance with the case of *Drosophila,* for which Huettner (1923) says that the polar nuclei apparently begin their migration a trifle earlier than the preblastema nuclei. As already suggested, the retarded arrival of the posterior nuclei in flea eggs may be ascribed to the fact that in certain cases the fusion nucleus is located somewhat anterior to the center of the egg when it divides.

The first appearance of protoplasmic pockets anticipating the constriction of germ cells, occurs soon after the nuclei have entered the periplasm to form the blastema. This is at the 128 nucleus stage, or following the seventh cleavage division. At this stage, some 15 nuclei are present in the periplasm in a sagittal section. The beginning of germ cell protrusion in flea eggs is relatively precocious when compared with *Drosophila.* In fact, the migration of the preblastema and pregerm cell nuclei is likewise earlier for the fleas than for the pomace fly. In the latter, according to Huettner (1923), it is not until after the next or eighth cleavage division that the nuclei begin to migrate toward the periphery.

The sections prepared for this study show that as the protrusions caused by the first germ cells become more prominent, others which are more lateral in position begin to make their appearance. Even when the most posterior cells are completely constricted, others may be just beginning to bulge out. There is consequently such a gradual transition from the ordinary blastema nuclei to the more median germ cells that it is impossible to determine, with any degree of accuracy, a boundary of distinction between the two types of nuclei. The first germ cells to be constricted appear to become complete during the second blastema substage, or after the eighth cleavage division. Constriction of those more lateral in position may continue into the early part of the third blastema substage following the subsequent or ninth cleavage division. The germ cells then reenter the nucleated periplasm during either the latter part of the third or during the fourth blastema substage before the cell territories of the blastoderm are delimited. Figure 48 (pl. 5) shows the germ cells after their return into the body of the egg. This reincorporation of the germ cells in the blastema surface is in accordance with the recent works of Butt (1936) on *Brachyrhinus,* and of Wray (1937) on *Calendra,* but at variance with the account of Hegner (1909a) for chrysomelid beetles. Hegner states that the germ cells of these insects remain outside the egg body

until blastulation is completed. Their entry later is by means of incompleted points of the blastoderm, termed pole canals. The situation in flea eggs differs likewise from that which Lassmann (1936) has found in *Melophagus*. In this form, the germ cells do not reenter the egg until much later. Instead, they lie for a time in the differentiating amnio-proctodaeal cavity and not until this has deepened considerably do they effect an entrance into the embryo.

By counting the total number of germ cells shown in the series from which the section for figure 46 (pl. 5) was taken, there were found to be eight. As a single nucleus, by three consecutive divisions produces eight nuclei, it might be inferred that these germ cells had a mononuclear origin. Such a circumstance could be harmonized with what occurs in such insects as *Miastor* or *Chironomus*. In the latter fly, according to Hasper (1911), a single primordial germ cell protrusion takes place. This divides into two cells as it constricts from the egg, and this division is followed by two others, so that, in all, eight germ cells are produced. If there were always eight such cells formed in flea eggs it might be assumed that they had originated from a single cleavage nucleus which could not be distinguished from the other nuclei in the egg, owing to the absence of posterior polar granules. An examination of other serial sections of eggs of the same stage, however, shows that in fleas the total number of germ cells produced is variable. Some eggs possess as few as 5, whereas others have as many as 12. Reference has already been made to the synchronous division of the cleavage and blastema nuclei. Because of the fact that the germ cells begin to push out as soon as the immigrating nuclei have entered the periplasm, it is apparent that all the germ nuclei were produced by the seventh cleavage. Obviously, therefore, they originated simultaneously with all of the other nuclei present in the egg. It is consequently impossible for any total number (also total progeny) of germ cells numbering between 5 and 15, other than 8 (2^3) to be produced from a single ancestral nucleus. The presence of variable numbers of germ cells in the eggs of fleas indicates for them a polynuclear origin.

The further history of germ cells in insects has until now been traced completely only in forms possessing differentiating posterior polar granules. For example, Hegner (1909a) found, for *Calligrapha*, that after reentering the eggs by the pole canals, these cells creep along between the yolk and the germ band to form, eventually, two groups near the developing coelomic sacs. These aggregates acquire follicular envelopes, probably mesodermal, and the gonads are completed. Likewise Lassmann (1936) was able to trace the germ cells of *Melophagus*

to their final position in the larval gonad. In this genus, as already stated, the germ cells lie for a time in the amnio-proctodaeal cavity and then pass through the embryonic wall. They then move along on the posterior mesenteron rudiment to the developing gonad.

Although the germ cells of fleas lack posterior polar granules, they are nevertheless distinguishable from the other early cells of the embryo by their larger size, their more prominent nuclei, and the usual clearness of their cytoplasm (pl. 5, fig. 48; pl. 12, figs. 89, 90). They enter the surface layer during the blastema stage, as described, and with the completion of blastulation are to be found incorporated in the blastoderm at the posterior end of the egg (pl. 5, fig. 48). With the appearance of the posterior mesenteron rudiment, the germ cells no longer form a part of the blastoderm, but come to lie on the inner surface of the rudiment (pl. 6, fig. 51). The whole egg section from which this enlargement was made is shown in figure 14 (pl. 2). When the posterior portion of the germ band is involuted into the yolk, the germ cells, together with the posterior mesenteron rudiment, are carried along. At this stage they lie on the embryonic tail at a point near which it is continuous with the amnion. From here they pass, at a later stage, into the epineural sinus where they form two aggregates. The two groups of germ cells move slowly forward, one on each side of the differentiating body cavity, where they are finally incorporated into the gonads as described in a later section.

Of the five workers who heretofore have written on the embryology of fleas, only two have observed the germ cells. One was Balbiani (1875) who, at a later date (1882) published an admirable work on the germ cells of *Chironomus*. In his paper on the development of the cat flea, he describes what he believes to be the germ cells in a stage somewhat later than that in which the embryonic membranes are completed. He says: "The organ of reproduction is already visible in the form of a small mass of clear cells located along the internal aspect of the abdomen immediately below the posterior margin of the vitellus. No envelope or cellular wall surrounds this group of germ cells." The fact that he describes them as clear cells substantiates the identification of the germ cells herein noted as well as the conclusion that the cytoplasm of flea germ cells is lacking in the polar granules common in those of other insects.

While Balbiani saw nothing of the early stages of the germ cells, it is apparent that Packard (1872) actually observed them at the time of their constriction. According to the terminology of his time he called them "pole cells." Although Metchnikoff (1866) had already

indicated the significance of corresponding cells in *Miastor,* Packard was content to state that they disintegrated when he lost track of them. His contributions to this subject are best shown by quoting his statements:

There was observed a vacant space between the yolk and the chorion at the posterior pole, the egg contents completely filling out the opposite end. Also at this time the end of the egg distinctly bulges out, and in this shallow sinus are four distinct polar cells and a small indistinct one in addition, they are distinctly nucleated just as in *Chironomus.* There seems to be a membrane (I suppose the vitelline membrane) retaining these polar cells in place. . . . . About two hours later, the vacant space at the posterior pole of the egg has disappeared, and the yolk and protoblastoderm have pushed up against the vitelline membrane and polar cells. In half an hour's time more, the yolk mass has advanced half way to the polar cells. At this time there were no signs of blastodermic cells. A few hours later, probably not over thirty after the egg had been laid, the blastoderm cells had appeared around the yolk. Soon after this the polar cells break down and disappear.

Weismann (1863) does not mention the germ cells in the dog flea eggs which he studied, yet he goes into some detail in his discussion of the "pole cells" which he observed in *Chironomus* and *Calliphora,* the other two insects considered in the same paper. This is accounted for by the fact that the rather opaque chorion in *Ctenocephalides canis* makes it impossible to observe the finer details of structure in whole mounts. As he was unsuccessful in his attempts to remove the chorion without injury to the vitellus and embryo, his discussion of the embryology of fleas is very limited. In *Calliphora,* and *Chironomus* particularly, the chorion is transparent.

It appears that the germ cells of fleas have not been studied by the section method heretofore, although both Tikhomirowa (1890) and Strindberg (1917) used this technique in their investigations on pulicid embryology. The former does not mention the germ cells, while Strindberg says that they are not disclosed in the eggs of *Archaeopsylla erinacei.*

FORMATION OF THE GERM BAND

At the conclusion of blastulation, the blastoderm cells are equally distributed over the surface of the egg. This condition is characteristic of the undifferentiated blastula. Soon, however, there occurs a concentration of cells toward the ventral midline and polar surfaces of the egg. This crowding of the cells toward the ventral midline is the first step in the formation of the ventral plate which in turn anticipates the germ band (pl. 6, fig. 50). The cells of the median dorsal region appear to be somewhat flattened by the lateral tension exerted upon

them. In contrast, the cells of the future ventral plate, owing to crowding, have elongated and become slightly columnar. The ventral region, therefore, is somewhat thickened. There is, however, no sharp line of demarcation between the thinned and thickened areas (pl. 1, fig. 6). Although somewhat differentiated by cell concentration and a consequential thickening appearing along the ventral surface, the egg is still bounded at all points by a single layer of cells. It is still to be regarded, therefore, as a blastula, and is specifically in the second blastula substage. In fact, another or third blastula substage follows this.

The transformation from the second to the third blastula substage is accomplished by a division of the cells constituting the thickened areas of the blastoderm. This is the eleventh mitotic division, numbering from the zygotic nucleus. The cells of the middorsal thinned region do not appear to participate in this division. Owing to the concentration of the cells in the thickened regions, particularly along the midventral line and its continuation around the poles, some of the nuclei may be forced inward, an illusion of a double cell layer thus being given. A somewhat similar apparently double-layered blastoderm has been described with different explanations in the eggs of certain other insects including *Apis* (Kowalevsky, 1871; Petrunkewitsch, 1901; and Nelson, 1915), *Polistes* (Marshall and Dernehl, 1905), and *Phormia* (Auten, 1934).

The multiplication of cells just described results in a still greater thickening of the germ band anlage. This is enhanced further by a change in shape of the cells of the ventral plate. By this change the cells lose their roughly triangular shape, become elongated, narrowed, and truly columnar. The narrowing allows space for the nuclei to reassume a single linear relationship, restoring the single layer appearance.

In contrast to the cells of the thickened portion of the blastoderm, those of the thin dorsal region seem to have remained quiescent and from the surface present a squamous epithelial appearance. Another striking difference between the cells of the two regions is that the majority of the columnar cells possess a large vacuole adjacent to the inner margin of the nucleus. This vacuole is absent in the flattened cells. Nelson (1915) describes a similar stage for *Apis*, but claims that in the development of this insect the thin dorsal strip regains a thickness equivalent to the remainder of the blastoderm, and then once again is reduced to a thin sheet of flattened cells. No such thickening and subsequent rethinning is evident in the eggs of fleas.

The remaining or thickened portion of the blastoderm is termed the ventral plate. As stated, it is thickest along the median line, extending to the anterior pole and somewhat around the posterior pole of the egg. While at first there is no sharp line of demarcation between the thinned dorsal area and the thickened ventral blastoderm, along the midline near anterior and posterior extremities of the ventral plate rather abrupt thickenings soon become evident. These are the first indications of the so-called mesenteron rudiments, which at a subsequent stage will form the midgut epithelium. They represent the first transformations of the single-cell-layered blastula to a multiple laminated condition. Figure 16 (pl. 2) shows the anterior mesenteron rudiment at the beginning of its formation, figure 15 (pl. 2) at a slightly later stage, and figures 23 (pl. 2), 53, 54 (pl. 6), and 25 (pl. 2) at correspondingly still later stages. The early development of the posterior mesenteron rudiment is indicated in figures 14 (pl. 2) and 51 (pl. 6). The details of their later history will be described below.

FORMATION OF EMBRYONIC MEMBRANES AND INVOLUTION OF THE EMBRYO

The embryos of fleas, like those of most other higher insects, become covered by two embryonic membranes at an early stage in the development of the germ band. The outer of these envelopes is known as the serosa; the inner one is termed the amnion. Both are cellular in nature and are produced from that portion of the blastoderm which is not involved in the formation of the ventral plate or embryonic rudiment. The amnion is continuous with the germ band, whereas the serosa, although during its formation continuous in turn with the amnion, ultimately loses even secondary connection with the embryo so as to form an entirely independent covering lying just inside the vitelline membrane.

The formation of the embryonic membranes in insects, although fundamentally similar, varies considerably in different forms as to its details. These variations are due to the relationship of the germ band to the vitellus.

The first indication of embryonic membrane formation in flea embryos is the appearance of two shallow indentations of the ventral plate, the beginnings of the amnio-serosal folds. Most authors call these simply the amniotic folds, but because of the dual fates of each, the compound name of amnio-serosal fold used by Wray (1937) has been adopted here.

One of these indentations is anterior in position, being located at a point on the germ band which is somewhat anterior to the location

of the anterior mesenteron rudiment (pl. 2, fig. 23). Because of the
extremely long embryonic rudiment that is developed in flea embryos,
this anterior depression lies at the anterior pole of the egg. Its anterior
margin soon grows out to form a double fold (pl. 6, figs. 53, 54), the
anterior amnio-serosal fold. Its inner layer, which is continuous with
the ventral plate, will become the anterior part of the amnion; its outer
layer is destined to become the anterior portion of the serosa. This
anterior amnio-serosal fold, as it increases in length, gradually extends
farther laterally (pl. 6, fig. 52), ventrally, and posteriorly. Because
of the greater width of the anterior portion of the germ band (pl. 7,
figs. 55, 57), due to the presence of the procephalic lobes in that
region, the anterior amnio-serosal fold is much wider than the cor-
responding posterior fold. Posterior to the cephalic region the anterior
fold narrows correspondingly to the width of the germ band. This
anterior amnio-serosal fold in flea embryos arises in the same manner
as do both the anterior and posterior folds in those insects which have
the germ band wholly superficial.

The posterior of the two primary amnio-serosal fold indentations
occurs on the ventral plate immediately posterior to the posterior
mesenteron rudiment. Its position lies somewhat around the posterior
pole of the egg toward the dorsal surface. At first the posterior amnio-
serosal indentation is identical to that of the anterior fold, but this
resemblance is transitory. It is true that the posterior fold is gradually
extended forward as its anterior counterpart is extended posteriorly,
but in the case of the posterior fold the indentation increases in depth
until it extends through the yolk about half way to the anterior pole
of the egg (pl. 7, figs. 55 to 57). This extensive deepening of the
posterior amnio-serosal indentation carries the posterior portion of
the germ band deep into the vitellus. This inward movement of the
embryo is to be regarded as a partial involution process corresponding
to the extreme type which is characteristic of odonate and hemipteran
embryos.

Coincident with the involution of the posterior end of the germ
band, the two amnio-serosal folds approach one another (pl. 7, fig. 56),
and finally join at the midventral region of the egg. When they
come into apposition their intervening walls rupture, and the inner or
amniotic layer and the outer or serosal portion of each fuse with the
corresponding parts of the other. This fusion occurs during the third
day of the embryonic period. In this way there occurs a complete
separation of the two embryonic envelopes, producing a double pro-
tective covering for the embryo over its ventral surface (pl. 7, fig. 57;

pl. 9, figs. 67, 70). The serosa is formed in part by the outer layers of the amnio-serosal folds and in part by the blastodermal covering over the vitellus. In some insect embryos such as those of *Leptinotarsa* (Wheeler, 1889), *Sciara* (Butt, 1934), and *Brachyrhinus* (Butt, 1936), a small portion of the dorsal blastoderm does not take part in the formation of the serosa. Instead, the cells of this portion clump together dorsally to form the so-called primary dorsal organ which is soon absorbed by the vitellus. In the embryos of fleas, however, no indication of such a primary dorsal organ is apparent, as the entire dorsal blastoderm is incorporated in the serosa. Soon after completion, the serosa becomes separated from the yolk surface and may for a time be observed as an entirely independent membrane composed of extremely squamous cells lying within the vitelline membrane.

In contrast to the serosa, the amnion covers only the surface of the germ band, its margins being continuous therewith. The space between this envelope and the embryo constitutes the amniotic cavity. Its anterior portion is formed by the overgrowth of the anterior amnio-serosal fold, its middle part by a similar growth of the lateral amnio-serosal folds (pl. 6, fig. 52), and the posterior part incorporates the lumen produced by the involution of the caudal end of the germ band (pl. 7, figs. 56, 59, 60). In accordance with the double nature of the posterior portion of the amniotic cavity at this stage, Lassmann (1936) has proposed the significant name of amnio-proctodaeal cavity. This term has been adopted here. It is worthy of note that the embryonic rudiment is not terminated at the inner end of this amnio-proctodaeal lumen as might be expected. Instead, it is continued around ventrally for a short distance before it connects with the amnion.

Histologically, the amnion and the serosa present a similar appearance. Both are composed of extremely flattened cells which, in section, appear as thin spindles. At the center of each such cell is a somewhat expanded area containing the nucleus (pl. 2, fig. 22). In the case of the serosa, as this envelope expands in drawing away from the surface of the vitellus, it becomes further attenuated so that its cells become even more compressed. Neither membrane shows any indications of cell division.

The movements of the germ band in flea development which correspond to the blastokinetic processes of hemipteran and odonate embryos are described at this point. Attention has already been called to the fact that the embryonic rudiment in fleas is partly superficial and partly involuted. At the end of the second day of the embryonic period the involution of the caudal region has reached its maximum, and the

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posterior third of the embryo lies completely surrounded by yolk and directed toward the anterior end of the egg (pl. 7, fig. 56). During the next 12 hours, however, there is a withdrawal of the involuted portion from the deutoplasm (pl. 7, fig. 55). This process corresponds to the more complex phase of blastokinesis known as revolution, which occurs in certain other insects, but the details are quite different. In the case of fleas it involves no rupture of the embryonic membranes. Furthermore, instead of withdrawing along the line of involution, the involuted caudal portion of the germ band in fleas merely pulls through the yolk toward the dorsal surface of the egg so that when the process is completed the entire embryo lies at the surface (pl. 10, figs. 73, 74, 75). During the processes of involution and that which corresponds to revolution of other forms, there has occurred a considerable increase in the length of the anterior end of the germ band. At this stage, therefore, it extends entirely around the anterior pole of the egg and is directed posteriorly on the anterior dorsal surface. With the withdrawal of the posterior end of the embryo from the yolk, its anterior and posterior extremities almost touch. The embryo at this stage is about 60 hours old.

FATES AND SIGNIFICANCE OF THE EMBRYONIC ENVELOPES

The embryonic envelopes of insects exhibit important differences as to their fates. However, the development of fleas includes a combination of fates for the amnion and serosa which appears to be unique.

Mention has been made of the fact that no structure, homologous to the primary dorsal organ of such forms as *Leptinotarsa, Sciara,* and *Brachyrhinus* is found in flea development. Likewise, there seems to be no primary dorsal organ of the type, described by Wray (1937) for *Calendra,* which has nothing to do with the embryonic membranes. Nevertheless, in order to avoid confusion with these two types of dorsal structures, the use of the term primary dorsal organ for the first dorsal body formed in fleas is avoided. Therefore, the first such structure differentiated in siphonapteran development is herein called the second dorsal organ.

In the embryology of fleas the serosa ruptures ventrally early in the fourth day, and is drawn dorsally to form an indistinct clump of cells which lies for a short time on the dorsal surface of the yolk as the second dorsal organ. It is gradually absorbed by the vitellus. This rupture of the serosa and its formation of the second dorsal organ corresponds to the fate of this membrane in the Trichoptera and *Chironomus* (Graber, 1888b). By contrast, the serosa in *Leptinotarsa*

(Wheeler, 1889) and *Calendra* (Wray, 1937) remains intact until the time of hatching.

In flea embryos, the amnion, after the rupture of the serosa, becomes completely detached from the embryo and forms a complete envelope enclosing the egg contents for a time. However, it remains for only a short time after the absorption of the serosa. Then it, too, ruptures ventrally and contracts dorsally to form the third dorsal organ (pl. 2, fig. 24; pl. 11, fig. 79). This occurs toward the end of the fourth day. The third dorsal organ is absorbed by the vitellus immediately before the dorsal closure of the embryo. The rupture and absorption of the amnion is similar to the fate of this membrane in *Leptinotarsa* (Wheeler, 1889) and *Calendra* (Wray, 1937). It differs from what occurs in the Trichoptera and *Chironomus* (Graber, 1888b) in which forms the amnion persists until eclosion. The rupture of both the serosa and amnion in Siphonaptera contrasts with the fates of these membranes in the Lepidoptera (Ganin, 1869) and in the Tenthredinidae (Graber, 1890), in which forms they both persist until hatching.

Strindberg (1917) alone, of those who have written on the embryology of fleas, mentions the fates of the embryonic envelopes. Apparently, however, he was handicapped by insufficient material and was therefore unable to determine the complete sequence of events. He notes that both the embryonic membranes cease to be conspicuous at one stage and then speaks of finding their degenerating cells inside the vitellus a little later. Nevertheless, he states that no dorsal organ is formed, thereby indicating that he missed the significant intermediate stages.

The question of the functional significance of the amniotic cavity to the development of the embryo is one which suggests itself at this point. Wheeler (1889), in discussing this problem for insects in general, regards this cavity as a place for the temporary deposition of excreted matter and therefore as an organ functionally analogous to the allantois of higher animals. He says: "It seems probable that while the inner ends of the ventral plate cells are absorbing and metabolizing the yolk, their outer ends are at the same time giving off into the amniotic cavity a less amount of liquid waste products." This appears to be a logical explanation especially applicable to the development of flea embryos for which Wheeler's hypothesis may be extended as follows: As already stated, the terminal portion of that part of the amniotic cavity which is formed during the involution of the embryo is connected with the proctodaeum to form the amnio-proctodaeal

cavity. The malpighian tubules which serve as excretory organs for the larva have an extremely early origin and arise as invaginations of the proctodaeum (pl. 9, fig. 71). It is possible that they may serve an excretory function in the embryo as well as in the larva. The opening of the proctodaeum into the amniotic cavity is at least significant, especially since in flea embryos the amnion remains intact until the embryonic period is more than half over. When its connections with the embryo are finally broken, the katabolistic products are still prevented from contaminating the deutoplasmic food supply by the provisional covering over the vitellus formed by the second dorsal organ. As the latter is absorbed, the amnion forms the third dorsal organ which in turn blocks the exposed surface of the yolk until the dorsal closure of the embryo. This appears to be the first suggested function for either an amniotic or serosal dorsal organ which has been advanced.

GERM LAYER DIFFERENTIATION

Coincident with the formation of the embryonic membranes and the involution of the posterior portion of the germ band, there occurs in the development of flea embryos what corresponds to the gastrulation process of other animals. In fleas, however, as in other insects, the ontogenetic process is so distorted by heterochrony and coenogenetic modification that it is difficult to recognize the relationship of this process to the less specialized and more fundamental method of gastrulation by invagination which is so general throughout the animal series.

The so-called entoderm of insects consists of those embryonic aggregates of cells which go to form the inner lining of the mesenteron. In flea development, these entoderm anlagen are two in number and consist of the mesenteron rudiments which have been mentioned in an earlier section. The appearance of these rudiments marks the initial step in the transformation of the organism from a simple monoblastic to a triploblastic stage. Both rudiments appear simultaneously, immediately following the concentration of the blastoderm cells toward the ventral surface of the egg.

The anterior mesenteron rudiment (pl. 2, figs. 15, 16, 23) originates at a point on the future embryonic midline which is ventral to the anterior pole of the egg mass. The first step in the formation of the rudiment is a change in the general appearance of the blastoderm cells over the region where the rudiment is to appear. Previously, all the cells comprising the ventral and lateral thickened areas of the blasto-

derm, excepting the germ cells, have been columnar in shape. At this stage, however, the cells which are to form the anterior mesenteron rudiment assume an irregularly cuboidal or spherical outline. A similar appearance of the cells comprising the mesenteron rudiments of *Leptinotarsa* (Wheeler, 1889) and *Apis* (Nelson, 1915) has been observed.

The mesenteron rudiments of fleas are not originated by a process of cellular proliferation from the blastoderm such as Carrière and Bürger (1897) have described for *Chalicodoma*. Neither is there an invagination of the blastodermic layer in its formation. Instead, the process is by migration of cells from the ventral blastoderm. With the changes in the appearance of the future mesenteron rudiment cells, which have been described above, the cells themselves tend to become loosely arranged. This loosening results in the appearance of numerous minute interstices between the cells of the rudiment anlage. It is probably responsible for the rounding out of the cells as well. The interstices are not apparent for long, as the cells soon migrate below the surface in an apparently confused mass and then become compactly arranged to form the rudiment proper. The loss incurred by the emigration of cells from the surface is gradually made up by the approximation of the blastoderm over the mesenteron rudiment. For some time previous to this, however, there remains an irregularly outlined and extremely shallow pit (pl. 6, fig. 53) over the mass of cells which may now be regarded as constituting the entoderm. The process up to this time is similar to that found in *Apis* (Nelson, 1915). In flea eggs, however, the shallow pit is finally closed over by the blastoderm (now ectoderm at this point on the surface of the egg mass) in contrast to the case of *Apis,* in the development of which Nelson observed that a plug of mesenteron cells reaching to the external surface remained after the approximation. In spite of the fact that it completely covers the rudiment of the mesenteron, the ectodermal sheet over this area in flea ova may be slightly indented to form a second shallow depression which is to be recognized as the location for the stomodaeal invagination.

While there is no evidence that cell proliferation has played any part in the formation of the anterior mesenteron rudiment up to this time, subsequently the rudiment increases in thickness by the multiplication of its cells. This is shown by the presence of mitotic figures among the cells of the lower portion of the entoderm mass. The anterior margin of this anterior mesenteron rudiment is thinned out rather abruptly and terminates at a point which is located slightly

posterior to the point of origin of the developing amnio-serosal fold. The short interval of undifferentiated blastoderm which separates the two is some dozen cells in length (pl. 2, fig. 25; pl. 6, fig. 54).

The origin of the posterior mesenteron rudiment, although essentially similar to that of its anterior counterpart, differs in certain details. The region of appearance in this case is dorsal to the pole rather than ventral. Furthermore, the length of the blastoderm concerned in its formation is considerably greater. The initial manifestation of the formation of the posterior mesenteron rudiment is a thickening, accompanied by a flattening of the blastoderm slightly dorsal to the posterior pole of the egg (pl. 2, fig. 14; pl. 6, fig. 51). Owing to the later involution of the posterior portion of the germ band, the anlage of this rudiment is drawn from its place of origin to a point yet farther around the posterior pole of the egg. The greater length of the posterior rudiment, as contrasted to the anterior one, is noticeable at all stages in its development. Furthermore, its margins on all sides are less abrupt. Although the posterior rudiment tends to be somewhat thinner than the anterior rudiment at corresponding stages of development, it ultimately attains an equivalent thickness. This thickening takes place during the later phases of the involution of the germ band and is not completed until the tail of the embryo has reached the center of the egg. Figures 59 and 60 (pl. 7) show this aggregate of mesenteron cells lying immediately within the ectoderm adjacent to the posterior terminus of the amnio-proctodaeal cavity. Although a short portion of the ventral plate is continued around this terminus to connect with the amnion, the cells of the posterior mesenteron rudiment are not included in this extension.

The appearance of the two mesenteron rudiments marks the first step in the differentiation of the germ layers in flea development, but there soon follows a change in the nature and thickness of the remaining parts of the ventral plate. This consists of the process usually called gastrulation. In the development of fleas, as of other insects, this process results in the transformation of the blastoderm into a double-layered germ band. The superficial layer in this case is continuous with the sheets which cover the two mesenteron rudiments and like those coverings is to be regarded as ectoderm. The lower layer is continuous with the mesenteron rudiments, there being no sharp line of demarcation between either of them and this lower layer. Because of the part played by this lower layer in the organogenesis of the embryo, it is to be identified as mesoderm.

The differentiation of the blastoderm of the ventral plate region in that section between the mesenteron rudiments is not identical in

all insects. An examination of the literature reveals descriptions of gastrulation in insects by the four following methods:

1. The lower layer is formed by an emigration or proliferation of cells from the blastoderm along the median line of the ventral plate. No obvious median groove is produced to assist in this process; in some instances all indications of such a groove are lacking. Korschelt and Heider (1899) discount the reports of the existence of this type, apparently because of their conviction that a tube, or groove at least, must be present as the remnant homolog of the gastrula-tube of other animals. There is no doubt, however, that such a method of lower layer formation actually exists in some insects. It has been observed by Heymons (1897) in *Lepisma* and by Uzel (1898) in *Campodea*. It is also described for the Collembola by Claypole (1898), Philliptschenko (1912), and Weber (1933). In addition, it has been reported in several of the Pterygota by a number of workers including Korotneff (1885) for *Gryllotalpa*, Wheeler (1889) for *Blatta*, and Hagan (1931) for *Hesperoctenes*.

2. The lower layer arises by an emigration of cells from the blastoderm along the median line of the ventral plate. In this case a distinct groove is formed, but its lips are not approximated to form a tube. This method has been described by Patten (1884) for Trichoptera, by Will (1888) for Aphidae, and more recently by Gambrell (1933) for *Simulium* and by Auten (1934) for *Phormia*.

3. There occurs an actual invagination of the ventral midline blastoderm to form a groove which changes into a closed tube by the approximation and fusion of its lips. This type of lower layer separation has been reported in several of the higher insects such as *Hydrophilus* (Kowalevsky, 1871, Heider, 1885 and 1889), *Leptinotarsa* (Wheeler, 1889), *Chalicodoma* (Carrière, 1890), *Calliphora* (Kowalevsky, 1886), and *Calendra* (Wray, 1937).

4. The middle portion of the ventral plate becomes separated from the lateral blastoderm. This middle plate then sinks inward while the lateral plates grow together and fuse over its outer surface. Like the third type described, this method has been reported only for certain higher insects. Among them are *Apis* (Nelson, 1915), *Sphinx* (Kowalevsky, 1871), and *Pieris* (Bobretzky, 1878).

In fleas the lower layer is produced by a combination of the first three methods listed above. In the anterior third of the ventral plate, including the brief section between the anterior mesenteron rudiment and the anterior amnio-serosal fold, there is a simple emigration of cells from the blastoderm, without the formation of a perceptible

groove (pl. 8, fig. 61 ; pl. 2, fig. 17). There is no proliferation of cells from the blastoderm by mitotic division such as Wheeler (1889) describes for *Blatta*. Instead, there is merely a sinking of the cells which are located at the midline. In certain cases this appears to be an irregular process similar to that which has been described for the formation of the mesenteron rudiments. Especially is this true in the region adjacent to the anterior rudiment where the transition from the cells of the lower layer to those of the rudiment is difficult to make out. Posteriorly, the migration of cells appears to become more regular. At the beginning of the second third of the ventral plate there is a gradual transition from this first method of lower layer formation to the second type which has been described above, viz, that in which the migration of cells is combined with the formation of a groove (pl. 8, figs. 62, 63, 64). This method is followed through-out the major portion of the germ band, and the groove is shallow in all places. The cells which form the sides of the groove are destined to form the lower layer. These cells separate from those which are to form the ectoderm, the breaks occurring at the points where the blastoderm turns in to form the walls of the groove. In the meantime, the sides of the groove approximate, but never to form a tube. In some cases the mesoderm-forming cells migrate to the lower layer level in two parallel columns. At a point somewhat ventral to the posterior pole of the egg, the groove deepens abruptly. From this region on to a position only slightly anterior to the posterior mesenteron rudiment, the sides of the groove widen out below the surface layer, after which the lips of the groove approach one another and fuse to form a true tube with a distinct lumen (pl. 2, figs. 18, 19 ; pl. 8, fig. 65). In the relatively short region at the posterior end of the embryo, between the posterior mesenteron rudiment and the section of the germ band where a tube is formed, a simple groove is again produced, the sides of which appose as in the middle embryonic region. Along the short length of germ band which forms the posterior extremity of the embryo and which is carried around the terminus of the invaginating proctodaeum, the method of lower layer formation is identical to that which occurs in the anterior third of the egg (pl. 7, fig. 58).

It has been stated that in the formation of the lower layer of flea embryos, proliferation of cells from the blastoderm does not play a part. Instead, the sunken cells arrange themselves so as to form a relatively simple row the full width of the germ band below the ecto-derm (pl. 9, figs. 69, 70). The details of this process vary according

to the method by which the cells of the lower layer reach their sub-
merged positions. In the anterior region of the embryonic rudiment
where the cells sink by an irregular migration, this lateral progress is
likewise by an apparently haphazard movement (pl. 9, fig. 67). In the
section where the shallow groove is formed and in those regions of
it in which the mesoderm-forming cells migrate in parallel columns,
these lines of cells separate sharply at the lower level midline and pass
in opposite directions toward the lateral limits of the germ band
(pl. 8, fig. 64; pl. 9, fig. 69). This is in agreement with the observa-
tions of Patten (1884) on *Neophylax*. In connection with that portion
of the embryonic rudiment where a distinct mesodermal tube is
formed, the beginning of the process is identical with that in which
no groove is formed. The first cells to leave the surface pass inward
with no space between them. The invagination takes place above this
solid mass so that the lower wall of the tube, when it is completed, is
two cells thick (pl. 8, fig. 65). With the compression of the tube
to obscure its lumen, these lower cells separate and migrate laterally
to form the extreme marginal parts of the unproliferated lower layer.
Figure 66 (pl. 8) shows this stage in a section cut at a level slightly
posterior to that from which the one shown in figure 65 was made.

In the three forms of lower layer formation which occur in the
development of the flea embryo, it is evident that the first cells to
leave the surface tend to form the most lateral portions of the meso-
derm. The relative positions of the cells in the lower layer with
respect to the midline are obviously the reverse to what they were
when these same cells were still a part of the blastoderm. This situa-
tion is the direct opposite of that occurring in *Apis* as described by
Kowalevsky (1871) and Nelson (1915). Although the lower layer,
when first formed in fleas, is typically only one cell in depth, it is
soon thickened by cellular proliferation. This is shown by the mitotic
figures which are evident among the mesoderm cells.

Of the five workers who have written on the embryology of fleas,
only those two who used the section method of study have mentioned
the germ layers. These are Tikhomirowa (1892) and Strindberg
(1917). However, the statements of both of them in this connection
are inaccurate. In respect to the origin of the lower layer (mesoderm)
Strindberg omits its consideration entirely, confining his remarks to
the mesenteron rudiments. He merely states that in *Archaeopsylla*
erinacei the epithelium of the mesenteron is derived from one anterior
and one posterior anlage originating from the lower layer. Since the
mesenteron rudiments of the forms investigated during this study

make their appearances as differentiated areas of the blastoderm before the lower layer is produced, his observation appears to have been in error.

While Tikhomirowa (1892) discusses the origin of the mesoderm at considerable length, her ideas are also inaccurate. Her paper is primarily a criticism of Patten's article on the embryology of *Neophylax* (1884) in which he describes the mesoderm as originating from cells which migrate inward from the midventral blastoderm, the same method as has been described above for the middle region of the germ band in the case of fleas. Tikhomirowa did not base her observations on any of the Trichoptera, but insisted that, contrary to Patten's observations, it must arise in *Neophylax* in the same manner she claimed to observe it originate in the flea and in *Chrysopa*. She is emphatic in her assertions that in both of these insects the mesoderm, and the lining of the mesenteron as well, are derived from the yolk cells (trophonuclei). Her views on this subject are clearly described in a translation of her own words:

In summing up my observations on the formation of the ectoderm and primary entoderm, viz, the blastoderm and yolk cells respectively, ... I must contend that here the process is different from that described by Patten in regard to the phryganids which he studied. In examining his figures and comparing them with my own, I find a great deal of resemblance between them. For this reason, it seems to me that that author was mistaken in affirming that all the cleavage cells go into the formation of the blastoderm. It is very possible that he did not notice the negligible number of segmentation elements which remain in the interior of the vitellus at the beginning of the formation of the blastoderm and which remain in the center multiplying rapidly and giving rise to the primary entoderm. In regard to the formation of the mesoderm I have several series of slides which show definitely that its cells are derived from the primary entoderm (vitelline cells). If we should study one of the preparations representing the germ band, we would remark that the primitive groove is very indistinct, so that in this stage there is not the slightest possibility of the separation of a part of the ectoderm (blastoderm) for the formation of the mesoderm. In sections of eggs of the same stage, we see that the mesoderm is very distinctly set off from the ectoderm. Directly below the ectoderm are situated cells of the mesoderm, one right next to the other. Here we can follow clearly all the transitions of the nuclei of the primary entoderm, or rather of the nuclei of the vitelline cells, by their size, form, and coloration, even to the mesoderm close by. On this point, also, my observations differ from those of Patten who states for the phryganids that all the mesoderm is derived from the blastoderm cells in the region of the primitive groove. . . . . I find in the earlier as well as later stages of *Pulex serraticeps,* incontestable proof of the fact that the mesoderm is derived from the primary entoderm or vitelline cells. The series of slides of *Pulex serraticeps* which I possess, shows the formation of the mesoderm from its initial stage and proves beyond doubt that the first cells of the mesoderm are nothing else than the cellular elements remaining behind in the interior of the egg after the

formation of the blastoderm and which approach little by little and one after the other to finally touch the blastoderm from underneath. The irregular form, the largeness and the pale color of the nuclei of the first mesoderm cells show a perfect resemblance to the primary entoderm cells on the one hand, and their absolute dissimilarity regarding the same characteristics to the cells of the ectoderm on the other. I believe, therefore, that the origin of the mesoderm from the primary entoderm in *Pulex serraticeps* is beyond doubt.

This quotation serves to illustrate the fact that the literature of insect embryology contains many errors. Frequently, as in the present case, the development of a certain form has been reinvestigated with opposite results, causing one to doubt the accuracy of much that has been written in this field. Such discrepancies are apparently due, in a large measure, to the difficulty of the technique involved. Fragmentary or otherwise poor sections prompt misinterpretations. The failure of Tikhomirowa to observe the origin of the mesoderm from the blastoderm may be ascribed to faulty preparations. It is a different matter, however, when one considers her derivation of the entoderm from the vitellophags. The origin of the cells which go to form the mesenteron epithelium has been one of the most debated questions concerned with insect development. In deriving the functional entoderm from the trophonuclei Tikhomirowa was merely following the lead of numerous other investigators of her period. Their opinions were doubtless motivated by a desire to harmonize the origin of the germ layers in insects with the germ layer theory and with what was known to occur in other groups. In the majority of animals the entoderm is differentiated with the formation of the gastrocoel by invagination. Consequently, in such forms the entoderm lies at the interior of the egg. These workers expected, therefore, to find the entoderm cells originating from the nuclei contained in the yolk. It has been pointed out already that in other insects, as well as fleas, the primary trophonuclei are augmented by cells migrating into the yolk after the completion of the blastoderm. Dohrn and his followers, seeing such cells intermediate between the vitellus and the surface layer, probably mistook the direction of their progress and added this observation to substantiate their belief. It may have been an attempt to carry the germ layer theory one step farther that prompted Tikhomirowa to derive the mesoderm from the same source as that from which she supposed the entoderm originated.

The mesenteron rudiments of flea embryos have been described in the present paper as originating from near the anterior and posterior extremities of the ventral plate blastoderm, making their initial appearance before the formation of the lower layer is evident. Such a

derivation, independent of the mesoderm, is not new to the literature of insect embryology. In 1897 Carrière and Bürger described such a method of entoderm origination in both *Chalicodoma* and *Tenebrio*. Noack (1901) says that in *Calliphora* the entoderm cells are produced in the same manner. It is of interest to note that Uzel (1897a, 1898) states that the entoderm of the apterygote forms *Lepisma* and *Campodea* is likewise formed from cells which migrate in from the blastoderm. Philiptschenko (1912) describes the same origin for *Isotoma*. However, in these last two cases, in addition to the anterior and posterior rudiments, entoderm is proliferated along the embryonic band.

EXTERNAL EVIDENCES OF SEGMENTATION AND CHANGES IN EMBRYONIC SHAPE

Very early in the development of flea embryos, at the beginning of the second day and even before the differentiation of the germ layers, there becomes evident what appears to be a precocious segmentation of the ventral plate. The manifestations of this pseudosegmentation take the form of incomplete transverse zones of the ventral blastoderm. They are incomplete in that the middle portion of the plate (consisting of the cells of the future mesoderm) is unaffected by this phenomenon. At first it appears that these precocious divisions correspond to the primary segmentation of the insect germ band which was first observed by Ayers (1884) in *Oecanthus* and later by Graber (1888a, 1890) in a number of different insects. These authors were able to distinguish four general regions in the early germ band. They were a primary cephalic, a maxillary, a thoracic, and an abdominal region. Graber attributed great phylogenetic significance to these divisions which he regarded as the primary body segments. Accordingly, he termed them macrosomites in contrast to the definitive body segments or microsomites into which they subsequently divide.

Careful examination of flea embryos indicates that these general zones are more numerous than the four regions observed by Ayers and Graber. In fact their number is not constant and what is more, they soon disappear. Therefore, they are to be regarded as pseudosegments which are due to some other factor than segmentation, perhaps a mechanical contraction. Wheeler (1889) describes a somewhat similar phenomenon in *Leptinotarsa* and ascribes it to a wrinkling of the ventral plate. The fact that such segmental appearance is not evident in longitudinal sections of flea eggs of this stage substantiates this explanation.

The true segmentation of the germ band of fleas does not take place until the third day of development when the germ layers are completely differentiated and revolution has taken place. This circumstance differs, therefore, from that which occurs in other insects such as *Hydrophilus* (Kowalevsky, 1871), *Chalicodoma* (Carrière, 1890), and *Apis* (Nelson, 1915), in which the definitive segments are marked off even before the lower layer is formed.

Preliminary to the segmentation of flea embryos, therefore, the double-layered germ band is produced. A description of this process as seen in sections has already been given. However, an understanding of its superficial appearances is requisite to the description of the external manifestations of segmentation. The superficial indications of the germ band become evident during the second day of development. The first of these consists of a flattening of the middle portion of the ventral plate preliminary to the immigration of its cells to form the entoderm and mesoderm. Next, the anterior region of the ventral plate expands perceptibly. This is coincident with the lateral expansion of the mesoderm. Evidencing this is the fact that the germ band becomes distinctly more opaque than the lateral regions of the blastoderm which are destined to form the embryonic envelopes. The lateral expansion of the anterior part of the germ band serves to differentiate the embryonic rudiment into two general regions (pl. 7, figs. 55, 57). The expansions of the anterior shorter one are the procephalic lobes (pl. 9, fig. 68). The longer narrower portion is the protocormic region. When the procephalic lobes first make their appearance, they are situated somewhat ventrad to the anterior pole of the egg. With the lengthening of the germ band, however, they come to lie dorsad to this pole, in which position they are to be found at the middle of the third day of development. At this time the caudal portion of the embryo has completely withdrawn from the yolk and the two ends of the germ band almost touch.

In some insects there is a pronounced difference in time between the appearance of the anterior and the posterior segments of the germ band. Those of the cephalic region have been reported to appear earlier in the majority of forms. Schnetter (1934) for *Apis* and Butt (1936) for *Brachyrhinus* have found that segmentation is initiated, not at the extreme anterior end, but in the future thoracic region from which point it extends both anteriorly and posteriorly. In flea embryos the difference in the time of appearance of the segments in the various portions of the germ band is negligible, but the anterior-posterior sequence is followed in this discussion for the sake of convenience. The procephalic lobes become divided into three

segmental regions. The most anterior of these soon develops a bilobed prominence which is the anlage of the labrum (pl. 3, fig. 34; pl. 10, fig. 75; pl. 11, fig. 82). It is known as the labral, ocular, or preantennal segment. The second segment gives rise to the prominent paired antennae and is therefore termed the antennal segment. The third procephalic segment, which really arises in the region of transition between the procephalic and protocormic regions, is much less prominent. It does, however, produce a pair of small evanescent rounded protuberances which may be regarded as homologs of the second antennae of crustaceans. The protocormic region gives rise to 17 segments plus an additional incomplete one which is to be regarded as the telson. The first three of these are called the gnathal segments as they produce appendages which are differentiated into the larval mouthparts (pl. 10, fig. 74). The most anterior is the mandibular segment and, as its name indicates, its paired appendages form the mandibles. Similarly the second or maxillary segment gives rise to the maxillae, and the third or labial segment produces the labium. This organ arises as a pair of appendages (the second maxillae) whose bases soon fuse to form the bilobed labium of the larva.

The three procephalic and the three protocormic segments described above soon lose their lines of demarcation and become closely fused to form the syncephalon of the developing larva. By contrast, all the remaining protocormic segments, except the most caudal one, retain their external identity even to the end of the larval period. The three segments immediately posterior to the gnathal ones become the prothoracic, the mesothoracic, and the metathoracic segments respectively.

The 11 segments of the protocormic region situated caudad to the thoracic region constitute the abdomen. The eleventh, which is considerably smaller than the rest, does not remain visible from the outside for long as it is soon carried inward by the invagination of the proctodaeum and becomes telescoped within the tenth segment.

The general changes in body shape may be outlined at this point. After 3 days of development, the germ band is so curved dorsally that the anterior and posterior ends nearly touch (pl. 10, figs. 73, 74, 75). During the fourth day the embryo shortens so that it reaches only to the poles of the egg. The two extremities of the embryo are still turned dorsally, however, and they remain so flexed until early in the fifth day, when the embryo straightens out and then curves ventrally (pl. 12, fig. 87). Coinciding with this ventral flexure, there is gradually an elongation of the embryo until it is so long that the curvatures of the ends are insufficient to allow its accommodation within the egg.

The anterior and posterior extremities, therefore, become so flexed that a cross-section of the egg may cut four complete sections through the embryo. The embryo retains its curled position until the time of hatching, after the sixth day of development.

DIGESTIVE TRACT

The fore-intestine, comprising the oesophagus and proventriculus in the late embryo, has its origin, in fleas as in other insects, in an ectodermal invagination known as the stomodaeum. This invagination arises directly over the position of the anterior mesenteron rudiment, becoming evident soon after the ectoderm has closed over the sunken entoderm. The stomodaeum deepens very gradually, keeping pace with the shrinkage of the yolk. As it deepens it pushes the entoderm of the anterior mesenteron rudiment before it. It ultimately extends posteriorly about one fourth of the length of the egg (pl. 3, fig. 34; pl. 10, fig. 75; pl. 11, fig. 82).

The hind-intestine arises in a somewhat similar manner although there are several differences to be noted. Like the fore-intestine, it is derived from an ectodermal invagination which in this instance is termed the proctodaeum (pl. 3, figs. 35, 36; pl. 10, fig. 75). It may be said that the relationship between the proctodaeum and posterior mesenteron rudiment is fundamentally the same as that which exists between the stomodaeum and anterior entodermal rudiment. In the case of the proctodaeum and posterior mesenteron rudiment, however, the relationship is made more obscure in the earlier stages of development by the involution of the posterior region of the germ band. The point at which the embryonic band sinks inward during this process is almost immediately over the position of the posterior mesenteron rudiment and therefore somewhat anterior to the posterior extremity of the germ band. This is also the point at which the proctodaeum pushed inward so that the first part of the resulting invagination to form appears to belong to both the proctodaeum and the amniotic cavity and has consequently been termed the amnio-proctodaeal cavity. However, since this first portion of the cavity of the invagination is bounded on both sides by the embryonic rudiment, it is definitely the lumen of the proctodaeum. Only later, as the involution of the posterior part of the germ band becomes more pronounced, is a portion of the extra-embryonic blastoderm drawn into the vitellus to form the internal (with reference to the yolk) section of the amnion (pl. 7, figs. 56, 59). That part of the cavity which is bounded on one side by the amnion is the only portion of the entire invagination which

belongs to the amniotic cavity alone. Its wide continuity with the lumen of the proctodaeum is responsible for the confusion of embryologists on this point. None of the five workers who have published on the embryology of fleas considers this relationship, but there is considerable difference of opinion among the authors who have written on the development of other insects possessing a partially involuted germ band. Weismann (1863) and Ritter (1890) both confuse the line of demarcation between the amniotic cavity proper and the proctodaeum. Other investigators working on the muscids, including Graber (1888b), Bütschli (1888), Voeltskow (1889), and Noack (1901), have made the same mistake. This error has been in the proper interpretation of that portion of the germ band which lies opposite the main part of the embryonic rudiment and which is continuous with the amniotic membrane. The above authors agree in regarding this short strip as a part of the amnion, and consequently suppose that the amnion forms one of the walls of the proctodaeum. Their identification of the early stage of the proctodaeum is, nevertheless, correct. Other workers, however, among whom are Hasper (1911) for *Chironomus,* Gambrell (1933) for *Simulium,* and Butt (1934) for *Sciara,* have not found the protodaeum in this early stage. For this reason, they all state that the stomodaeum is discernible much earlier than the proctodaeum.

In the development of flea embryos, the early stages of the proctodaeum are less obscure, owing to the fact that observation is not further complicated by the tendency of the posterior portion of the germ band to roll into a spiral as it is in the dipterans mentioned above. In fleas, therefore, it is possible to follow the development of the proctodaeum from the time of its first appearance. This appearance is simultaneous with that of the stomodaeum and with the involution of the posterior part of the embryo. With the withdrawal of the posterior end of the germ band from the yolk to the dorsal surface of the egg, the proctodaeum already shows a tendency to become directed anteriorly, for at this stage it is perpendicular to the adjacent part of the embryonic rudiment (pl. 10, fig. 75). In respect to the embryo it is now pointing dorsally instead of posteriorly as before. The majority of the posterior mesenteron rudiment cells lie anterior to it (toward the posterior pole of the egg) at this stage.

The hind-intestine of the late embryo is not a straight tube. It extends anteriorly from the anus to about the anterior margin of the sixth segment and then curves ventrally upon itself and passes posteriorly to the region of the eighth segment. Here it again turns sharply ventrally and anteriorly to unite with the mesenteron. The

elongated nature of the hind-intestine becomes evident rather early in development. As a consequence of this elongation, the procto-daeum, unlike the stomodaeum, soon becomes coiled and at some stages is curved somewhat laterally to the midline. This is evidenced by the fact that certain sagittal sections, as well as transverse ones, cut the proctodaeum at more than one place (pl. 11, figs. 80, 84). Strindberg (1917) pointed out this elongated nature of the procto-daeum in his brief paper on *Archaeopsylla*. He also notes that the hind-intestine is considerably thickened near the anal opening, and that it is one of the most conspicuous of the embryonic organs.

Associated with the hind-intestine are the malpighian tubules. There are four of these formed in the flea embryo. As in other insects, they arise as diverticula of the proctodaeum proximal to its blind end. Their first evidences make their appearance rather early, in fact while the proctodaeum still lies perpendicular to the germ band. By the time the proctodaeum becomes directed anteriorly they may be easily seen (pl. 9, fig. 71). They grow very rapidly and soon show as thick-walled tubes in cross-section (pl. 3, fig. 32; pl. 11, fig. 84), lying laterad to the developing hind-intestine. Their blind ends remain free in the haemocoel.

The mesenteron derives its inner lining from the two entodermal rudiments whose differentiations have been described in a previous section. As in the majority of insects whose embryology has been studied, this epithelium is entirely bipolar in origin, in contrast to the condition described by Strindberg (1913) for Isoptera, in which he says the mesenteron rudiment is single and proliferated from the entire length of the germ band. This same author (1917), however, derives the entoderm of fleas solely from an anterior and a posterior mesen-teron rudiment. His error in deriving these rudiments from the lower layer has already been pointed out.

With the shrinkage of the yolk and the invagination of the stomo-daeum and proctodaeum, the two entodermal rudiments are carried into the interior of the embryo. At first they lie as small clumps of cells against the blind ends of the ectodermal invaginations which have pushed them inward. Because of this apposed relationship to the ectoderm, many workers have come to the mistaken conclusion that the inner lining of the midintestine is ectodermal in its derivation.

The mesenteron rudiments, as already stated, lie slightly ventrad to the blind ends of the stomodaeum and proctodaeum. From each rudiment there is proliferated a pair of laterally placed tonguelike processes, those of the anterior rudiment directed posteriorly (pl. 11, figs. 81, 83), and those of the posterior one directed anteriorly. These

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are the mesenteron ribbons. The ribbons of each side gradually approach one another and fuse, forming a complete band of entoderm connecting the stomodaeum and proctodaeum (pl. 11, fig. 80). The two bands formed in this manner then widen gradually both dorsally and ventrally. Their ventral growth is much more rapid than their dorsal expansion, and as their initial position is somewhat ventral, they meet and fuse ventrally first so that the closure of the ventral wall of the midintestine occurs considerably earlier than its dorsal closure. The dorsal closure is delayed until the third dorsal organ, composed of the massed cells of the ruptured amnion, has sunk into the vitellus (pl. 2, fig. 24; pl. 11, fig. 79). Then the dorsal margins of the widened mesenteron ribbons fuse along the dorsal midline and the epithelial lining of the midintestine is completed.

The method of the enclosure of the vitellus by the cells of the entoderm as here described for flea embryos corresponds essentially to that which has been described for all other insects thus far studied, possessing bipolar entodermal rudiments, except *Apis*. In the honey-bee, by contrast, and according to the observations of both Grassi (1884) and Nelson (1915), the two mesenteron rudiments each form a median dorsal ribbon instead of a pair of ventrolateral ones. This results in the dorsal surface of the yolk being covered first.

The final steps in the embryological development of the intestinal tract of fleas are the breaking through of the blind ends of the stomodaeum and proctodaeum to make the lumen of the digestive tube continuous from the mouth to the anus, and the investiture of the entire tract with its muscular layer.

NERVOUS SYSTEM

Very soon after the separation of the lower layer cells from the ectoderm, the neural groove appears along the entire midventral line of the embryo (pl. 9, figs. 69, 70). This is the first step in the development of the nervous system, all of which is produced by the ectoderm. The neural groove makes its appearance even before the first manifestations of the cephalic appendages. Instead of originating by invagination, the neural groove appears to be produced by two longitudinal thickenings of the ectoderm, one on each side of the midline of the germ band (pl. 10, fig. 78), the median unthickened portion becoming the groove. The two ridges continue the full length of the germ band, one passing laterally on each side of the depression which marks the position of the stomodaeal invagination. They are continued, therefore, on the cephalic segments and unite in the head

region. The appearance of the neural ridges is due to the active pro-
liferation of specialized ectodermal cells below the surface layer.
These primary nerve cells are called neuroblasts (pl. 9, fig. 72). The
neuroblastic thickenings along the lengths of the neural ridges consti-
tute the so-called lateral cords. Neuroblasts are also proliferated
from the floor of the neural groove, and these form the less prominent
middle cord.

With the segmentation of the germ band, all of its layers are
affected except the entoderm. By this process the lateral nerve cords
are metamerically constricted into segmental divisions. The superficial
layer of ectoderm over the neuroblasts gives rise to the epidermis;
therefore its cells are called the dermatoblasts (pl. 9, fig. 72). The
neuroblasts on the other hand proliferate the definitive nerve cells.
Those of the lateral cords are particularly active in the intrasegmental
regions where they give rise to the masses of nerve cells constituting
the ganglia. Because of the fact that the lateral cords are paired, two
ganglia are produced in each segment. The ganglia of successive
somites are joined by the less thickened interganglionic portions of the
lateral cords, the connectives. Figure 27 (pl. 3) shows the fused
paired ganglia and their connectives in horizontal section, and like-
wise indicates that the two ganglia of such a fused pair are trans-
versely connected by two commissural neuropile tracts. These com-
missures appear to be formed from ganglion cells proliferated by
the neuroblasts of the middle cord. The paired nature of the com-
missures of a fused ganglionic pair is shown clearly by the two neuro-
pile tracts in each such definitive ganglion (pl. 12, fig. 85). From its
first appearance, this middle cord is segmented into chainlike thick-
enings of neuroblasts which correspond in position to the future
ganglionic areas. They are strictly intrasegmental, therefore, so that
there are no median intersegmental connections produced between
the connectives, such as Wray (1937) describes for *Calendra*. In this
respect the development of flea embryos corresponds to Schaefer's
(1938) observation on *Phormia*. The neuropile of fleas, or the central
mass of fibrous tissue which is evident in the nerve tracts, appears to
be composed of the attenuated ends of the ganglionic cells closely
packed together.

Later in embryological development, as described above, the two
ganglia of each segment become approximated to fuse closely at the
midline with the commissures and thus form a composite definitive
ganglion. The connectives retain their individuality, however, thus
preserving intersegmental evidence of the bilateral origin of the
ventral nerve cord.

The above account agrees quite closely with the observations of Hatschek (1877) who pioneered in the embryological study of the nervous system of insects, and also corresponds to the writings of most insect embryologists. No observations were made in the present study on the origin of the delicate ganglionic covering, the neurilemma. Wheeler (1893) for *Xiphidium* and Eastham (1930) for *Pieris* are of the opinion that it is produced by cells of the middle cord.

The ganglionic swellings remain evident on the surface until about the end of the fourth day of development. Their disappearance is gradual and is due to the sinking of the ganglia to a lower level (pl. 3, fig. 26; pl. 12, fig. 87). This sinking also serves to obliterate the neural groove.

The central nervous system of a flea embryo consists of 19 pairs of ganglia. Figure 87 (pl. 12) shows the complete nerve chain, the thoracic and abdominal ganglia having retained their identity, while the cephalic ones are coalesced as described below. The first two of these go to form the greater part of the definitive brain or supra-oesophageal ganglion. The anterior pair, which at first forms the independent protocerebral lobes, soon unites to form the bilobed protocerebrum of the ocular segment. An apparently single commissural mass connects the two halves of this neuromere. The component parts of the second pair of cephalic ganglia likewise fuse to form a bilobed neuromere which in this case is known as the deutocerebrum. It has as its function the innervation of the antennae. It also has a single commissure. These first two pairs of ganglia are the only ones of the entire nerve chain which have their origins anterior to the position of the stomodaeal invagination. Although the lateral cords continue anteriorly to this region, the proliferation of the ganglion cells of the protocerebrum and deutocerebrum is difficult to follow. In this area the proliferation is very irregular. The origin of the commissures of these neuromeres was not determined definitely, but no indication of a middle cord was evident anterior to the stomodaeum. This last observation is in agreement with the account of Schaefer (1938) for *Phormia*. It seems, therefore, that the connecting cell masses do not have an independent origin in these cases but are produced merely by the approximation and fusion of their respective pairs of ganglia.

The brain of the flea embryo, like those of other insects, includes a third pair of ganglia, which has a different origin from those of the first two segments. This pair is derived from the lateral cords just posterior to the stomodaeal invagination. Because of this origin, therefore, the elements of this pair are to be regarded as ventral ganglia

which secondarily move anteriorly and dorsally to unite with the deutocerebrum and protocerebrum in the formation of the supra-oesophageal ganglion or definitive brain which lies dorsad to the oesophagus (pl. 12, figs. 87, 88). This ventral origin is evidenced by the fact that the transverse commissures (fused so as to appear one) pass below the oesophagus (pl. 12, fig. 86). Ventrally, they unite with the connectives of the most anterior of the trunk ganglia and in this way help to form the so-called circumoesophageal connectives. This third pair of cephalic ganglia appears as paired swellings lying immediately posterior to and below the deutocerebral lobes. These swellings are the tritocerebral lobes and the segment in which they lie is known accordingly as the tritocerebral segment.

The fourth, fifth, and sixth pairs of ganglia belong to the gnatho-cephalon which includes the mandibular, the maxillary, and the labial segments (pl. 3, fig. 33). They are all typical ventral chain ganglia in their origin, each pair being united by two prominent commissures in the early stages. Although arising separately, these three pairs of ganglia ultimately fuse to form the single suboesophageal ganglion of the late embryo and larva (pl. 3, fig. 26; pl. 12, figs. 86, 87, 88).

The seventh, eighth, and ninth pairs of ganglia are developed in the three thoracic segments. Unlike the coalesced neuromeres of the gnathocephalon, these preserve their identity and are distinct in the larva (pl. 12, fig. 87).

The 10 posterior pairs of ganglia belong to the abdominal region, one pair originating in each segment thereof except the most caudal one. During development there is a shortening of the ventral cord and a resultant anterior movement of these ganglia so that they do not all remain in the segments of their origin. The definitive number of abdominal ganglia present in the late embryo is eight. The first of these remains in the first segment, but moves to its anterior region. The second finally extends somewhat over into the first segment. The third ganglion has its final position partly in the second somite and partly in the third. The fourth ganglion takes up a similar position between the third and fourth segments. The fifth ganglion entirely leaves the segment of its origin and in the late embryo lies in the posterior region of the fourth segment, In a similar manner the sixth ganglion of the abdomen migrates into the posterior part of the fifth segment. The seventh ganglion is even less conservative, as it moves into the middle portion of the sixth segment. The eighth defini-tive ganglion of the abdomen is somewhat longer than the others of this region and is really a composite structure formed by the coa-

lescence of the last three ganglionic pairs, viz, those originating in the eighth, ninth, and tenth segments respectively. This eighth definitive ganglion of the late embryo lies in the anterior part of the seventh somite.

COELOMIC SACS

The segmental arrangement of the coelomic sacs characteristic of annelids and arthropods is manifested in the development of flea embryos (pl. 10, fig. 77). The lateral migration of the immigrated cells of the lower layer will be recalled from the account given in a previous section. By this migration a complete layer of mesoderm is formed below the ectoderm. This extends the full width of the germ band. Soon the extreme lateral margins of this lower layer become thickened to form the mesodermal bands. The mesoderm, like the superficial layer, is divided into metameres by the segmentation process. In most of the mesodermal somites formed by this process, there is developed a pair of coelomic sacs (pl. 2, fig. 21; pl. 10, fig. 76). These arise as small cavities within the intrasegmental regions of the mesodermal bands. In flea embryos the lumina of these cavities are bounded by thick walls and are similar to those described by Heider (1889) for *Hydrophilus*. There is no communication between the sacs of adjacent segments to form mesodermal tubes such as Nelson (1915) found in the embryos of *Apis*. In regard to the nature of these rudimentary coelomic primordia in fleas it may be stated that it is intermediate between the condition manifested in *Apis* and that which occurs in many Diptera. In the embryonic development of the Muscidae there is no indication of coelomic sacs according to Graber (1889). Similarly, Gambrell (1933) and Butt (1934) found no coelomic cavities in the embryos of *Simulium* and *Sciara* respectively. Moreover, owing to the small size and the thick walls of the coelomic sacs occurring in fleas, these structures in the embryos of the Siphonaptera are very different from their homologs in such primitive insects as *Lepisma* and the Orthoptera. In all of these forms, Heymons (1895, 1897) discovered that the primitive mesodermal cavities are very extensive and possess thin walls, approximating the type found in such lower arthropods as *Peripatus*. As in *Peripatus,* they extend into the appendage rudiments and ultimately the appendicular portion of each sac is constricted off, leaving the larger dorsal part to partake in the formation of the definitive body cavity.

Heider (1889) is of the opinion that in *Hydrophilus* the coelomic sacs represent the divided original lumen of the tube formed during

the differentiation of the lower layer. *Carrière* (1890) believes these cavities of the mesodermal somites originate in the same manner in the development of *Chalicodoma*. According to the supposition of these authors, the lumen of the original tube is incompletely and temporarily closed by a dorsoventral compression. This closure flattens the mesoderm into two distinct layers which are said to separate at a later time in the regions of their lateral margins to form the coelomic sacs. This explanation is in harmony with their designation of the mesodermal tube as an elongated gastrocoel. Apparently, it was an attempt to parallel the derivation of the coelomic pouches from the archenteron, such as occurs in certain other animals, and to harmonize the situation with the view of Hertwig and Hertwig (1881) which prompted their hypothesis. Graber (1890) was not able to substantiate Heider's statements as to the case of *Hydrophilus,* and the work of Carrière has not been confirmed. In the case of flea embryos there is no possibility of such an origin for the coelomic sacs even in the posterior region of the embryonic rudiment where a distinct mesodermal tube is formed, for the lower layer, when it reaches the lateral margins of the germ band, is only one cell thick. It is not until later that the paired mesodermal bands are produced, and their appearance is due to cellular proliferation of the originally single-layered mesoderm. In the Siphonaptera, therefore, the coelomic sacs arise as independent clefts in the thickened and solid lateral regions of the lower layer.

The number of the pairs of coelomic sacs occurring in the embryos of fleas appears to be 16. The most anterior of these lies in the deuto-cerebral segment. No indication of their presence was observed in either the preantennal or the intercalary segment. It appears that such cavities have been detected in the preantennal segment of only one insect form, viz, *Carausius morosus* (Wiesmann, 1926), and even in this generalized species they are rudimentary. As for paired meso-dermal cavities occurring in the intercalary segment, they have been described in a few of the lower insects among which is *Carausius morosus* according to the work of Wiesmann cited above.

The 15 remaining pairs of coelomic sacs which occur in flea embryos are located in the first 15 segments posterior to the stomodaeal invagination. Three belong to the gnathocephalon, three to the thoracic region, and the remaining nine pairs are found in the first nine abdominal somites. The lumina of the most posterior pair are very small and rudimentary. The last two segments of the abdomen appear never to produce even such rudimentary cavities.

THE BODY CAVITY

The definitive body cavity of fleas, as in other insects, is chiefly a secondary one in that it is derived for the most part from the epineural sinus, rather than from the lumina of the coelomic sacs. The epineural sinus, in turn, has its origin as a space resulting from the reduction in size of the deutoplasmic mass as the development of the embryo proceeds (pl. 2, fig. 21). As the vitelline mass shrinks, it withdraws from the germ band, first along the midventral region, so that the resulting cavity lies immediately above the nerve cord. In its origin, therefore, the cavity is specifically epineural. Later the epineural sinus is extended laterally and dorsally on both sides until finally, with the dorsal closure of the embryo, it entirely surrounds the mesenteron which has formed in the meantime about the remaining yolk. The cavities of the relatively small coelomic sacs are added to the epineural sinus. The splanchnic walls of these sacs break through (pl. 10, fig. 78), bringing their respective lumina into communication with the extensive epineural sinus, thereby establishing the definitive body cavity (pl. 2, fig. 24; pl. 11, fig. 79).

MUSCLES

Even in the newly hatched larva the muscles are not sufficiently differentiated to facilitate identification. Therefore, no attempt was made to study their individual embryological origins. It may be said, however, that they have two general sources. The muscles of the body wall and those which are associated with the mouthparts and antennae are derived from the outer or somatic mesoderm. Some of the body wall muscles are longitudinal in arrangement, whereas others are oblique. In contrast to these somatic muscles, the muscles of the digestive tract are derived from the splanchnic mesoderm (pl. 11, fig. 80). These are either longitudinal or circular, and their arrangement appears to vary with the particular enteric region in question, as is known to be the case in adult insects.

FAT CELLS

The fat cells of the flea embryo are not closely grouped to form compact masses such as form the fat bodies of the older larvae. Instead, they appear singly or as small irregular clumps of cells scattered throughout the definitive body cavity. Some of them lie dorsal to the dorsal diaphragm within the pericardial sinus and for this reason may be called pericardial fat cells. Others which lie ventral

to this diaphragm and within the perivisceral sinus, are the perivisceral fat cells. No fat cells were observed in the perineural sinus.

The embryonic fat cells of fleas are very similar to the adipose cells of the higher animals. They appear to become distended with oil at a very early age. As in the adipose cells of mammals, the nucleus is displaced to the surface where it appears like the set in a ring. This condition differs from the observations of Nelson (1915) on the newly hatched larva of the honeybee in which form the fat cells only occasionally were found to possess a minute fat globule.

The embryonic origin of the irregularly placed fat cells in the flea embryo is difficult to follow in detail. They are definitely mesodermal derivitives, however. From their positions, it appears that the pericardial fat cells come from the somatic layer, whereas the perivisceral ones are derived from the splanchnic mesoderm adjacent to the developing enteric muscles.

CIRCULATORY SYSTEM

The circulatory system of the flea larva is composed of the dorsal blood vessel (heart) and the various sinuses of the haemocoel. The dorsal blood vessel is one of the last structures to be formed. It is derived, as in other insect embryos, from cells which are known as cardioblasts. At the time of their first appearance, they constitute a pair of narrow longitudinal bands, one band located dorsolaterally on each side of the body along the line of junction between the somatic and splanchnic portions of the mesoderm. With the dorsal growth of the mesoderm and the corresponding extension of the body cavity as the vitelline mass shrinks away from the upper surface of the egg, the cardioblastic bands gradually approach one another and ultimately meet along the dorsal midline. The apposition of the splanchnic mesoderm to the surface of the already completed midintestine epithelium is simultaneous with this process. Some time before the two heart-forming bands of cells unite, these become separated from the enteric muscle-producing portion of the mesoderm. The cardioblasts never lose their connection with the somatic mesoderm, however. The actual union of the two cardioblastic bands, to form the dorsal blood vessel, occurs shortly afterward. This tube extends well into the head region.

The open portion of the circulatory system in the flea larva is similar to that of other forms. It consists of the definitive haemocoel which is formed from the completed and partially partitioned epineural sinus plus the coelomic sacs. This partitioning takes place by

the formation of two indistinct horizontal septa. The dorsal dia-
phragm is produced by the somatic mesoderm which, as stated above,
remains attached to the cardioblastic strands. The ventral diaphragm,
which is even less distinct than its dorsal counterpart, appears to be
developed from the ventral somatic mesoderm.

Like the other structures of the circulatory system, the haematocytes
of flea embryos appear to be mesodermal in origin. They are all
nucleated cells. Wigglesworth (1934) is of the opinion that haemato-
cytes may play a part in the organogenesis of insects by producing
connective tissue membranes to cover the internal organs. In the case
of flea development the enclosing membranes, of the gonads at least,
are not formed in this manner.

The embryonic blood cells in fleas seem to take their origin by pro-
liferation from the intermediate mesoderm between the longitudinal
mesodermal bands. Snodgrass (1935) suggests, however, that because
of their later phagocytic and digestive activities, it is possible that the
haematocytes of insects are really derived from the intermediate strand
of entoderm which is differentiated in some insects such as the
Isoptera (Strindberg, 1913). If this supposition is correct, they are
to be regarded as genetically related to the secondary trophonuclei and
the epithelial cells of the mesenteron.

GONADS

The origin of the germ cells and their migration to the epineural
sinus has been described in a previous section. Having arrived in the
region of the fifth abdominal segment, the germ cells, now gonia,
become apposed to the inner surface of the splanchnic mesoderm to
form two groups of cells, one on each side of the body. With the
rupture of the coelomic sacs and the inward movement of the splanch-
nic mesoderm toward the mesenteron the germ cells are carried along
farther toward the interior. Then again, with the dorsal migration of
the cardioblasts and until the separation of the splanchnic mesoderm
from the cardioblastic bands, the clumps of germ cells are moved
somewhat dorsally. During these changes in position the sex cells on
each side become enclosed by a covering of the splanchnic mesodermal
cells with which they are associated (pl. 12, fig. 89). These covering
cells gradually become flattened to form the follicular epithelium of
the gonad. Posteriorly, splanchnic mesodermal cells, similar to those
which form the epithelial envelope, differentiate into a strand which
is continuous with the gonad (pl. 12, fig. 90). This tube is the anlage
of the oviduct or vas deferens depending upon the sex of the larva.

There is some variation as to the definitive position of the gonads in the flea larva. During development, the body of the sex organ is observed to lie in parts of three different segments. These are the fourth, fifth, and sixth abdominal segments. The definitive larval gonads lie in one of these somites. Lass (1905) says that the larval ovaries are located in the sixth abdominal segment whereas the testes are situated further anterior. If his differentiation is correct, it may be assumed that the gonad anlagen of the female contract to form the larval ovaries which are restricted to the sixth abdominal segment. Likewise, the anlagen of the testes contract to occupy positions anterior to their female counterparts. These are in the region of the fourth abdominal segment.

TRACHEAL SYSTEM

The tracheae of fleas, like those of other insects, arise as paired invaginations of the ectoderm. These are segmental in arrangement. They first become evident about the time the coelomic sacs make their appearance and while the neural groove is still open (pl. 2, fig. 28). They are situated near the lateral margins of the germ band. The mouths of the invaginations ultimately become the spiracles, while the invaginations themselves deepen, branch, and anastomose to form the complex respiratory system of the larva. A longitudinal section along one of the tracheae is shown in figure 30 (pl. 2). The definitive number of spiracles found in the fully developed flea embryo is 10 pairs. These are located on the prothorax, the metathorax, and the first eight abdominal segments. All of them arise in their definitive position except the first, which originates on the mesothorax and migrates to its larval position during embryonic development. Its position near the posterior margin of the prothoracic segment is indicative of its mesothoracic origin. Evanescent tracheal invaginations, such as have been observed in *Leptinotarsa* (Wheeler, 1889) and *Calendra* (Wray, 1937), originating in the prothoracic segment, were not found in the flea embryo. Likewise, no transitory tracheal pits corresponding to those described by Nelson (1915) as occurring on the labial segment of the embryo of *Apis* were discovered in this study. Tracheal invaginations of a rudimentary nature, arising on abdominal segments posterior to the eighth like those reported in *Lepisma* (Heymons, 1897), *Leptinotarsa* (Wheeler, 1889), and *Calendra* (Wray, 1937), also appear to be lacking in the development of fleas.

OENOCYTES

These cells, whose function is as yet not definitely known, have been observed in a number of insect forms. In flea embryos they are enormous in size in comparison to the other cells about them (pl. 2, fig. 30). Their nuclei are large, regular, and oval. The origin of the oenocytes has been traced in *Melolontha, Lina,* and *Hydrophilus* by Graber (1891). He found that in the embryos of these beetles the oenocytes arise from paired metastigmatic invaginations of the ectoderm. Wheeler (1892) found similar invaginations in *Blatta* and *Xiphidium,* but is of the opinion that they are of minor importance in the differentiation of these cells. He also studied the origin of the oenocytes in a wide variety of embryos including representatives of the Hemiptera, the Ephemerida, the Neuroptera, and the Lepidoptera. In these forms, metastigmatic invaginations were not found and the oenocytes were observed to arise by simple delamination from the lateral ectoderm. During the present study oenocytes were seen in several stages of development in the flea embryo. As in *Blatta* and *Xiphidium,* the oenocytes arise in fleas from the lateral ectoderm of the anterior region of the abdomen, and metastigmatic invaginations are differentiated. When first proliferated, the oenocytes are similar in size and appearance to the other ectodermal cells. They grow rapidly, however, and soon become readily distinguishable. They were observed only in the abdominal region where they are frequently associated with the cells of the fat body within the body cavity. They often lie adjacent to tracheal invaginations.

OTHER ECTODERMAL DERIVATIVES

In addition to the ectodermal derivatives which have been discussed already, the ectoderm gives rise to a number of other structures which have not been considered in detail during this study. These include the epidermis (hypodermis), the tentorium and apodemes of the endoskeleton, the corpora allata, the labial (salivary) glands, the silk glands, and the anlagen of the posterior portions of the genital tract. The ectodermal origin of these structures in fleas is in agreement with the work done on other insects.

In regard to the epidermis it may be added that this layer of the body wall is derived from what remains of the ectoderm in its superficial embryonic position after all the invaginations and delaminations to form the internal ectodermal organs have occurred. Furthermore, in flea development, no extra-embryonic dorsal sheet of ectoderm, such as Nelson (1915) describes for *Apis,* is differentiated. The

cuticula makes its appearance during the sixth or final day of development. This forms a sclerotized covering, the exoskeleton of the larva. It is secreted by the cells of the epidermis, and, as it is impermeable, its presence becomes manifest in attempts to stain late embryos for whole mounts. Unhatched larvae, after being kept in alcoholic borax carmine for several days, remained absolutely unstained except for a light coloration of the ectodermal regions of the digestive tract. This coloration was apparently due to seepage of the stain through the mouth and anal openings.

The setae also appear during the final day of embryonic development. As in other insects, they are produced as elongated hardened processes of certain of the epidermal cells.

The hatching spine of the flea embryo likewise makes its appearance during the final day of development. It, too, is a secondary epidermal structure and occurs as a sharp ridge located on the middorsal line of the head (pl. 3, fig. 31).

HATCHING

Unlike certain other insects such as *Leptinotarsa* (Wheeler, 1889), *Lina* (Graber, 1877), the Neuroptera (Smith, 1922), and *Tenebrio* (Sikes and Wigglesworth, 1931), the flea larva is not invested in a cuticular envelope at the time of hatching.

The normal hatching process of fleas has been described by Sikes and Wigglesworth (1931) and agrees in most points with the observations made during this study. Late in the last or sixth day of its life within the egg shell, the now fully differentiated larva expands to fill completely the lumen of the egg. This increase in size is due to a distention of the larva brought about by its swallowing the amniotic fluid. Since the withdrawal of the serosa and amnion into the vitellus prior to the dorsal closure, the amniotic fluid has filled the space between the vitelline membrane and the embryo. The ingestion of this fluid may be observed clearly through the relatively transparent chorion of *Ctenocephalides felis*. When the larva swallows the amniotic fluid its spiracles become exposed to the air, and following this exposure the liquid contents of the tracheal system is absorbed and air takes its place. After a time the larva begins to move about within the egg, and it is at this point that the hatching spine which has been described above comes into use. The spine acts as a can-opener and in due time normally pierces both the vitelline and chorionic membranes. As it moves about inside the shell, the larva ordinarily travels in a longitudinal direction. Almost always, therefore, the slit made

by the hatching spine is longitudinal. If the larva happens to cut such a slit along one entire side of the egg between the curvatures of the poles, it is able to crawl out. If, however, the hatching spine slips out of the cut and the slit in the chorion is too small to allow an escape, muscular contractions of the larva's body may be used to tear the shell open far enough to allow an escape. The hatching spine of fleas is definitely a part of the first instar larval cuticula and is retained, consequently, until the first ecdysis.

SUMMARY

Ctenocephalides felis (Bouché), *Nosopsyllus fasciatus* (Bosc.), and *Hystrichopsylla dippiei* Roths. are the species studied. Except for variations in the external characteristics of the eggs, no specific differences were observed.

Satisfactory sections of the eggs at all stages of development were obtained by the use of a double imbedding combination technique involving modifications of Boycott's clove oil celloidin and Wall's hot celloidin methods. Tertiary butyl alcohol was used for all dehydration series.

The eggs are centrolecithal. The periplasm of the egg is reached by spermatozoa by means of micropylar openings which are arranged in circular areas, one at each pole.

Maturation of the female pronucleus occurs in the anterior periplasm. Syngamy occurs in the central region of the egg, as a rule somewhat toward the anterior pole. The periplasm is very thin except at the posterior pole where it is widened to form the posterior polarplasmic cap. An inner protoplasmic reticulum is continuous with the periplasmic layer and ramifies throughout the vitellus.

Cleavage is meroblastic peripheral. The periplasm is first supplied with nuclei following the seventh cleavage division. The nuclei usually reach the periplasm at all points simultaneously. Four blastema substages are recognized. Blastulation consists of the delimitation of the nucleated periplasm of the last blastema substage into cell territories. Three blastula substages are evident. The second blastula substage is produced by a concentration of cells toward the ventral surface. The third blastula substage follows the eleventh mitotic division which involves only the cells of the thickened blastoderm, thus producing the anlage of the ventral plate. Cell accumulations produced by emigration from the blastoderm appear near the anterior and posterior extremities of the ventral plate. These are the mesenteron rudiments.

The trophonuclei have two origins. The primary trophonuclei are formed from cleavage nuclei which remain within the vitellus when the first blastema stage is produced. The secondary trophonuclei are derived from cells which return into the vitellus from the blastoderm or mesoderm of the germ band. Secondary yolk cleavage does not occur.

Posterior polar granules are not evident. The germ cells are poly-nuclear in origin. Their number varies from 5 to 12 in different eggs. The first protoplasmic pockets appear at the posterior pole during the first blastema substage. The germ cells produced thereby complete their constriction during the second blastema substage. More lateral ones may be somewhat delayed. The germ cells reenter the egg before blastulation.

The mesoderm originates by three methods. In the anterior third of the germ band this is by simple emigration of cells from the blasto-derm. In the middle region a shallow median longitudinal groove assists in the process. Along the sharp bend of the germ band, where the embryonic rudiment is involuted into the vitellus, the groove is transformed into an invaginated mesodermal tube. In the short region between the section of tube formation and the posterior mesenteron rudiment, a simple groove is again produced. Along the short length of the germ band which is carried around the terminus of the in-vaginating proctodaeum, the mesoderm is again formed by simple emigration.

The germ band is partially superficial and partially involuted at the time of mesodermal differentiation. The amnion and serosa are formed by the overgrowth and fusion of the amnio-serosal folds, aided in the posterior region by the involution process. Both embryonic membranes disappear before eclosion, the serosa first and the amnion later, each rupturing and forming a dorsal organ. The dorsal organs are absorbed by the vitellus.

The growth and movements of the germ band or embryo are as follows. The formation of the ventral plate occurs on the first day of development. Involution of the posterior portion of the embryonic rudiment takes place on the second day. Withdrawal of the involuted section of the germ band follows during the first part of the third day. The anterior and posterior extremities lie close together on the dorsal surface at this stage. On the fourth day the embryo shortens toward the poles of the egg. On the fifth day it flexes ventrally and begins to lengthen. This elongation is continued during the sixth or final day of development so that the extremities coil upon themselves, one on either side of the middle portion of the body.

Segmentation becomes externally evident during the latter part of the third day of development. The early distinguishable procephalic lobes form three segments: the labral, the antennal, and the intercalary. The protocormic region gives rise to 17 segments. The first three are the gnathal segments and the next three are those belonging to the thorax. The remaining 11 form the abdomen.

The digestive tract arises partly from anterior and posterior invaginations of the ectoderm. These are the stomodaeum and proctodaeum respectively. They grow toward each other pushing the mesenteron rudiments before them. The proctodaeum arises in conjunction with the amniotic cavity. The stomodaeal invagination produces the foreintestine and the proctodaeal invagination gives rise to the hindintestine. The anterior and posterior mesenteron rudiments each proliferate to form two lateroventral ribbons. The mesenteron ribbons of each end grow toward and ultimately fuse with those of the opposite end. They also widen until they completely enclose the remaining yolk mass, thus forming the epithelium of the midintestine. The ventral completion of this lining is accomplished first, dorsal closure being delayed until after the absorption of both dorsal organs. The four malpighian tubules develop as diverticula of the proctodaeum. They are evident by the end of the third day of development.

The central nervous system arises from two lateral cords, one below each neural ridge, and from a middle cord which is located below the neural groove. Two pairs of ganglia, the protocerebral and deutocerebral, form anterior to the stomodaeum. A third pair, the tritocerebral, originating posterior to this invagination, moves forward and fuses with the first two pairs to form the definitive brain or supraoesophageal ganglion. The three pairs of ganglia which originate in the gnathal segments coalesce to produce the suboesophageal ganglion. The three thoracic ganglia remain distinct. Of the 10 abdominal ganglia, the last 3 fuse to form one definitive ganglion. The nerve cord shortens in the late embryo so that not all of the abdominal ganglia lie in the segments of their origin.

Sixteen pairs of coelomic sacs are formed. They arise in the intrasegmental portions of the mesodermal bands, one pair in the deutocerebral segment and in each of the first 15 segments posterior to the stomodaeal invagination. These sacs are thick-walled and possess small lumina. They soon break open and join their cavities to the epineural sinus to form the definitive body cavity or haemocoel.

The gonads are prominent embryonic organs. They are produced by the formation of a splanchnic mesodermal sheath about the gonial

cells which have separated into two groups and migrated laterally and anteriorly during the development of the embryo. The gonads lie in abdominal segments four to six. A mesodermal strand, the genital duct anlage, passes posteriorly from the body of the gonad.

Other mesodermal derivatives are the muscles, fat cells, haematocytes, and the limiting walls of the circulatory system. From the ectoderm are derived such additional structures as the tracheae and tracheoles, the oenocytes, the endoskeleton, the labial and silk glands, the corpora allata, parts of the genital tract, and the epidermis together with its secondary structures such as the cuticle, the setae, and the hatching spine.

Hatching occurs after 6 days of development under the temperature and relative humidity standards followed in this study. After swallowing the amniotic fluid, the young larva escapes from the egg through a slit in the shell made either by the hatching spine alone or by this spine aided by muscular contractions of the body.

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EXPLANATION OF PLATES

PLATE 1

FIG. 1. The micropylar apparatus at the posterior pole of an egg of *Nosopsyllus fasciatus* showing a tendency toward a spiral arrangement of the openings. × 200.

FIG. 2. Longitudinal section through a portion of the chorion at one end of an egg of *Hystrichopsylla dippiei* showing the volcanolike raised processes and the thick rigid shell of this species. × 250.

FIG. 3. Transverse section through a *Ctenocephalides felis* egg in the third blastema substage. Enlargements of this section are shown in figures 10 and 11. × 100.

FIG. 4. Margin of a section of a *Ctenocephalides felis* egg in the first blastema substage showing the vitelline spheres with their contained refringent vitelline bodies. Two nuclei may be seen in the periplasm. × 450.

FIG. 5. Margin of a section of a *Ctenocephalides felis* egg in the second blastema substage. This is an enlargement of a portion of the section shown in figure 40. × 450.

FIG. 6. Posterior margin of a section of a *Ctenocephalides felis* egg in the second blastula substage showing the transition from the thinned dorsal blastoderm to the thicker ventral blastoderm. A sagittal section through an entire egg at this stage is shown in figure 50. × 450.

FIG. 7. Longitudinal section through a *Ctenocephalides felis* egg in the first blastula substage. × 100.

FIG. 8. Anterior margin of a section of a *Ctenocephalides felis* egg in the fourth blastema substage showing the nuclei crowded together in the periplasm. × 450.

Fig. 9.   Enlargement of a portion of the section shown in figure 7 demonstrating the division of the periplasm into cell territories. × 450.

Fig. 10.   Enlargement of a portion of the section shown in figure 3. Four nuclei may be seen in the periplasm. × 450.

Fig. 11.   Similar to figure 10. × 450.

Fig. 12.   Longitudinal section through the anterior half of a *Ctenocephalides felis* egg in a stage just prior to syngamy showing one polar body (PB) lying in the periplasm and the sperm (SN) and egg (EN) nuclei among the vitelline spheres. Only one more nucleus, the other polar body, showed in sections made from this egg. × 400.

Fig. 13.   Portion of a section through a *Nosopsyllus fasciatus* egg before cleavage showing the effect of Heidenhain's iron haematoxylin on the vitelline spheres. Some of the strands of the protoplasmic reticulum may be seen between these black spheres. × 400.

PLATE 2

Fig. 14.   Sagittal section through an egg of *Ctenocephalides felis* showing the early formations of the mesenteron rudiments. The flattening of the germ band at the posterior pole, the first step in the formation of the posterior mesenteron rudiment, is clearly demonstrated. The beginning of the anterior mesenteron rudiment may be seen as a slight thickening of the germ band just ventral to the anterior pole. An enlargement of the posterior portion of this section is shown in figure 51. × 100.

Fig. 15.   Portion of the anterior margin of a *Ctenocephalides felis* egg showing the anterior mesenteron rudiment at a stage slightly later than that shown in figure 16. As yet there is no indication of the amnio-serosal fold. × 450.

Fig. 16.   Portion of the anterior margin of a *Ctenocephalides felis* egg showing the anterior mesenteron rudiment at the beginning of its formation. This stage corresponds to that shown in figure 14. × 450.

Fig. 17.   Transverse section through the anterior third of the germ band of a *Ctenocephalides felis* egg at a stage somewhat later than that shown in figure 61, showing formation of the mesoderm by simple emigration of cells from the blastoderm. The embryonic membranes have completed their formation above the germ band at this stage. × 400.

Fig. 18.   Horizontal section through a *Ctenocephalides felis* egg cutting the germ band through the procephalic lobes at the anterior end and through the region of mesodermal formation by the differentiation of a distinct tube at the posterior pole. The amniotic cavity is apparent above the tube. × 100.

Fig. 19.   Enlargement of the posterior portion of the section shown in figure 18. This section is similar to that which is shown in figure 65. × 400.

Fig. 20.   Horizontal section through a *Ctenocephalides felis* egg at a stage similar to that shown in figure 18 but at a somewhat more dorsal level. The procephalic lobes show to better advantage in this section. The plane of the cut follows the amnio-proctodaeal cavity for a short distance where the tail of the embryo turns into the vitellus at the posterior pole of the egg. × 100.

FIG. 21.   Transverse section through the germ band of a *Nosopsyllus fasciatus* egg at the level of one of the pairs of coelomic sacs (CS). The epineural sinus is also shown, forming as a space between the embryonic band and the receding yolk mass. × 400.

FIG. 22.   Transverse section through the procephalic lobes of a *Ctenocephalides felis* embryo showing details of the anterior region of the amniotic cavity together with the cellular nature of the amniotic and serosal membranes which cover it. × 450.

FIG. 23.   Portion of a sagittal section through a *Ctenocephalides felis* egg with the anterior amnio-serosal fold beginning to form. The anterior mesenteron rudiment, at a stage a little later than that shown in figure 15, may be seen to the left of the fold. × 400.

FIG. 24.   Transverse section through a *Ctenocephalides felis* embryo just prior to dorsal closure. The cells of the third dorsal organ may be seen sinking into the vitelline mass. At the left a portion of the definitive body cavity is evident. This section is at a stage similar to that shown in figure 79. × 450.

FIG. 25.   Portion of a sagittal section through a *Ctenocephalides felis* egg at a stage a little later than that shown in figure 54. The amniotic membrane with its attenuated cells, the anterior mesenteron rudiment, the ectoderm, and the mesodermal layer are all clearly shown. × 400.

PLATE 3

FIG. 26.   Parasagittal section of a *Ctenocephalides felis* embryo during the fifth day of development showing the body curved ventrally. At this plane the supraoesophageal and suboesophageal ganglia are connected by one of the circumoesophageal connectives. The ganglionic thickenings of the ventral nerve cord are no longer evident on the surface. × 100.

FIG. 27.   Horizontal section through a portion of the ventral nerve cord of a *Ctenocephalides felis* embryo showing the paired ganglia fused to form definitive ganglia. In two of these ganglia the two cross-commissural neuropile tracts are demonstrated. The interganglionic connectives are also shown. × 450.

FIG. 28.   Portion of a transverse section through a *Nosopsyllus fasciatus* embryo showing a tracheal invagination in longitudinal section. × 450.

FIG. 29.   Portion of a sagittal section through a *Ctenocephalides felis* embryo cutting the proctodaeum in longitudinal direction. This stage is a little later than that shown in figure 35. × 450.

FIG. 30.   Section along a tracheal invagination of *Hystrichopsylla dippiei* showing two large oenocytes lying adjacent to its wall. In this section a metastigmatic invagination parallels the tracheal invagination for a short distance. × 300.

FIG. 31.   Transverse section showing a portion of the head of a late embryo of *Hystrichopsylla dippiei*. The developing hatching spine is a prominent structure. × 250.

FIG. 32.   Transverse section through the proctodaeum of a *Nosopsyllus fasciatus* embryo showing the four developing malpighian tubules. × 400.

Fig. 33. Sagittal section through the head of a *Ctenocephalides felis* embryo showing the blind end of the stomodaeum at this stage. Below the stomodaeum, the three fused ganglia of the gnathocephalon are demonstrated. × 450.

Fig. 34. Sagittal section through the stomodaeum of a *Ctenocephalides felis* embryo. × 450.

Fig. 35. Sagittal section through the proctodaeum of a *Ctenocephalides felis* embryo at a stage somewhat younger than that from which figure 29 was made. × 450.

Fig. 36. Portion of a section through a *Ctenocephalides felis* embryo at a stage somewhat older than that from which figure 35 was made. The proctodaeal invagination is considerably deeper in this stage.

PLATE 4

Fig. 37. Section through the middle anterior region of a *Ctenocephalides felis* egg showing the male and female gametic nuclei fusing in syngamy. Their peripheral processes are continuous with the reticular protoplasm which ramifies throughout the egg. × 400.

Fig. 38. Section through the middle anterior region of a *Ctenocephalides felis* egg at a stage immediately following syngamy. The star-shaped zygotic nucleus lies in the center of the field. The complete section from which this enlargement was made is shown in figure 39. The vitelline spheres and their enclosed vitelline bodies show very clearly. × 400.

Fig. 39. Longitudinal section through an unsegmented egg of *Ctenocephalides felis* showing the zygotic nucleus lying in the middle anterior region of the vitellus. This nucleus is shown enlarged in figure 38. The thin chorion of this species shows clearly in this section. × 115.

Fig. 40. Longitudinal section through a *Ctenocephalides felis* egg in the second blastema substage of development. As is usual in early-stage preparations, the nuclei are only faintly visible. × 115.

Fig. 41. Longitudinal section through a *Ctenocephalides felis* egg in the third blastema substage of development, showing the nuclei in the periplasm. × 115.

Fig. 42. Longitudinal section through the same egg as that from which figure 41 was made, showing three germ cells lying outside the egg at the posterior pole. These cells are shown enlarged in figure 46. × 115.

PLATE 5

Fig. 43. Posterior portion of a longitudinal section through a *Ctenocephalides felis* egg prior to cleavage, showing the periplasm widened at the posterior pole to form a distinct cap, the posterior protoplasmic cap. × 400.

Fig. 44. Longitudinal section through the posterior region of a *Ctenocephalides felis* egg in the first blastema substage of development, showing four germ nuclei in the periplasm at the posterior pole. × 400.

FIG. 45.   Longitudinal section through the posterior region of a *Ctenocephalides felis* egg at a stage immediately following that shown in figure 44, showing three germ cells bulging out preparatory to constriction. The granular appearance of the interior of the egg and of a part of the germ-cell cytoplasm is possibly due to Blochmann's corpuscles. × 500.

FIG. 46.   Posterior portion of the section shown in figure 42, showing primordial germ cells which have been extruded from the egg mass at the posterior pole. They lie between the periplasm and the vitelline membrane. × 400.

FIG. 47.   Longitudinal section through the posterior region of an egg of *Ctenocephalides felis,* also in the third blastema substage of development. Six germ cells are shown at the posterior pole, lying outside of the periplasm. × 400.

FIG. 48.   Longitudinal section through the posterior region of a *Ctenocephalides felis* egg in the first blastula substage of development. The periplasm is distinctly divided into cell territories. This section also shows a small group of germ cells which have reentered the egg and are lying just within the blastoderm. × 400.

PLATE 6

FIG. 49.   Longitudinal section through a *Ctenocephalides felis* egg in the fourth blastema substage of development. The full quota of first blastula substage nuclei is present but the cell territories characteristic of the blastula stage have not been delimited. × 115.

FIG. 50.   Sagittal section through a *Ctenocephalides felis* egg in the second blastula substage showing the crowding of the cells toward the ventral midline which is the first step in the formation of the ventral plate. The thinned dorsal region and the thickened ventral region are clearly distinguishable. × 115.

FIG. 51.   Posterior portion of the section shown in figure 14, showing the flattening of the posterior pole of the germ band, the first step in the formation of the posterior mesenteron rudiment. A group of germ cells may be seen lying inside this flattened region. × 400.

FIG. 52.   Transverse section through the germ band of *Ctenocephalides felis* showing the beginning of the formation of the paired lateral amnio-serosal folds. × 400.

FIG. 53.   Sagittal section through the anterior region of a *Ctenocephalides felis* egg in a stage slightly more advanced than the one shown in figure 23. The anterior amnio-serosal fold and the anterior mesenteron rudiment are both very clearly shown as is also the shallow pit at the point of emigration of the mesenteron rudiment cells. The chorionic and vitelline membranes also show to advantage in this figure. × 400.

FIG. 54.   Sagittal section through the anterior region of a *Ctenocephalides felis* egg at a stage somewhat more advanced than that shown in figure 53, showing the double nature of the amnio-serosal fold which has grown farther posteriorly. The ectoderm has entirely closed over the pit which existed above the mesenteron rudiment. × 400.

PLATE 7

FIG. 55. Whole mount of a *Ctenocephalides felis* embryo dissected from the egg early in the third day of development. The germ band is unsegmented and the tail region is not entirely withdrawn from the vitellus. At this stage the embryo consists of two general regions, an anterior one widened to form the procephalic lobes and a posterior unwidened one forming the protocormic region. × 115.

FIG. 56. Sagittal section through a *Ctenocephalides felis* egg at a stage slightly younger than the one shown in figure 55 and corresponding to the stage shown in figure 54. The posterior amnio-serosal fold grows more rapidly than its anterior counterpart and may here be seen approaching the midventral region of the egg. This stage illustrates the maximum involution of the caudal region with the amnio-proctodaeal cavity lying at the center of the vitellus. It also shows how the germ band is carried around the inner extremity of this lumen. × 115.

FIG. 57. Horizontal section through a *Ctenocephalides felis* egg in the same stage of development as that shown in figure 55. The germ band is cut transversely in three places, at the anterior end through the procephalic lobes, at the middle of the egg through the tail where the embryonic rudiment rounds the amnio-proctodaeal cavity, and at the posterior end of the egg where the tail piece was originally invaginated into the vitellus. The amniotic and serosal membranes are complete and show at both poles of the egg. × 115.

FIG. 58. Section through a *Ctenocephalides felis* egg showing the involuted posterior portion of the germ band in transverse section at a plane posterior to the posterior mesenteron rudiment and at the center of the egg. The mesodermal cells may be seen migrating inward from the blastoderm. The lumen shown is the inner extremity of the amnio-proctodaeal cavity. × 400.

FIG. 59. Sagittal section through the posterior region of the germ band of *Ctenocephalides felis* at a stage similar to that shown in figures 56, 58, and 60. The posterior mesenteron rudiment cells are to be seen lying below the ectoderm adjacent to the posterior terminus of the amnio-proctodaeal cavity. × 400.

FIG. 60. Parasagittal section similar to the sagittal one shown in figure 59. × 400.

PLATE 8

FIG. 61. Transverse section through the germ band of *Ctenocephalides felis* at a point between the anterior amnio-serosal fold and the anterior mesenteron rudiment showing mesoderm formation by simple emigration of cells from the blastoderm. × 400.

FIG. 62. Transverse section through the germ band of *Ctenocephalides felis* at a point in the anterior portion of its second third in that region of the embryonic rudiment where mesoderm formation is by the migration of cells from the blastoderm together with the formation of a groove. × 400.

Fig. 63. Transverse section through the middle portion of the germ band of *Ctenocephalides felis* where the method of mesoderm formation is similar to that described for figure 62. × 400.

Fig. 64. Transverse section through the germ band of *Ctenocephalides felis* at a point in the posterior extremity of the second-third region, showing mesoderm formation by cellular migration following shallow groove formation. The regular course of the mesodermal cells as they move laterally below the ectoderm is also shown. That this stage is somewhat later than that shown in figures 62 and 63 is indicated by the fact that the amnion is formed and that the ectoderm has closed over the point of emigration of the mesodermal cells, thereby practically eliminating the groove. × 400.

Fig. 65. Posterior portion of a horizontal section through a *Ctenocephalides felis* egg showing the germ band cut transversely at a point just anterior to the region of its involution into the vitellus. This figure illustrates mesodermal differentiation by the formation of a distinct tube and also shows the amniotic cavity in the region where it is about to be transformed into the amnio-proctodaeal cavity. The amnion and serosa are clearly indicated ventral to the amniotic cavity. This section corresponds to that shown in figure 19. × 400.

Fig. 66. Transverse section through the germ band of *Ctenocephalides felis* at a level slightly posterior to that shown in figure 65 and at a somewhat later stage. The mesodermal tube is compressed and its lumen is obscured. The amnio-proctodaeal cavity and the amnion are both clearly shown, and some of the attenuated cells of the serosa may be seen lying against the surface of the vitellus. A region of deutoplasm consequently separates the two embryonic membranes. × 400.

PLATE 9

Fig. 67. Transverse section through the anterior region of the germ band of *Ctenocephalides felis* in that part which is differentiating into the procephalic lobes. The haphazard lateral progress of the mesodermal cells is shown. The amniotic cavity, amnion, and serosa may be seen above the germ band. × 400.

Fig. 68. Transverse section through the procephalic lobes of *Ctenocephalides felis* at a stage somewhat later than that shown in figure 67. × 400.

Fig. 69. Transverse section through the middle region of the germ band of *Ctenocephalides felis* showing the arrangement of the mesodermal cells at the completion of their migration from the blastoderm. The neural groove is beginning to form. × 400.

Fig. 70. Transverse section through a *Ctenocephalides felis* egg showing the germ band cut at two places. The completed amniotic and serosal membranes appear above the upper section. In both sections the neural groove and the layer of mesodermal cells lying below the ectoderm may be seen. × 375.

Fig. 71.   Sagittal section through the posterior region of the germ band of a
            Ctenocephalides felis embryo showing the lumen of the proctodaeum
            together with two diverticula at its inner end representing the
            beginnings of two of the malpighian tubules. × 400.

Fig. 72.   Transverse section through the germ band of a *Ctenocephalides felis*
            embryo showing the early differentiation of the ventral nerve cord.
            The neural groove is shown at the middle of the band and to the left
            of this groove, just below the ectoderm, several neuroblasts may be
            seen. These neuroblasts are larger and rounder than the mesoderm
            cells which lie below them. The elongated ectodermal cells are
            dermatoblasts. × 400.

PLATE 10

Fig. 73.   Whole mount of a *Ctenocephalides felis* embryo dissected from the
            egg after 3 days of development, when withdrawal of the tail from
            the deutoplasm is complete. Both the anterior and the posterior
            extremities of the germ band have grown around their respective
            poles of the egg and approximate each other on the dorsal surface.
            The segmentation is distinct. × 115.

Fig. 74.   Parasagittal section of a *Ctenocephalides felis* embryo at a stage
            similar to that shown in figure 73. Segmentation is distinct. The
            oral appendages appear in order: labral, mandibular, maxillary,
            and labial. Because of the parasagittal plane of the section, the
            stomodaeum does not show, and on the maxilla is an apparent
            ventral projection. A deep cleft separates the maxilla from the
            labium. The three thoracic segments follow the labium and posterior
            to these come the abdominal segments. The eleventh abdominal
            segment has already been carried in from the surface by the
            invagination of the proctodaeum. × 115.

Fig. 75.   Sagittal section through a *Ctenocephalides felis* embryo at a stage
            corresponding to that shown in figures 73 and 74. The stomodaeal
            and proctodaeal invaginations are clearly shown. The labrum lies
            anterior to the stomodaeum. Immediately posterior to this in-
            vagination the three gnathal segments appear very much fused.
            × 115.

Fig. 76.   Transverse section through the germ band of a *Ctenocephalides felis*
            embryo showing the neural groove and coelomic sacs. The walls
            of the sacs are thick and their lumina very small. This stage corre-
            sponds to that shown in figure 77. × 400.

Fig. 77.   Portion of a parasagittal section through the germ band of a *Cteno-
            cephalides felis* embryo showing the segmental arrangement of the
            coelomic sacs. × 400.

Fig. 78.   Transverse section through the germ band of a *Ctenocephalides felis*
            embryo showing how the neural groove is formed by the production
            of paired longitudinal thickenings, one such thickening on each side
            of it. The definitive body cavity or haemocoel, formed by the fusion
            of the epineural sinus and the ruptured coelomic sacs, is also
            shown. × 400.

PLATE 11

FIG. 79.   Dorsal portion of a transverse section through an embryo of *Cteno-cephalides felis* at a stage just prior to dorsal closure showing the absorption of the third dorsal organ by the vitellus. At the left a layer of entodermal cells may be seen lining the yolk mass. To the left of these is a portion of the definitive body cavity. Parts of the chorionic and vitelline membranes are shown in the upper part of the figure. This stage is similar to that shown in figure 24. × 400.

FIG. 80.   Sagittal section through a *Ctenocephalides felis* embryo at a stage somewhat more advanced than that shown in figure 75. The coiled hind-intestine of this stage is shown cut through in several places and the anterior mesenteron ribbons have formed a complete floor of entoderm for the yolk mass. This entodermal lining is covered by the splanchnic mesoderm and below this lies the haemocoel. A strip of somatic mesoderm appears in the midventral region of the embryo. × 115.

FIG. 81.   Sagittal section through the anterior region of a *Ctenocephalides felis* embryo showing the inner end of the stomodaeum. The paired anterior mesenteron rudiments have fused ventrally and their cells are shown in this section lining the vitellus below the stomodaeum. The fusion of these ribbons dorsal to the stomodaeum is just beginning. × 400.

FIG. 82.   Sagittal section through the anterior portion of a *Ctenocephalides felis* embryo showing the stomodaeal invagination and the labrum. × 400.

FIG. 83.   Horizontal section through the anterior region of a *Ctenocephalides felis* embryo showing the paired lateral mesenteron ribbons (MR) lying adjacent to the yolk. × 400.

FIG. 84.   Portion of a section through a *Ctenocephalides felis* embryo showing the hind-intestine cut at two places. Three of the developing malpighian tubules may be seen communicating with the procto-daeum in the lower one of the cuts shown here. × 400.

PLATE 12

FIG. 85.   Portion of a sagittal section through a *Ctenocephalides felis* embryo showing several ganglia of the ventral nerve cord, each with its two transverse neuropile tracts. × 400.

FIG. 86.   Transverse section through the head region of a *Ctenocephalides felis* embryo in a late stage of development. In the upper portion of this figure, and on either side of the oesophagus, are to be seen the paired posterior extremities of the supraoesophageal ganglion. The suboesophageal ganglion lies below the oesophagus and in this section is shown connected with the supraoesophageal ganglion by the neuropile tracts of the circumoesophageal connectives. This stage corresponds with that shown in figures 26 and 87. × 400.

FIG. 87.   Sagittal section through a *Ctenocephalides felis* embryo in the fifth day of development shortly after ventral flexion. The proctodaeum is a particularly conspicuous structure. The supraoesophageal ganglion, the suboesophageal ganglion, and the chain of ventral

nerve ganglia also show to advantage. The ventral ganglionic thickenings are no longer evident on the surface. A parasagittal section of this same embryo is shown in figure 26. × 115.

FIG. 88.   Parasagittal section through the head region of a *Ctenocephalides felis* embryo at a stage similar to that shown in figures 26 and 87 showing the supraoesophageal and suboesophageal ganglia connected by the circumoesophageal connective of the side. The lateral nature of the section is further indicated by the lack of transverse commissural tracts such as appear in the ganglia shown in figure 85. The definitive nature of the mandible is also evident in this figure. × 400.

FIG. 89.   Portion of a sagittal section through a late embryo of *Ctenocephalides felis* showing the gonad cut longitudinally, revealing the prominent germ cells inside it. × 400.

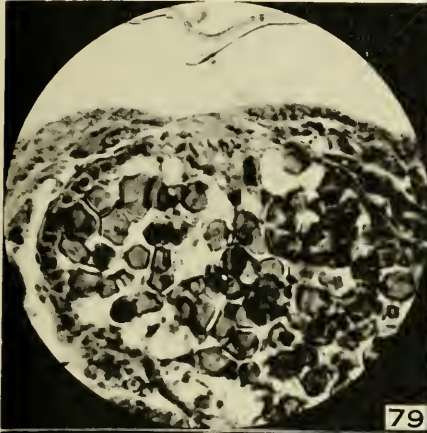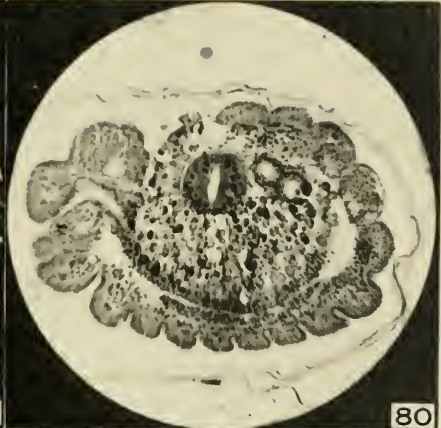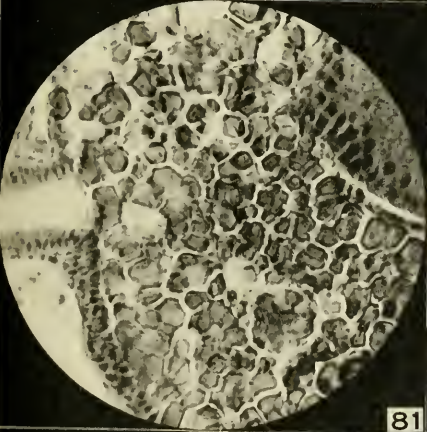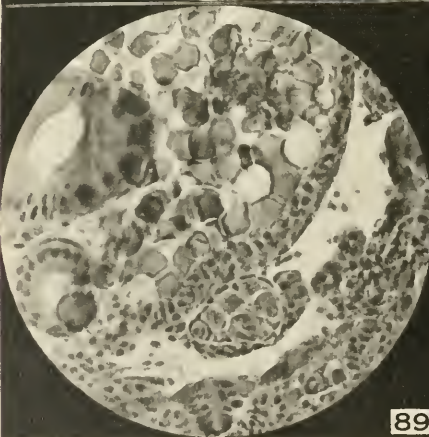FIG. 90.   A similar section to that shown in figure 89 but showing the anlage of the genital duct leading posteriorly from the gonad. × 400.

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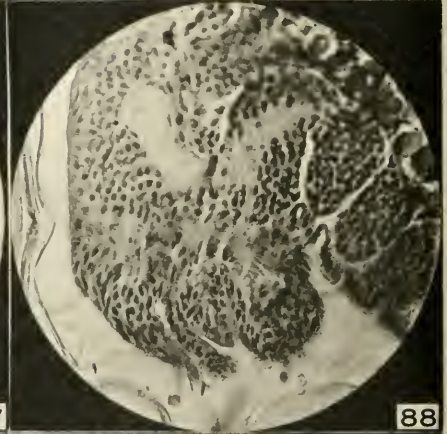(See explanation of plates at end of text.)

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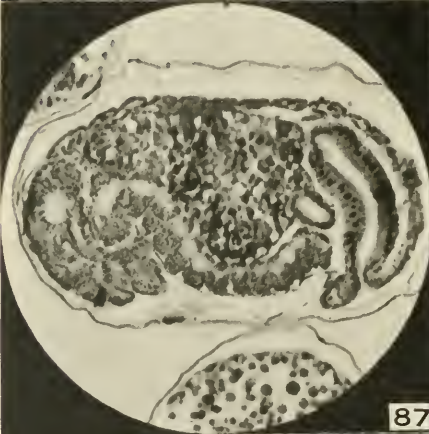(See explanation of plates at end of text.)

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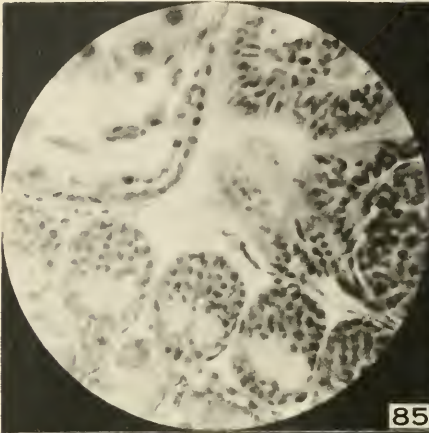**EMBRYOLOGY OF FLEAS**
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