V—The Secretory Phenomena in the Oviduct of the Fowl, including the Process of Shell Formation Examined by the Microincineration Technique

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I—Introduction

The present studies were commenced primarily to investigate the cytological phenomena in glandular tissues in which the secretion consists mainly of inorganic salts in solution. In selecting convenient material, the fowl uterus was chosen as a vertebrate organ liberating a secretion which, in view of the chemical composition of the egg shell, must contain calcium salts in high concentration with a trace of magnesium carbonate, but without significant amounts of iron, silicon, or aluminium. From previous investigations, it has been established that at least two types of gland cells exist in the lining epithelium of the uterus and that of the tubular glands of the uterine corium, both of which contribute to the final stages of egg-shell formation in the fowl. The addition of half the total egg-white, in the form of thin fluid albumen, has been conclusively attributed to the activity of the uterine glands, but no convincing localization of the egg-shell secretion has been made owing to the remarkably low concentration of calcium salts in the actively secreting uterus ; a concentration for which the ordinary histo-chemical tests are ineffective.

The technique of microincineration, which hitherto has been confined largely to problems of a histological or embryological nature, owing to the difficulty of obtaining delicate cytological detail in the ash residues, was examined as a possible means of identifying the storage and elaboration of inorganic material in the uterine gland cells and its later extrusion as the mature secretion. A comparative examination of the mineral content of regions adjacent to the uterus and the cytology of heavy albumen secretion in the cranial sections of the oviduct, led finally to a study of secretory processes in the entire organ. The more important phases of the glandular activity in the infundibulum, albumen region, and the isthmus have been re-investigated, particularly where lack of agreement exists, in recent literature, on details of major importance. A final solution, however, of many aspects of this problem naturally rests on the histo-chemical analysis of material fixed and sectioned without the use of protein precipitants or solvents. In this connection the new freezing-drying technique of Gersh (1932) might prove to be most suitable.

The literature on the chemistry of egg-white, the shell and its membranes is extensive, but little of it has been correlated with the previous histological studies of the secretory processes in the fowl oviduct. In some instances only presumptive evidence has been used in interpreting the functions of the glandular structures in this organ. The earlier literature on the fowl oviduct is summarized by Surface (1912), who made a detailed study of the histology of this organ, limited by the use of an insufficient variety of fixatives and stains, but extended by Bradley (1928), particularly with reference to the identification of the goblet and other mucin producing cells in the epithelium. A further paper by Giersberg (1922) was devoted to the histology of the reptilian and avian oviducts, and the formation of the shell membranes from keratin fibres was dealt with in detail. Cushny (1902) was the first to refer to the region immediately cranial to the isthmus, as being a specialized portion of the albumen region with an almost continuous mucous

secreting surface. His paper was unknown to SURFACE and GIERSBERG, while BRADLEY made an independent reference to this region.

Many of the conclusions drawn by these authors rest upon the work of PEARL and CURTIS (1912), in which the proportions of egg-white added to the egg, in different regions of the oviduct, and the time periods taken by the egg in passing through these regions, are carefully analysed. TURCHINI (1924) alone has made a special histological study of the calcium secretion of the uterus by microincineration. His results with this technique were confined to low power observations of mineral residues on platinum foil. The only cytological studies of the fowl oviduct are those of BRAMBELL (1925), who gave a detailed account of the behaviour of the Golgi apparatus, more particularly in the uterine epithelium.

The large size of the fowl oviduct appears to have limited the investigations of these workers to isolated portions of the oviducal wall, fixed apart from the adjacent developing egg. Of great interest, therefore, is the more recent work of FROBÖSE (1928), who demonstrated the curious modification in the epithelium covering the uterine folds which lie actually in contact with the developing shell surface ; observations which he made by fixing the entire uterus and egg *in situ* after ligaturing the severed isthmal and vaginal regions.

Finally, an excellent summary of the anatomy and histology of the oviduct has appeared recently in the " Handbuch der vergleichenden Anatomie der Wirbeltiere," by A. J. P. VAN DER BROEK (1933), and a review of the literature concerning the chemistry of the egg-white, the shell and shell membranes, may be found in various chapters of NEEDHAM's " Chemical Embryology " (1931).

In mammals certain cytological phenomena described by MOREAUX (1913), and SNYDER (1923), and the more extensive work of BARTLEMEZ and BENSLEY (1932) on the human uterine glands, are referred to in this investigation where similarity is evident. But detailed comparison between mammalian and avian material seems useless where such widely divergent functions exist in regions such as the uterus.* The recent paper of HILL and HILL (1933), on the Monotreme oviduct, contains a more detailed account of the secretory phenomena associated with the formation of the egg-envelopes than has been given for any other oviparous vertebrate. The findings in this paper are of the utmost value, because close correlation has been made between (1) the secretory state of the various gland cells, (2) the secretory material present in the oviducal lumen, and (3) the structure of the egg-envelopes, observed coincidentally in a graded series of Platypus and Echidna material. No previous literature deals with the Sauropsidan oviduct in this carefully controlled manner. In the fowl it cannot be decided at present whether all the tubular glands in the oviducal corium contribute their share of secretions to the passing egg or whether scattered groups of glands secrete while others are quiescent and awaiting the passage

* The term uterus, as applied to the expanded caudal part of the fowl oviduct, which might be more clearly described as the shell gland, is universally accepted, though earlier comparative anatomists have emphasized its inappropriateness.

of a succeeding egg. This and other aspects of secretory activity still in doubt, might be investigated by using material comprising oviducts with an egg in successive levels of the various anatomical regions, and sectioned with the egg *in situ*.

II—Material and Technique

The selection of oviducts containing eggs in stages representing each successive secretory process, from the naked yolk to the completed egg, is conditioned largely by chance whilst the organ itself is inconveniently large for serial sectioning. In no previous studies on this subject has the material been sufficiently complete, and, even in the present investigation, additional material might have been collected to illustrate the maximum secretory phase of the isthmo-uterine junction. A series consisting of newly formed shell membranes or partly calcified shells, fixed together with an adjacent portion of the corresponding oviducal wall, might also yield further information. From a total of thirty-two fowls (White Leghorn and Rhode Island breeds), sufficient material has been obtained to enable a study to be made of the stages of secretory activity, rest, and preparation for a new cycle in the albumen region, the isthmus and uterus, whilst two examples of the infundibulum, following maximum secretion, have been examined. In addition, two specimens of the isthmo-uterine junction, immediately following the passage of an egg into the uterus proper, and two oviducts in a partly atrophied state, at the conclusion of an egg-laying period, have been available.

The fixatives and stains which have been used on this material are listed below, and reasons for their use will be discussed in various sections of the paper ; for it has been realized that no single fixative can be used to preserve, with equal clarity, the wide variety of substances secreted by the tubular glands and the ciliated epithelium. Critical notes on the microincineration technique are given in the discussion on the uterus.

Fixatives—Zenker-formol without acetic acid, Regaud, Schridde, Ludford's Mann-Kopsch, Da Fano silver impregnation, absolute alcohol and formalin (micro-incineration), mercuric chloride and acetic acid (Feulgen), 5% mercuric chloride in saline (mucin).

Stains—Heidenhain's iron hæmatoxylin, Delafield's hæmatoxylin, Feulgen, muci-carmine, thionin blue, Best's carmine, eosin.

The effect of the fixing fluid reagents on solutions of egg albumen and on pure egg-white, has been investigated, as suggested in Baker's monograph (1933), but the fixation and staining behaviour of these mature secretions of the oviduct offer little indication of the reactions finally to be obtained in the intact tissues, where pro-secretion material exists in variable physical states and rests uncombined with the secretions of other cell-types. The speed of penetration of a protein precipitant, such as mercuric chloride, appears to have an important bearing on the appearance of the secretory material in an albumen producing cell. And it must be remembered, as Bensley and Gersh (1933) have clearly stated, that " the reagent is acting not

on the individual constituents of the cell, whose distribution is sought, but upon the cytoplasm as a whole, and the result is a composite one depending on the interaction of all these several factors."

Technical difficulties have been experienced in choosing suitable means for preserving cytological detail in the material that has been fixed in bulk with the egg *in situ*. The structure of the distended oviducal wall actually in contact with a developing egg is important, and the injection of even small amounts of fixative into the lumen involves a marked alteration in the natural position of the mucosal folds. In all types of fixation, the intimate contact between the epithelium and the egg surface is difficult to preserve. In view of the poor fixation obtained by allowing the fixative to penetrate the oviducal wall through the musculature, after immersion in the solution, most of the material used in this investigation has been taken after dissection of small areas of the oviduct, following removal of its contents. When studying the shell secretion in particular, specimens containing eggs at various stages of calcification were immersed *in toto* in the fixative after ligaturing the unexpanded portions of the oviduct immediately cranial and caudal to the egg. Subsequently a small window was cut through to the egg interior to release its contents. After decalcification in 5% nitric acid in 70% alcohol, the shell invariably collapsed within the uterus. To prevent this it was necessary to fill the egg interior with soft paraffin wax before decalcification. In this condition the shell remained expanded but the gas resulting from the action of the acid tended to destroy the relationship between the structures. The entire uterus, after embedding in a large block of paraffin wax, was cut with a fret-saw in planes passing through the isthmal and vaginal canals and then across areas in which contact between the shell and the uterine wall was visibly preserved. Small portions of these special areas were removed and re-embedded. Sections were easily obtained which were excellent, apart from the poor preservation of the uterine epithelium. The double embedding process appears to have no advantages with this material owing to the impermeability of the calcified shell to celloidin.

III—Observations

1—*Anatomy of the Oviduct*, fig. 1, Plate 12

No detailed description of the anatomy of the fowl oviduct need be given, but, as it has been found necessary to refer to histological regions not previously recognized by a constant terminology, the following details are important. The oviduct, in different breeds, varies considerably in length, as shown by Giersberg (1922), quoting from previous literature and his own material, in which 42·5 cm and 86 cm are recorded as the shortest and longest examples in the active egg-laying condition. The variation in the non-laying birds (between 14·2 cm and 19 cm) is not so marked. The specimens used in the present studies are approximately 50 cm in length and the dimensions given for special regions refer to this average measurement.

In its general histological structure the oviducal wall consists of external peritoneal and muscular coats and an internal mucosa. The mucosa is thrown into folds which vary in size and arrangement in different regions, fig. 1, Plate 12. A ciliated and glandular epithelium covers the folds and forms an internal lining to the entire oviduct. The corium of the folds may contain merely connective tissue and blood vessels or a dense packing of tubular glands. The open spaces between small folds are termed " pits." Where the folds are broad and their lateral surfaces tend to come in contact with those of adjacent folds, the recesses between them are called " crypts."

Five major subdivisions of the oviduct, (1) infundibulum, (2) albumen region, (3) isthmus, (4) uterus, and (5) vagina, have been generally recognized for a considerable time. The classification, apart from macroscopic boundaries, is based principally upon the structure of the tubular glands of the corium. The first glands appearing in the cranial end of the oviduct form an indefinite boundary between the infundibulum and albumen region of previous authors. GIERSBERG (1922) distinguishes the open, translucent walled " Trichter " from the tubular, thicker walled region which he calls the " tube." From my own observations it appears preferable to define the infundibulum as the entire anterior portion of the oviduct (length 8 cm) which extends caudally as far as the first typical albumen secreting glands, *x*, fig. 1, Plate 12. The infundibulum may then be subdivided approximately into two halves. The cranial half (including the fimbriated margin of the infundibulum) has no glands in the corium and is more appropriately termed the " funnel," *f*. The remaining caudal half of the infundibulum possesses characteristic chalaziferous glands which are quite distinct from albumen glands, and this region I have called the " chalaziferous region."

The increased epithelial height in the last 4 cm of the albumen region (length 20 cm) accompanies a remarkable development of mucous cells, first described by CUSHNY (1902) in the lining epithelium. The term " mucous region " is used in this paper to denote the part in question, though its cranial boundary is ill defined, *mr*, fig. 1, Plate 12. A group of special glands exists in the junctional region between the isthmus (length 7 cm) and uterus. These become intermingled cranially with isthmal glands and caudally with the uterine type. The junctional region (length approximately 2 cm) is referred to as the isthmo-uterine junction, *ij*, fig. 1, Plate 12. The uterus averages 7 cm in length, and the vagina, which is devoid of tubular glands, extends for 6 cm.

2—*The Infundibulum*, figs. 2–5, Plate 13

The chief interest of this region, in the present work, lies in the fact that it is the source of the heavy chalaziferous secretion which forms a thin layer around the mature ovum, internal to the dense albumen. Several oviducts, containing ova in the upper section of the albumen region, form the material on which the following description is based. They represent the end-point of secretion in the infundibulum.

A uniform ciliated epithelium (height 30 μ), in which no glandular cells are evident, covers the funnel wall, with the exception of the shallow pits between the

major folds, *gr*, fig. 2, Plate 13. Here, groups of non-ciliated cells of a glandular type, with regular basal nuclei and an occasional increase in cell-size towards the centre of the pit, may be observed. These structures were called glandular grooves by SURFACE (1912), and his description of them is confirmed in all details. No secretion has been identified as issuing from their epithelium and the individual cells remain curiously constant in appearance throughout the present material.

At a point approximately 3·5 cm from the funnel lips, mucous cells alternate with ciliated cells (height 25 μ) over the entire epithelium, apart from the glandular grooves. The bleb-like apical ends of the mucous cells stain heavily with mucicarmine, thionin, and Delafield's hæmatoxylin, *g*, fig. 3, Plate 13.

At the point where the funnel region ends and in the chalaziferous region which follows it, a gradual transformation of the glandular grooves into short tubular glands accompanies the appearance of the mucous cells. Fig. 3, Plate 13, shows the early formation of these tubules in a section at the cranial limit of the chalaziferous region. To the left, a typical glandular groove, *gr*, is illustrated and beneath it is a small evagination from the groove *e* cut transversely. An elongate tubular gland, in longitudinal section, *tb*, appears on the right. In all details the tubule epithelium is similar to that forming the primitive grooves, apart from minor variations in cell-size and nuclear position. The mucous cells, readily recognizable by their deeply staining tips, are seen to extend as far as the margins of the grooves and the apertures of the tubular glands. In the larger grooves, the central epithelial cells may be both ciliated and mucous in character.

Branching of the folds in the infundibular wall and further tubular gland formation, with a retention of all the previous features in the lining epithelium, may be found in the more caudal levels of the chalaziferous region. In identifying tubular glands cut in section, care has been taken to avoid confusing a true gland with a small pit or glandular groove in oblique section. The folding of the mucous epithelium in the chalaziferous region somewhat resembles that of the mammalian Fallopian tube, where a false appearance of tubular glands in the corium is often obtained. The dark mucous cells clearly indicate an obliquely sectioned pit or crypt in the case of the fowl (*g*, fig. 4, Plate 13), whilst serial sections show the continuity of individual glands with their glandular grooves.

In the larger chalaziferous glands with dilated lumina, an irregularly staining, viscous secretion collects which may also be seen lying free in the oviducal lumen between the smaller folds. Where the secretion occurs, the epithelium is hypertrophied and the apical cell walls are ruptured (*tc*, fig. 4, Plate 13), whereas in empty glands, the cells have clear unbroken margins (*te*, fig. 4, Plate 13). The mucous secretion from the ciliated epithelium of the larger grooves in the chalaziferous region is not preserved with Zenker-formol fixation and the remaining glandular grooves, which have not transformed into tubules, are invariably empty. Though the chalaziferous glands are well developed throughout 4 cm of the infundibulum, they are not so closely packed as the glands in succeeding regions of the oviduct.

At the commencement of the albumen region, the folds become simpler and the glandular grooves disappear but, before the broad longitudinal folds of the typical albumen region are developed, albumen secreting glands are differentiated in the corium. Groups of them lie mingled with the remaining glands of the chalaziferous type, *a*, fig. 5, Plate 13. With Zenker-formol fixation, a clear distinction exists between the two types of glands in the active secretory condition. The darkly staining shrunken nuclei of the cells of the albumen glands lie in contact with the cell membrane, and the cytoplasm is alveolar and filled with secretion which stains deeply with iron hæmatoxylin. In the lumina, the secretion is homogeneous and quite different from that of the chalaziferous glands, *cf. a* and *ta*, fig. 5, Plate 13. An albumen gland in the cranial boundary of the albumen region may be lined entirely with typical albumen-secreting epithelium, but occasionally the proximal section of a tubule, near its outlet into the oviducal lumen, may be formed from chalaziferous or glandular groove cells, *tm*, fig. 5, Plate 13. Mixed glands with distal albumen cells and proximal chalaziferous cells are, however, not common.

The musculature and connective tissue of the infundibulum have been accurately described by previous authors, but I can find no reference to the unusual numbers of plasma cells in this region. Large nests of these cells, resembling lymph nodes, occur in the loose connective tissue between the glands, and occasional masses of them lie in contact with the larger blood vessels. These plasma cell groups appear to provide very useful material for studying the origin of this cell-type.

3—*The Albumen Region*, Plates 14 and 15, fig. 14, Plate 16, and fig. 21, Plate 17

The following observations on this portion of the oviduct serve only to confirm those of previous authors and to show that individual differences of opinion may be explained, in several instances, as due to the variable reaction of the secretion antecedents of albumen to different fixatives. Portions of the albumen region of an oviduct, containing an ovum surrounded with egg-white and situated just caudal to the infundibulum, were used to show the maximum phase of albumen secretion. The preparatory or pre-secretion stages of the albumen region were demonstrated in portions of the oviducal wall anterior to the site of the ovum. Preparations showing the end point of albumen secretion were selected from an oviduct in which the egg had reached the uterus. From this material it has been possible to demonstrate the phases of activity and rest typical of both the tubular glands and the lining epithelium.

In studying the albumen region, the method of fixation is of the greatest importance. It has been found that solutions containing acetic acid, or mercuric chloride alone, almost entirely destroy the earlier stages of albumen formation. They produce such a coarse precipitation of the free egg-white in the oviducal lumen that it is either distorted or partly removed during washing and embedding. Zenker-formol or Regaud's fixative, followed by iron hæmatoxylin, have proved most suitable in elucidating the stages of albumen formation, for they preserve the albumen

in homogeneous masses both intracellularly and after it is expelled from the tubular glands.

(*a*) *The tubular glands during secretion*—In sections from an oviduct secreting albumen at the time of fixation, the glands are seen to vary in their degree of staining. More commonly one finds a central group of glands in each fold to be coarsely granular with large black globules of secretion in the epithelium. Adjacent to these are areas of more uniform darker staining glands, grading in staining intensity to the periphery of the fold, where the granules are faintly grey. Detailed examination of these areas of variable staining intensity showed that they were not artefacts resulting from unequal penetration of the fixative but an indication of the variable physical or chemical states of the secretion, associated with (1) storage in the resting glands, (2) active secretion, (3) the cessation of secretion followed by regeneration of the epithelium. In a resting oviduct, moreover, the position of the coarsely granular and lighter staining glands becomes reversed, so that the central areas in the fold contain lightly staining glands and the peripheral glands undergo the darker staining phase.

A typical gland of the coarsely granular type found in the central areas of the folds in actively secreting oviducts and in the periphery of the folds during rest, *tl*, fig. 10, Plate 15, has its outer margins well defined, but the individual cells are not clearly differentiated. The lightly staining nuclei, *tn*, are peripheral and lie each in a small area of clear cytoplasm. The rest of the cytoplasm is filled with spherical secretion granules, *bg*, ranging in diameter from 3 μ to very small dimensions. As a rule, the larger granules appear ebony black after iron hæmatoxylin, whilst the smaller stain less intensely. The secretory material is aggregated towards the gland lumen. An irregular group of deeply staining granules situated in the centre of each gland is often the only indication of the position of the gland lumen.

Where the glands are observed, under low magnification, to change from the coarsely granular type to a more homogeneous dark brown, the appearance of the epithelium, under high power, is strikingly different from that just described. The boundaries of the individual tubular glands are obscured, except where a capillary or a mass of connective tissue intervenes. Few cellular details are apparent (fig. 11, Plate 15, where glands, separated in sectioning, are selected to emphasize these details). The nucleus, *tn*, is invariably peripheral in each cell. It is shrunken and stains uniformly black. The clear surrounding zone of cytoplasm, found in the previous type of gland, is absent. The entire cytoplasm is occupied by dark brown granules, *d*, not always spherical in shape and lying within vacuolar spaces of slightly greater size. Between the vacuoles are fragments of deeply staining cytoplasmic reticulum. But, although these glands appear, under low magnification, to be heavily stained throughout, the secretory granules do not stain uniformly ; a few appear ebony black, others are almost unstained. In the gland lumina there is generally an abundant secretion which dilates them. Large lakes of albumen indicate an exceptional enlargement of the glands as they lead to the surface of the oviducal lining.

In the still lighter staining areas of the folds, which occur more frequently towards their tips, during active secretion, each gland is clearly defined and the limits of its individual cells are much more evident. The latter consist merely of a cytoplasmic network, *cy*, containing a shrunken nucleus, fig. 12, Plate 15. The albumen masses, *ad*, within the cytoplasm are pale and at times are clearly distinguishable only by the vacuoles in which they lie or by slight variations in their density.

In anticipation of the discussion which is to be given later (p. 180) on the functional significance of these three phases in the tubular glands of the albumen region, it may be stated here that :—

(1) The coarsely granular type of gland represents the resting albumen gland.
(2) Where the secretion is more homogeneous and dark brown in staining reaction, a phase of active secretion is occurring.
(3) The lighter staining secretion is present in the glands that have ceased secreting and are about to regenerate.

After extrusion from the epithelium, the albumen retains its staining characteristics. Where individual glands are separated by loose connective tissue, particularly those which lie immediately beneath the ciliated epithelium, a curious extravasation of albumen into the inter-tubular spaces was observed. The peripheral walls of the epithelial cells adjoining such spaces appear to break down, and so the secretion is released into them. This material, fused into relatively homogeneous masses, completely fills all the available space between the glands and included in it are to be seen plasma cells, fibroblasts, and capillaries. This phenomenon provides a striking demonstration of the excessive activity of the glands of the albumen region.

Immediately following a phase of active secretion, when the egg has passed to a lower level in the oviduct, low power observation shows that the peripheral glands have developed into the coarsely granular type of the storage phase, fig. 10, Plate 15. They lie beneath the epithelium, particularly near the tips of the folds. As the egg shell is completed, the albumen glands, in close proximity to the crypts, also undergo this transformation. In the core of each fold the glands become lightly staining during regeneration and are very clearly indicated by their black, shrunken nuclei. This change in the staining reaction of the peripheral glands is a most constant feature, whilst the transformation in the more deeply placed glands is variable in the individual folds of a single transverse section through the oviduct. Apparently the secretory rhythm in the glands of any fold is such that the peripheral glands are the first to enter the maximum secretory phase. As the egg is transferred to the uterus, these glands have reached the storage phase in preparation for the passage of a new ovum. The centrally placed glands appear to lag behind so that they are in the storage phase even when the new ovum is actually in the albumen region.

The extruded albumen lying in the crypts between the folds appears as homogeneous masses. Possibly owing to physical or chemical changes which take place in the secretion as it flows towards the oviducal lumen, the staining reactions of the

albumen are variable. In the deepest parts of the crypts, it stains intensely, but in their upper parts it appears as a light grey material. As it exudes from the glands, the albumen forms a continuous stream or a series of droplets which later coalesce, fig. 14, Plate 16. Dense mucous-like strings occur in the lighter staining albumen in the crypts, either situated in apposition with the cilia of the epithelial cells or completely surrounded by albumen. These strings do not occur near the apertures of the tubular glands, but are closely associated with the lining epithelium.

(*b*) *The tubular glands during shell formation and during rest, after egg-laying*—The presence of albumen in the crypts and glands of the albumen region, during shell formation, indicates that the secretion is still produced in quantities that are far from negligible. At this time the peripheral glands are invariably granular in type and serve as reliable indicators of the completion of a maximum secretory phase, at least several hours previously. In the resting state, prior to the recommencement of a fresh cycle of egg-formation, the majority of the glands in the folds stain lightly, since the gland epithelium is in active process of regeneration. In this process, the nuclei enlarge and the adjacent cytoplasm reorganizes about the residual secretory granules, which never entirely disappear, *tn*^{2}, *rl*, fig. 13, Plate 15. It is doubtful whether complete regeneration is effected in all glands before a fresh secretory phase is reached. The deeper glands appear to regenerate as the peripheral ones are secreting, suggesting clearly that the albumen region has glands always in the process of secreting, though the majority undergo maximum activity only when the ovum is actually within the albumen region of the oviduct.

(*c*) *The ciliated epithelium during active secretion.*—Ciliated and mucin secreting cells can be distinguished throughout all stages in the albumen region. Owing to the great expansion of the tubular glands, the epithelium covering the folds is stretched in varying degrees so that measurements of the cell-height may not be entirely reliable. In comparison, the isthmal and uterine regions are lined with a more stable epithelium. In the upper part of the albumen region, during active secretion, the epithelium is reduced almost to 10 μ in thickness but, before the middle of this region is reached, a gradual thickening commences, reaching to a maximum of 25 μ in the mucous region. This is retained as the average height of the isthmal epithelial cells. In addition, areas of the albumen region appear to regenerate during the secretory cycle, while others remain disrupted and the process of regeneration involves an increase in the height of the individual cells.

The glandular cells of the epithelium may be described as goblet cells because in all essential cytological details of their secretory processes, they resemble the latter. But the great numbers of these cells in an active epithelium and the cellular distortion occurring during maximum secretion of the tubular glands, make the characteristic goblet shape often indistinguishable. The cranial part of the albumen region, with turgid albumen glands, may have an epithelium so stretched that its cells are cubical, with centrally situated nuclei, fig. 10, Plate 15. Here, almost every cell may be

ciliated and the development of goblet cells is scanty. In the epithelium of the albumen region, alternate cells are not necessarily involved in the transformation to a secretory type, and adjacent cells may retain their cilia or they may collectively become goblet cells. In this latter case, the cilia are cast off and the nuclei move towards the basement membrane. The alveolar cytoplasm, containing mucigen granules, sheds its apical cell membrane and the nucleus finally shrinks and stains deeply as the secretion commences, *gn*, fig. 6, Plate 14.

The process of regeneration of the goblet cells has a definite sequence which is similar to that in the tubular gland epithelium. It commences with the reorganization of the nucleus which, as it swells, becomes surrounded by newly organized cytoplasm. The epithelium may increase in height slightly and, as more cytoplasm is regenerated apically, the ciliated cells regain their columnar form, figs. 7, 8, and 9, Plate 14. Finally the apical cell walls of the goblet type regenerate and cilia reappear in most instances. This regeneration of the epithelium begins usually at the tip of each fold. The epithelium lining the crypts regenerates at a later interval.

A close harmony between the secretory and resting phases of the ciliated epithelium, and of the tubular glands beneath it, has been observed. Where the gland epithelium shows active secretion, the ciliated epithelium is producing mucin and regeneration follows simultaneously in both. In figs. 7, 8, and 9, Plate 14, it may be noted that the tubular glands contain dark granular albumen, *bg*, fig. 9, typical of the resting phase, only when the ciliated epithelium is approaching the end point of regeneration.

A small caudal section of the albumen region, at least 3 cm in length, has been referred to as the mucous region. In this portion of the oviduct, the tubular albumen glands cease activity before the lining epithelium regenerates. The crypts do not contain albumen when the goblet cells appear to reach their phase of maximum secretion. It is interesting to note that the degree of mucous production in this special region, immediately cranial to the isthmus, probably exceeds that in any other higher Vertebrate epithelium. After fixation in 5% mercuric chloride, the mucin may be preserved exuding between the ciliated cells in great masses, fig. 21, Plate 17 in which state the ciliated cells undergo great compression.

As the epithelium of the albumen region, in general, ceases secreting, plasma cells pass through it in great abundance, from spaces beneath the basement membrane. In fact, during the resting phases of the oviduct, plasma cells increase in number throughout the connective tissue associated with the tubular glands. Close examination reveals nests of these cells, which are absent when the glands are active and the connective tissue spaces are diminished. In several instances, plasma cells have been found between the bases of ciliated epithelial cells (*pl*, fig. 9, Plate 14), and the possibility occurs that these cells, migrating through the epithelium, may be so crushed and distorted as to resemble lymphocytes, which are the cell-type usually described in connection with the similar mammalian mucous membranes (Bartlemez and Bensley (1932)).

4—*The Isthmus*, figs. 15–17, Plate 16, fig. 18, Plate 17, fig. 27, Plate 19.

(*a*) *The tubular glands during secretion*—Unlike the condition in the albumen region, the glandular epithelium in the isthmus does not undergo three distinct secretory phases and it never appears to reach the same excessive degree of secretion. Under low power, the isthmal tubular glands are remarkably homogeneous in staining, during both activity and rest. The peripheral glands are not noticeably different from those in other regions of the same fold.

In the isthmus, the tubular glands may be identified, even during the resting phase, as different from those of the albumen region, unless mercuric chloride-acetic acid fixation has been used. With the latter fixative, the albumen and ovokeratin secretions may be so coarsely precipitated that they lose their distinctive staining reactions. The following account is based upon material fixed with Zenker-formol.

As the shell membrane is secreted by the isthmus, the gland epithelium shows maximum activity. Each epithelial cell becomes crowded with spherical, deeply staining granules of variable size, *go*, figs. 15 and 16, Plate 16. The gland lumina are filled with similar granules or with fused secretory masses, which appear to migrate unchanged towards the lumen of the oviduct ; but it is difficult to trace the secretion beyond the entrance of the gland ducts or within the deeper recesses of the crypts. Small rod-like or granular, deeply staining masses are only occasionally preserved in the isthmal lumen. Although each epithelial cell becomes crowded with secretion, there is no marked compression of its nucleus. The nucleus is clearly defined, apart from instances where granules may lie above it in the section. The cytoplasm nowhere reaches an intensely alveolar stage with the secretion contained in vacuoles, nor are the nuclei shrunken or deeply staining. These latter conditions are found only in the albumen region.

During rest, all the glands stain evenly, whilst a reduction in the number of their granules leads to the appearance of clear cytoplasmic areas, which give the whole section a more uniformly grey appearance, fig. 18, Plate 17. Apart from these changes the tubular glands remain strikingly similar in each individual fold whether they are in the active or the quiescent phases. Unlike the albumen region, it is difficult to estimate the exact secretory stage of an isthmus from stained sections, unless information on the state of the oviduct in general is available.

In the isthmus, the greatest interest lies in the manner in which the secretion of the tubular glands enters the oviducal lumen, since this secretion is undoubtedly the ovokeratin material forming the fibrous, two-layered shell membrane. In the free state the secretion retains the same staining properties, as when originally formed in the epithelial cytoplasm, and it does not appear to flow out of the tubular glands in large droplets or as deeply staining masses to fill available spaces in the crypts. There is no staining evidence of physical or chemical changes during its formation. The secretion exudes in the form of fine granules which coalesce into threads or it may form twisted fibre-like strands immediately it leaves the glands, *z*, figs. 15 and 16, Plate 16. These fibres project from the glands into the interior of the oviduct

and, after staining with iron hæmatoxylin, resemble black bristles inserted in the ducts.

During fixation, the secretion appears to remain undistorted, and it exhibits no visible variation with all the fluids used in this investigation (*cf.* the albumen). These facts, taken in conjunction with the viscosity of the secretion in the free state, suggest that the secretion is quite different from albumen. It must be noted, however, that the appearance of the glands at any secretory stage in the isthmus is closely similar, after Zenker-formol fixation, to the single resting phase of the albumen glands, *cf.* fig. 15, Plate 16 with fig. 10, Plate 15. The identification of the isthmal glands as albumen glands, by several previous authors, is possibly due to a confusion of these individual stages.

(*b*) *The ciliated epithelium during secretion*—The individual isthmal folds have a less evenly rounded form in section, which is possibly due to the fact that the tubular glands of this region do not expand to the same extent as in the albumen region. The tips of the folds are characteristically angular, and the apertures of the tubular glands, situated in marked depressions of the ciliated epithelium, are very much more numerous than in other regions of the oviduct. The crypts are generally entirely open so that their deeper recesses are rarely obscured by close contact between adjacent folds. Some of the cells in the lining epithelium remain ciliated and others transform into gland cells which are peculiar to this region. Under low power, however, no stretching or extreme mucous activity in the epithelium, is noticeable, so that the columnar cells are uniformly higher (25 μ) than they are in the albumen region. The ciliated cells retain their form throughout the secretory cycle of the isthmus, being unconstricted by adjacent glandular cells. Their nuclei are usually apical in position, figs. 15 and 17, Plate 16.

Just before an egg reaches the isthmus, the cells with basal nuclei lose their cilia and the cytoplasm becomes faintly alveolar. It stains a cream colour with iron hæmatoxylin as compared with the clear grey cytoplasm of the ciliated type. All the preliminary stages in goblet cell formation, entailing nuclear shrinkage and the destruction of the apical cell membrane, ensue; but the cell never becomes a typical goblet cell in contour or in the final phases of its secretory cycle. In the cytoplasm a viscous secretion collects, which is unaffected by Zenker-formol, Regaud, or alcohol fixation. Following the formation of this secretion, conspicuous blebs, which alternate between the groups of cilia, arise on the apical margins of the gland cells, *bl*, fig. 17, Plate 16. Yet on reaching this stage, the secretion appears, from fixed material, to remain intact and there is no evidence that it accumulates in fused masses or as threads entangled in the cilia. It is likely that the secretion, with its positive reactions to mucin stains, is progressively withdrawn from the cytoplasm, possibly by an instantaneous liquefaction on the surface of the bleb. During the maximum secretory stage, vacuoles, *v*, form on the apical and basal aspects of the bleb cell nucleus, with a resulting reduction in the volume of the secretion, *v*, fig. 17, Plate 16. Later, the nucleus enlarges and a gradual reduction in the

secretion follows. The glandular cycle of this cell type is completed by the atrophy of the blebs and the reappearance of the cilia. Regeneration is nowhere so complete, however, as in the albumen region. Certain cells remain in the glandular phase, with blebs, between shell membrane forming cycles, and several days after the cessation of egg-laying, these characteristic cells are still numerous. The vacuoles may contain deeply staining secretory masses.

5—*The Isthmo-Uterine Junction*, fig. 20, Plate 17.

(*a*) *The tubular glands*—The curious histological structure of the oviducal wall in the isthmo-uterine junction has not been widely investigated and BRADLEY (1928) was the first to describe it in detail. It should be emphasized, in the first place, that there is no abrupt discontinuity between the glands of the isthmus and those of the junctional region, such as occurs between the glands of the isthmus and the albumen region. Instead, a gradual change takes place in the corium from the typical isthmal glands to the lightly staining glands of the uterus, so that a single fold in the isthmo-uterine junction generally contains a mixed collection of glands. This arrangement does not consist, however, of typical isthmal glands intermingled only with the uterine type. A third variety of gland appears in the junctional region which requires detailed description.

These special glands, fig. 20, Plate 17, are closely packed in groups between the isthmal and uterine types, but they are readily distinguished by several marked cytological characters. The epithelial cytoplasm is paler than in the isthmal type and it contains granules, *gs*, which have similar staining reactions to those in the isthmal glands. The granules, however, are never so abundant and they vary in numbers from several to a dozen or so in each cell. In the present material, the gland epithelium of the isthmo-uterine junction in the active or quiescent state never appears to be completely filled with secretion as it may be in the albumen region or isthmus. A characteristic vacuolization of the cytoplasm, *ve*, suggesting a disruption during fixation, is invariably present. This phenomenon is also encountered, to a lesser degree, in the uterine glands, and it has been interpreted as a fixation effect caused by the fluid nature of the secretion. Finally, a marked thickening of the cell membranes in the special junctional glands stands in contrast with the thin-walled uterine epithelium. No indication exists that this special type of gland may undergo a phase of secretion storage similar to that in the isthmus. The sparse granulation and intensely vacuolated cytoplasm of the junctional epithelial cells are indeed sufficient evidence for regarding them as a distinct variety of gland.

(*b*) *The ciliated epithelium*—Even in longitudinal section, no change in the appearance of the ciliated epithelium of the junctional region, as a recognizable transition from the isthmal to the uterine type, is perceptible in the present material, though the average cell height, over 30 μ, is greater than in preceding regions of the oviduct. In most features, the epithelium does not change from the isthmal type. It possesses the bleb-like mucous cells and ciliated cells in the same abundance,

fig. 20, Plate 17. The appearance of masses of fine granules in the apical cytoplasm of some of the ciliated cells and an occasional hypertrophy of this cell type, leading to compression of the mucous cells, are the only significant changes to be recorded. At intervals, a reduction in the height of the junctional epithelium may be noted, particularly where the underlying glands are uterine in character ; but to state that groups of isthmal or of uterine glands are covered by their own type of epithelium, seems unwarranted in the present material. Vacuolation is common in the blebbed cells on both aspects of the basal nucleus, fig. 20, Plate 17. In the ciliated cells, vacuoles may occur in the area usually occupied by the granules.

6—*The Uterus*, fig. 19, Plate 17 ; Plate 18 ; Plate 19, figs. 26, 28, 29 ; Plate 20, and Plate 21

Oviducts have been selected containing an egg in the isthmus, with the uterus therefore in a pre-secretion phase ; with an egg in the uterus, showing a faint deposit of calcium on the completed shell membranes ; with eggs in various stages of calcium deposition, indicated by the complete flexibility, or rigidity of the shell. In addition, stages were taken in which the egg was either retained for several hours, up to one day, after completion, or had been laid some hours previously. A description will first be given of the glandular tissues from material fixed in small pieces apart from the surface of the egg.

(*a*) *The tubular glands*—Despite the absence of a discontinuity between the glands of the isthmus and uterus, those in the latter region are widely dissimilar in their cellular structure from any so far described, fig. 19, Plate 17. They are noticeably smaller in diameter. Closely packed together in the active state, with hypertrophied capillaries and a greatly reduced connective tissue stroma between them, the boundaries of the glands are recognized with difficulty. The epithelium, in transverse section, consists of five to seven or more polygonal cells, enclosing a central lumen which is invariably patent and empty. The cytoplasm stains a pale grey and is filled with lightly staining granules. Some of these granules may be imperfectly fixed mitochondria in the Zenker-formol material. In the Schridde preparations, the mitochondria are filamentous and so abundant that few other cytoplasmic details can be distinguished. A relatively large nucleus lies towards the cell base, *tn*, fig. 19, Plate 17.

During the maximum secretory phase, the cytoplasm remains relatively homogeneous but, following the completion of the egg shell, when the epithelium is exhausted, the latter is difficult to preserve. It seems evident that the uterine gland cytoplasm is partly fluid owing to the large irregular spaces which develop in it after all types of fixation. In Schridde preparations, this disruption is less pronounced and sharper granule differentiation is obtained. A slight hypertrophy of the epithelium is noticeable during the maximum secretory phase.

The lack of cytological phenomena, clearly indicating the pre-secretion, secretion, and storage stages in the uterine glands, and the difficulty in demonstrating the

granular antecedents of the secretion in the epithelium and tubule lumina, suggest that the secretion is a thin fluid. The curious reaction to the fixative implies that substances, which are greatly altered by protein precipitants, are contained in the secretion as it rests in the epithelial cytoplasm.

(*b*) *The ciliated epithelium*—Unlike the albumen region, the uterus is lined by a relatively even epithelium, approximately 30 μ in height, and the cilia are shed to a lesser degree throughout secretory activity. For the most part the epithelium consists of a single layer of cells, with alternating apical and basal nuclei. The cell with the basal nucleus possesses a very reduced apical cytoplasm which, in some instances, is difficult to demonstrate as reaching to the internal surface of the uterine wall. These two cell-types will be referred to as apical and basal cells. Occasionally the basal cells proliferate and produce additional cellular layers against the basement membrane, but such instances of pseudo-stratification are rare. Under moderate magnification, three principal phases in the epithelium can be distinguished with sufficient regularity to justify the assumption that they are indicative of separate states of activity.

(i) *Granule formation in the apical ciliated cells*—The appearance of numerous granules (after fixation in Zenker-formol, *gv*, fig. 29, Plate 19 ; alcohol-formalin, *gv*, fig. 28, Plate 19 ; Regaud and Schridde, and staining with iron hæmatoxylin), lying between the cilia and the nucleus, or scattered more irregularly throughout the cell, coincides with the onset of shell matrix secretion. Sections from oviducts, containing a uterine egg with incomplete shell formation, are never without these granules, whereas material taken several hours after egg-laying or while the egg is in the albumen region, contains apical cells devoid of granules. No granules have been noted in a specimen which atypically retained a completed egg until a second egg had commenced shell membrane formation.

The granules usually lie in the cytoplasm adjacent to the Golgi apparatus or between it and the nucleus. After Da Fano silver impregnation, the granules stain clearly with iron hæmatoxylin, *gv*, fig. 31A, Plate 20. During active shell secretion, they may fill the cytoplasm and extend to the ciliated cell membrane. Here, they appear to pass through the latter and to accumulate as a granular material entangled in the cilia, *gv* and *gx*, fig. 28, Plate 19. The granules are variable in size and frequently clump together as they leave the cell. The basal epithelial cells do not possess these granules during shell secretion. In fact, the epithelium as a whole consists of ciliated cells with an apical nucleus, about which deeply staining granules are grouped, alternating with basal cells, which appear to be in a state of rest. An interesting example of excessive secretory activity in the apical cells is illustrated in fig. 34, Plate 21. The darkly staining nuclei serve to mark out an area, *ep*, as a distinct patch amongst the more normal epithelial cells.

(ii) *Vacuole formation in the basal cells*—After completion of the calcification of the egg shell and the disappearance of secretory phenomena in the apical cells, the basal cells undergo characteristic changes. They enlarge and partly compress

the apical cells lying between them. Large vacuoles appear above or below the nuclei, *bcv*, fig. 36, Plate 21, resembling the condition described in the isthmal epithelium. In most instances two vacuoles occur in each cell, so that the nucleus is left more or less isolated from the rest of the cytoplasm. The absence of a vacuole, in a position apical to the basal nucleus, is rare, though individual vacuoles vary considerably in size.

On the superficial aspect of the apical vacuole, a clumped mass of deeply staining granules may arise. These occupy the area which contains a reduced Golgi apparatus in silver impregnations. The granules may be seen also to disperse throughout the cytoplasm and to extend as far as the ciliated cell membrane. During this transformation, the cell remains ciliated, but stages may be distinguished where the cilia become reduced to short tufts, alternating with the long, fully regenerated cilia of the apical cells. Specimens taken from fowls, which had retained an egg for more than eight hours after the shell had been completed, showed these vacuoles fully developed.

The granular material was still present in the epithelium several days after the last egg-laying, forming a conspicuous darkly staining mass closely similar to the basophil material found by BARTLEMEZ and BENSLEY (1932) in the human uterine gland epithelium.

(iii) *Mucous secretion from basal cells*—Over a limited area immediately related to the isthmo-uterine junction, but covering folds of typical uterine corium, the uterine epithelium contains mucous cells typical of the isthmal and junctional regions. Apart from this area, it has never been possible to trace the activity of the basal cells of the entire uterine epithelium as ending in a mucous secretory phase with the formation of bleb cells. A production of mucin from the areas of the uterine wall surrounding the isthmal and vaginal apertures seems to be sufficient for the lubrication of the egg surface during movement within the uterine lumen.

From the present material it would appear that the maximum secretory stage of the basal cells in the cranial part of the uterus coincides with the passage of the egg from the isthmus into this region. As will be noted later, the cells providing a lubricating secretion for the completed egg, in its movements during egg-laying, are arranged in tubular glands in the more cranially situated vaginal folds. It is possible, therefore, to interpret the blebbed mucous cells of the isthmus, the isthmo-uterine junction and the cranial end of the uterus, as producing a secretion of a more fluid character, specially adapted to the surface texture of the naked shell membranes.

The three stages of secretory activity in the uterine epithelium will now be correlated with other phenomena which are visible when the uterine wall has been fixed and sectioned in close contact with the egg envelopes.

7—*Changes in the Uterine Mucosa in Contact with the Egg Surface, studied* in situ

Making allowances for shrinkage during fixation, decalcification, and embedding, there appears to be every reason to suppose that normally the uterine mucosa lies

always in intimate contact with the developing shell during the secretory phases, apart from transient periods when muscular activity may lead to a reorientation of different areas of the uterine epithelium in relation to the surface of the membrane. It is a matter of common observation that the oviduct undergoes great distension as the egg increases in size and, in the uterus particularly, the musculature contracts in a most remarkable way when the uterine wall is cut and the egg is released from the lumen. In his explanation of the ovoid form of the hen's egg, D'ARCY THOMPSON (1917) assumes that muscular tension and peristaltic contraction are the principal factors in determining its shape, though the details of his theory are limited by the fact that he was not aware that the egg proceeds through the oviduct with its pointed end foremost, and then undergoes rotation within the uterus just before laying, so that the blunt end is finally directed caudalwards.

In all the material fixed with an egg *in situ* and not injected with fixative through the uterine wall, areas in each section may be found where the larger uterine folds are flattened into intimate contact with the surface of the egg, figs. 32 and 33, Plate 20. A specimen was fortunately obtained in which the first stages of shell-matrix deposition had begun and others have been studied in which the shell-matrix had advanced to the final stage of shell-cuticle formation, fig. 36, Plate 21.

(*a*) *The secretion of Mammillæ*—As soon as the egg enters the uterus, important modifications of the uterine folds take place. The largest of them become bent at various angles so that extensive areas of their epithelium are pressed into close apposition with the shell membrane. This contact surface is interrupted only by the relatively few apertures of the tubular glands and at varying intervals by the spaces existing between individual folds, fig. 32, Plate 20. The shell membrane then lies in close relation with modified patches of uterine epithelium which are discontinuous, and it must be assumed that the muscular contractions of the uterine wall, resulting in rotatory movement of the egg itself, would lead to a rhythmic alteration in the shape of the flattened uterine folds. Finally a stage would be reached in which the shell membrane had been in contact with areas of the uterine epithelium for a period sufficient for the even deposition of secretions on its entire surface.

Detailed investigation of the uterine epithelium lying in contact with the shell membrane reveals the development in it of small pit-like areas, each roofing over a small, dense, ovoid body which stains rather heavily with eosin, *ma*, fig. 32, Plate 20. These bodies are easily identified as the mammillæ of the shell matrix, owing to their shape and their concentrically layered structure. The fact that the mammillæ lie partly embedded in the peripheral fibres of the outer shell membrane is strongly in favour of the contention that close contact, under pressure, between the uterine epithelium and the shell membrane, is the normal relationship between these structures. Owing to this pressure and the intense secretory activity of the epithelial cells at this stage, there is considerable alteration in the appearance of the ciliated epithelium in the areas of contact, *ep*, fig. 32, Plate 20, as compared

with the remainder of the epithelium lining the crypts and spaces between the smaller and larger folds.

The individual epithelial cells of the contact areas become darkly staining and distorted to such a degree that the normal, simple columnar character of the inactive uterine epithelium is often lost. The apical and basal nuclei stain intensely and the cytoplasm is both granular and disrupted, particularly in the small pits surrounding the mammillæ. But, owing to the poor fixation which results from the preservation of an entire uterus with an egg *in situ*, by infiltration of the fixative through the muscle layers to the internal glandular tissues, it is not possible to study the cytological details associated with the secretion of the mammillæ from the epithelial pits.

Viewing the lining epithelium of the uterus as a whole, the modified epithelial areas in contact with the egg are seen to be abruptly discontinuous on the surface of the larger folds. In other words, each large fold is covered proximally, near its point of continuity with the general uterine wall, by simple epithelium containing apical and basal cells, and, distally, the epithelium changes suddenly to the compressed type which covers all portions of the fold actually in contact with the egg as well as parts of the fold closely related to these contact areas, fig. 32, Plate 20. During egg-shell formation, often at least one-third of the total length of the folds, in the expanded state, is covered with compressed, heavily staining epithelium. The rest of the uterine epithelium shows apical cells with granules and basal cells. These form the entire covering of the shorter folds which do not come into contact with the egg. A detailed cytological description of the simple apical and basal epithelial cells, has already been given (*ante* p. 165).

(*b*) *Secretion of the organic shell matrix*—Following the deposition of mammillæ in the shell membrane, a new type of secretion appears in the uterine lumen in the form of rounded masses which stain particularly brilliantly with acid fuchsin and remain a dark, purplish blue in sections stained with Delafield's hæmatoxylin and eosin. With such staining reactions, it is impossible to confuse this secretion with any other material derived from the entire oviducal wall. The secretion is clearly denser than albumen though not so viscous as ovokeratin, which in any case is strongly eosinophilic. From the position of the secretory masses, which vary in size from small globules to bodies 40 μ in diameter, they would appear to be derived from the free uterine mucous membrane and not particularly, if at all, from the modified epithelial areas on the flattened folds. In fig. 35, Plate 21, a mass of this secretion is shown lying in a depression of the epithelium covering a short fold some distance from the actual uterine lumen. It will be noted that the epithelium in contact with the secretion is characteristically simple in structure ; not modified as in the case of the epithelium which secretes the mammillæ. If the apical cells are studied in detail (not shown at the magnification of fig. 35), the granules lying between the cuticular border and the nuclei appear closely similar in composition to the free secretion and, since no such secretion appears to be formed in the tubular glands or is extruded from them at their apertures into the uterine lumen, the only

interpretation possible is that here we have the secretion of the apical cells (*ante* p. 15) in free form in the uterine lumen. In a later part of this communication, this secretion will be shown to possess a heavy inorganic content (*vide* p. 173 and figs. 24 and 25, Plate 18). In certain areas of the section where the mammillæ are completely differentiated, the slightly less dense secretion of the apical cells of the uterine epithelium may now be seen to have migrated to a position in between the flattened folds and the surface of the shell membrane. In fig. 33, Plate 20, a compact mass of this secretion, *sm*, is shown pressed against the shell membrane in indented areas of the darkly staining epithelium. There is no difficulty in distinguishing the new material from the mammillæ, owing to the differences in staining properties, size, and general microscopical texture. The photomicrograph illustrates also the slightly granular yet homogeneous staining of the secretion as compared with the albumen and mammillæ in the same section. By a process of condensation, this material appears to be organized about the mammillæ and on the free surface of the shell membrane, to form a characteristic new layer of the egg envelopes, which is obviously the shell matrix. The shell at this stage is not calcified to the extent of brittleness or rigidity. With the material available it is not possible to trace the increasing development of this secretion, but it is present in large amounts in the uterine spaces in specimens containing an egg with a completely calcified shell and an intact cuticular layer.

(*c*) *Final stages in egg shell formation following calcification of the matrix*—In material which has been decalcified, it is very difficult to preserve intimate contact between the uterine epithelium and the shell surface. This is due to shrinkage in preparation and to the lack of any secretory material which would form a delicate cementing substance between the uterine tissues and the polished, relatively impermeable shell. After decalcification the shell matrix stains deeply with hæmatoxylin and is rather fibrous in appearance. The mammillæ become strongly eosinophilic.

Of more importance, however, is the striking recovery of the ciliated epithelium in contact with the shell. Having regained its more normal, lighter staining reaction and the strictly apical and basal arrangement of its nuclei, evidence is now shown of special secretory activity. A phase, which has already been described in detail as vacuole formation in the basal cells (*ante* p. 165), commences.

Coincident with the appearance of vacuoles and deeply staining basophilic secretion within the basal cell cytoplasm, a distinct aggregation of granular masses, *sc*, fig. 36, Plate 21, outside the epithelium leads to the formation of a dense, thin layer on the surface of the shell matrix. This material stains more intensely than any other secretion derived from the uterine wall. It forms what can only be regarded as the cuticle of the egg shell. In this instance also, the vacuolated epithelial cells can be distinguished only over the " contact " areas of the flattened uterine folds. It must be emphasized, however, that shell matrix globules, which stain less densely than the cuticular granules, are still present throughout the uterine spaces and, in the absence of fine cytological detail, it is not possible to decide

conclusively that the final layer of the shell is the product only of the vacuolated basal cells. Residual shell matrix secretion may take some part in the formation of the cuticle but this is unlikely.

8—*The Inorganic Structure of the Uterine Epithelium and Corium following Microincineration*

It seems advisable for certain general observations, made by the author on the microincineration technique, to be recorded, with the addition of the opinions of previous workers, before reference is made to the results and conclusions reached from the study of incinerated sections of the special tissues with which we are here concerned.

At the present time it cannot be maintained that real chemical accuracy is possible with this method, though the ultimate aims of histologists, in using microincineration, appear to lie in the direction of making inorganic analyses of individual cells and tissues. To take an individual cell and to hope to define the exact regions of the cytoplasm where certain inorganic elements are located after incineration is not to be expected ; only approximate results on a few of the inorganic constituents of protoplasm can be obtained. The ease with which ions may migrate from one portion of a cell to another during any type of histological fixation, except possibly with the freezing-drying procedure, is sufficient to justify extreme caution.

After incineration, the identification of individual ions in the ash rests, at present, on a few simple chemical tests and a series of assumptions based upon the colour of the ash deposit as seen principally by dark ground illumination. Chemically speaking, the colour values indicate little apart from the fact that thick clumps of ash, containing calcium, are likely to be whitish in appearance and may be mixed with traces of magnesium, silicon, and aluminium. Relatively large concentrations of iron may impart a reddish hue of iron oxide to ash in which it is mixed. This iron can be seen readily in the developing nervous system of the chick, as described by Horning and Scott (1932). In individual cells, particularly in erythrocytes, the iron can be detected with less certainty, though it undoubtedly gives a yellow-reddish coloration in the ash of clumped masses of blood cells.

Cowdry (1933) in reference to a paper by Mason, qualifies the opinion that sodium may be identified " by its bluish-white sheen " as being probably caused by physical and not chemical properties. Deposits of ash, of known chemical composition, which may be obtained by evaporating a solution of Ca_3PO_4 on a glass slide, followed by incineration, show a gradation in colour from blue to bluish-white and even to opaque white ; a phenomenon obviously depending upon the amount of ash present in any particular area of the preparation. Sufficiently finely deposited KH_2PO_4 will yield even a prussian blue coloration, under dark ground illumination, after incineration. It may be argued that results obtained in this manner should not be compared with those from incinerated tissue sections, but they serve to emphasize that only strictly chemical reactions may be used to identify elements such as sodium which, in finely divided ash, may show varying coloration, not clearly distinguishable optically from other inorganic substances.

The application of reagents giving specific reactions with such ions as calcium, iron, and sodium has not proved very helpful. One test alone seems reliable ; the application of dilute H_2SO_4 to the ash, resulting in the formation of gypsum crystals. This can be applied to a microscopic area of ash with a micropipette, but the technical difficulties of confining the reaction to the ash of a single cell are evident. One cannot apply the test to an area of intra-cellular ash and localize the calcium in any special region of the cell. The reagent tends to spread on the glass slide and to react with adjacent ash deposits. A thin coating of celloidin over the incinerated section may minimize the spreading of the reagent but, unfortunately, it completely obscures the microscopical structure of the ash in dark field. There appears no doubt, however, that the major portion of the white ash in incinerated sections consists of calcium salts mixed with other oxides in rarer concentration.

Apart from this one reagent, no success has been obtained with standard microchemical reactions applied to ash deposits. It must be emphasized that, to preserve clear cytological detail, it is essential to use sections not more than 5 μ in thickness. Only broad cellular layers are well defined in the ash of thicker sections. MOREAU (1931) has estimated the approximate amount of calcium present in a section of tissue (kidney or liver), 0·02 mm thick and 1 square centimetre in area, as probably 0·0002 mmg. A single cell in such a section has an ash content falling far outside the limits of reactions used in microchemistry ; or, if a reaction does take place with a microscopic amount of the reagent itself, the new compound formed is invisible. The gallo-formic reaction of Cretin, which gives a brilliant blue coloration in the presence of minute concentrations of calcium, is not successful when applied to the ash of cell groups except possibly in bone. The blue compound formed in this reaction is colourless in microscopic amounts. The sodium cobalti-nitrite test for potassium similarly breaks down with even large areas of ash.

In general, then, the only element which can be detected chemically in a mass of ash resulting from incineration of thin tissue sections is calcium. The localization of iron, by its colour, and of silica by its bi-refringence under polarized light, depend on the degree of mixing of these substances with the ash as a whole. Iron, in a finely divided form, does not appear to be detectable by its colour, if mixed with a large concentration of calcium and other substances.

The process of incineration itself can be controlled only by aiming at preparations with a maximum of cell detail in the final ash. The electric quartz oven, originally designed by POLICARD, consisting of a cylindrical quartz tube surrounded by a heating element, controlled by a rheostat, can be shown to vary in temperature from the central regions of the tube, outwards to each open end, where the temperature is much lower owing to atmospheric contact. Tests were made with the oven used in this investigation, by means of a thermocouple, and the temperature recorded at different points along the length of the tube, while in a steady state of 690° C at its central point, for which a current of 2·72 amp was necessary. A graph of these temperatures indicated that variations in temperature existed between 485°C, 2 cm from one end to the central point, and that these fluctuations were not strictly symmetrical, owing to irregularities in the winding of the heating element and variations in the physical nature of the quartz.* Recently, HORNING (1934) has figured an improved incinerator, in which the tube is both longer and narrower than in previous types. A more even distribution of heat results and, from his illustrations and personal examination of his preparations, much finer structure in the ash has been obtained.

Theoretically the most perfect incineration is that effected by raising the temperature between the final charring and final oxidation phase no higher than is absolutely necessary to complete the process. The existing technique does not allow the investigator to determine the exact point of complete oxidation or to control the temperature by time periods as an indication of final oxidation. The only criteria for determining successful incineration are the production of ash showing clear cytological structure, involving cell walls, nuclear membranes, and other characteristic cell details. The absence of traces of unoxidized material, as seen by direct illumination, and the solubility or removability of the ash from the slide by common reagents, indicating the absence of fusion with the glass, complete these conditions.

A further factor influencing successful incineration from a cytological standpoint, is the thickness of the original section. Even with thin sections, it is obvious that slight variations in actual thickness occur, both in different regions of the same section and in adjacent sections in a paraffin ribbon. These are due to the varying conditions surrounding microtome sectioning and, though not apparent with direct illumination in a stained preparation, they are very noticeable after incineration. Any quantitative work leading to the estimation of the absolute amount of ash in different cells, made by taking its relative distribution, will depend on the assumption that the section has been cut evenly to

* I am indebted to Dr. G. BRIGGS, Department of Physics, University of Sydney, for kindly conducting these tests.

a specified thickness. Finally it has been found that, in floating out the sections in absolute ethyl or methyl alcohol, greater cytological detail results if the alcohol is allowed to come in contact with both sides of the paraffin ribbon. The explanation of this phenomenon is unknown. It probably occurs accidentally in the technique of most workers owing to the ease with which absolute alcohol penetrates through small cracks and tears in the ribbon to its upper surface. The alcohol does not appear to influence the distribution of the inorganic material, as the peripheral regions of tissue sections are still clearly defined without any obvious diffusion of salts into the clear spaces on the slide. Such a diffusion readily occurs if the sections are floated out with water.

Details of chemical analyses of the fowl egg-shell, tabulated by NEEDHAM (1931) from various authors and from the figures of LANGWORTHY, show that 93·7% is represented by $CaCO_3$ (calcite), 1·3% by $MgCO_3$, 0·8% by $Ca_3(PO_4)_2$, while organic matrix accounts for 4·2% of the total. It follows, then, that identification of the shell secretions of the uterus, involves, in part, the detection mainly of calcium salts with a small trace of magnesium. No microchemical tests are sufficiently delicate to locate the magnesium mixed with calcium in the ash of sectioned fowl uterus. In the following account the term " calcium " will be used conveniently to describe the products of incineration of $CaCO_3$ and $Ca_3(PO_4)_2$, with the additional qualification that traces of magnesium are likely to be present. Fortunately for this investigation, silicon and aluminium, which are found in small amounts in the ostrich egg, have not been identified in the hen's egg and presumably they are absent in the uterine secretions.

Portions of uterine wall were taken from a number of hens in different stages of egg production or rest, for fixation in the usual mixture of 9 parts absolute alcohol and 1 part commercial neutralized formalin. Sections were cut at 3 μ and 5 μ both for incineration (floated on the slide with absolute alcohol) and for controls stained with iron hæmatoxylin.

Where no egg formation had occurred for several days, though the oviduct was still unatrophied, an examination of incinerated sections was made to determine the mineral content of the uterine tissues not engaged in active calcium secretion. Sections 5 μ in thickness showed an even epithelial margin, *x*, fig. 22, Plate 18, with, here and there, a fine haze of ash representing the remains of cilia and cuticular margins which were too delicate to record in a photomicrograph. The ash of the cell walls was present at intervals, while the nuclei, *bn* and *an*, incinerated as rings of ash (probably the nuclear membrane and immediate periphery of the nucleoplasm) containing clumps of ash from the nuclear contents. The cytoplasm consisted of finely divided greyish ash, somewhat irregularly distributed. No ash deposit remained representing the connective tissue spaces, but the capillary endothelium was clearly defined and contained the remains of an occasional blood corpuscle. Singularly small traces of ash were found in the gland epithelium apart from the delicate cell walls and a heavy ash deposit in the nuclei.

The low power appearance, in dark ground illumination, is important in emphasizing that the ciliated epithelium in a non-secreting uterus contains more ash than the corium. This appears to be due to the relative cell crowding, since each gland cell in the corium covers a larger area in section than the individual cells

of the lining epithelium, fig. 22, Plate 18. The cytoplasm in the latter epithelium is homogeneous and denser as compared with that of the tubular glands. Thicker sections therefore increase the visible disparity in ash content between the two tissues, owing to the looser packing in the corium.

Material from fowls with an egg in the uterus and possessing a partly calcified and still pliable shell, was taken as indicating the phase of maximum calcium secretion. Incinerated sections of this material, figs. 23 and 24, Plate 18, showed a striking difference in the ash content. In the secretory state the ciliated epithelium stands out as a brilliant white band against the corium. It is possible accurately to identify the resting and actively secreting stages of the uterus merely by low power observation of incinerated sections. Under oil immersion the details of the epithelium are sharply defined to the extent of distinguishing cell walls, apical and basal nuclei, and the basement membrane, fig. 25, Plate 18. Occasional opaque white ridges on the surface of the epithelium appear to be artefacts due to section folding and clumping of the cilia. But, in addition, during active secretion there is a general increase in the epithelial cytoplasmic ash together with the new appearance of large, opaque granular deposits, *gv*. These are found generally in a supranuclear position and are confined to the apical cells, so that they are spaced in clumps at fairly regular intervals along the epithelium. There can be little doubt that these characteristic aggregations of ash, situated between the nuclei and the cilia, and present only in the epithelium during active shell secretion, are the remains of the special granules previously described in the ciliated cells after Zenker-formol fixation, *ante*, p. 165, *gv*, fig. 29, Plate 19. They are also present in the control sections after alcohol-formalin fixation, *gv*, fig. 28, Plate 19.

In the corium, a slight increase in whitish ash is noticeable in the tubular glands, fig. 23, Plate 18, fig. 26, Plate 19. This consists of a heavier deposit in the cell membranes and of greyish ash scattered in the cytoplasm. No special granular masses can be identified and, for the most part, the gland cytoplasm is ash free. Without the ciliated epithelium, it would not be easy to identify the secretory state of a uterus from the appearance of its tubular glands in incinerated sections.

9—*The Golgi Apparatus and other Cytoplasmic Phenomena in the Ciliated Oviducal Epithelium*

An examination of silver and osmium impregnations of the uterine epithelium, in particular, has been made in view of BRAMBELL's contention (1925) that secretory processes in the columnar cells possibly involve the extrusion of Golgi material from the apical cell cytoplasm, followed by its reformation in the basal cells. It has been impossible to confirm this phenomenon in the present material, which includes a large series of preparations, representing the entire secretory cycle in the uterus and isthmus. BOWEN (1926) in his discussion on the Golgi apparatus in gland tissues, refers, in a footnote, to the lack of thorough and critical evidence supporting the origin of the Golgi apparatus *de novo*, and points out that BRAMBELL's observations are at variance with well-established facts in other secretory cells.

In the first place, BRAMBELL assumes that all the epithelial cells are ciliated and similar in type throughout the uterine secretory cycle. He fails to distinguish the basal cells as passing through a short phase of special secretion (probably associated with cuticle formation) during which vacuoles and heavily staining pro-secretion granules appear in the cytoplasm. In an addendum to his paper, he refers to his disagreement with TURCHINI (1924) that two separate cell-types are present and, in fact, states elsewhere that the entire epithelium of the oviduct is made up of one type of cell which may degenerate and desquamate during the passage of the egg. More recently, BRADLEY (1928) has independently shown the existence of the non-ciliated gland cells in the cranial portion of the uterus, as elsewhere in the oviduct.

Quite apart from his non-recognition of the existence of two cell-types with distinct glandular phases in the uterine epithelium, BRAMBELL's view that the Golgi apparatus is extruded from the cell is difficult to confirm. He describes a progressive migration of the nucleus in the ciliated cells, from a central position to one near the cell membrane, followed, after extrusion of the Golgi apparatus, by a reversed migration of the nucleus towards the basement membrane, and thus he assumes that a form of kinetic energy is present in the nucleus. In most gland cells, quite apart from the fact that so-called nuclear migration is in an opposite direction to that of secretory elimination, it is more reasonable to interpret the movements of such cell-structures as occurring under the influence of an increase in volume of the pro-secretion products. GIROUD (1926), in discussing the variable position of the Golgi apparatus in gland cells, has shown that insufficient attention has been given to the mechanical effects of an increase in secretory material in the cytoplasm, on the disposition of associated cell structures. It is not legitimate, apart from observations on the living cell, to assume that such changes in position depend on an inherent motility of the cell-structure in question.

If, however, such migratory activity in the nuclei of the uterine epithelium really does occur, one would expect to find the nuclei widely scattered during secretion, occupying indeed any position between the apical and basal cell margins, according to the exact point in the process which each individual cell happened to be in at the time of fixation. Feulgen staining, which emphasizes nuclear position so clearly, demonstrates, on the contrary, that the majority of the cells can be accurately described as apical or basal (as previously defined, p. 165), in a thin section during secretory stages. In addition it is important to note that the apical nucleus rarely approaches the cilia within a distance less than its own diameter. There is always cytoplasm on its apical aspect, sufficient to contain the expanded Golgi apparatus and associated granules, without the appearance of undue crowding. In the atrophying uterus, after a cycle of egg-laying, the dissolution of the tubular glands leads to shrinkage of the ciliated epithelium and here the nuclei crowd together in a confused manner, the constant apical and basal polarity of the two cell types being lost only under these conditions.

The condition, described by BRAMBELL, entailing the appearance of argentophile deposits on the ciliated cell membrane, as in fig. 30, Plate 20, and outside it in

contact with the cilia, *z'*, has been observed occasionally, but it is not a constant feature of the secretory cycle. Almost any silver impregnation of the oviducal epithelium contains artefacts in the lumen, which appear to form in association with coagulated secretion or cell debris amongst the cilia. These occur at random and it would be impossible to trace them as the secretory products of any individual cell, even if they are derived from Golgi material. In the uterus the high concentration of calcium increases the conditions favourable to the formation of artefacts with silver nitrate, which is a well-known, though unreliable, reagent for calcium identification in tissues. With osmium impregnation one finds far less blackened material outside the cell, but, where it is present, one has still the difficulty of distinguishing between blackened secretion masses and supposed Golgi material. In such a position, on the exposed surface of a block of uterine wall, the ciliated epithelium and free secretions in contact with it are likely to show more variable reactions with osmium tetroxide than cells not in such free contact with the reagent (*e.g.*, in the corium).

The curious form of the Golgi apparatus, noted by BRAMBELL in the basal cells of the uterine epithelium, where it exists as a reduced compact mass, has been confirmed, *gb*, fig. 30, Plate 20. But variations in its position, not shown previously, are common. It may be subdivided into several bodies in contact with the nuclear membrane at its apical or basal poles, or it may take up a position mid-way between these extremes. The reduction in size of the Golgi apparatus in the basal cells, which alone is constant, is a common feature of other types of ciliated epithelium. An illustration by KOPSCH (1930) shows a reduced granule-like Golgi apparatus in human tracheal epithelium, and LUDFORD (1925) has described similar stages in the epididymis. In the fowl uterus it probably indicates a phase of inactivity of the basal cells, which have been shown to undergo a restricted secretory cycle leading to cuticle formation.

In the ciliated apical cells, the granules stainable with hæmatoxylin are revealed in Da Fano preparations, lying in close relationship with the Golgi apparatus or scattered towards the cell margin, *gv*, fig. 31A, Plate 20. Occasionally the network is associated with small vacuoles within its meshes, *vg*, fig. 31B, Plate 20, similar to those figured in the pancreas by GATENBY (1931) and others. It has been noted that the basal cells are more active in the isthmus during the entire egg-laying cycle than in the uterine epithelium. The hypertrophy of the Golgi material in these cells during secretion is followed by its reduction to a few granules or an elongate filament, only when the oviduct is functionally at rest and atrophied. Despite the excessive disruption of the cytoplasm in the goblet cells of the albumen region, and the severe distortion of the nucleus, a large Golgi reticulum may be demonstrated between the latter and the secretion. It is larger than the Golgi apparatus of the ciliated cells but soon regains equality in size as regeneration of the mucous cells is completed.

As in other types of glands, it may be assumed that the Golgi apparatus has some relation with the various secretions of the fowl oviduct. The excessive activity of this

organ and the chemical nature of the secretions, appear, however, to render it less suitable as material for the study of the intimate cytology of secretory phenomena than many other types of gland tissues. Broadly speaking the size of the Golgi apparatus is an indicator of the stages of activity and rest in the individual cells of the ciliated and gland epithelia.

A detailed examination of the mitochondria has not been attempted in the present material, but preparations with Regaud and Schridde fixation have been made to avoid confusion with granules revealed by other techniques. It is quite clear that the special granules of the ciliated cells in the uterus and the isthmus are different from mitochondria. In the uterine glands the mitochondria are in the form of elongate filaments and rods, quite unlike the pale granules described in these cells with Zenker-formol fixation. It has been stated elsewhere in this paper, that attempts to compare avian and mammalian oviducal epithelia have little significance in view of their diversity of function. But, even in the human uterine glands, of which a detailed cytological description is given by BARTLEMEZ and BENSLEY (1932), certain cell structures exhibit a behaviour which is closely paralleled in the fowl. I shall refer to these here as a group of cell phenomena for which no interpretation is at present available.

In the ciliated epithelium during shell secretion and for some hours following egg-laying, the arrangement of the mitochondria in the basal cells is strikingly similar to the zoning described by BARTLEMEZ in the human glands, seven days after the onset of menstrual bleeding. A dense clump of elongate filaments is concentrated between the basement membrane and the basal nucleus. In the midst of these, a vacuole may appear in contact with the nucleus. The curious cells " loaded with mitochondria," in BARTLEMEZ' paper (Plate III), are not at all rare in the fowl uterus and the cell hypertrophy, which he terms hydrophic degeneration, found during the second day of menstrual flow, is illustrated in the present paper during calcium secretion, *x'*, fig. 30, Plate 20. Cells resembling the " Stiftchenzellen," which BARTLEMEZ found abundantly during menstruation, occur in the uterine epithelium after egg-laying, They appear to represent degenerate basal cells, filled with heavily staining secretion or disintegration products. The vacuolation common in the ciliated epithelium of the isthmus and uterus is similar to that which BARTLEMEZ ascribes to glycogen plasmolysis. Despite the known glycogen content of avian egg-white, attempts to stain glycogen with Best's carmine or iodine have been unsuccessful in the present material.

10—*The Vagina*

The material used in these studies provides ample confirmation of the view that the vagina plays no part in the secretory processes leading to the formation of the shell. Anatomically the lumen of this portion of the oviduct does not lie directly in the same axis as the rest. It bends to one side and the walls of both regions are closely bound together by connective tissue so that the vaginal canal forms an inseparable thickening over part of the expanded uterine wall. Where the vaginal

lumen connects with the uterine cavity, a restricted number of vaginal folds, which are characteristically leaf shaped and arranged in parallel rows, *va*, fig. 1, Plate 12, come into contact with the uterine egg. Despite this relationship, the vaginal epithelium does not show any evidence of the secretory phases described in the uterus. In the corium of the vaginal folds a few scattered tubular glands appear, *mg*, fig. 37, Plate 21, which, in the present material, have been studied only in the pro-secretion stages. They bear a marked resemblance to mucous glands. Their lubricating function seems evident and probably their secretion coincides with egg-laying.

Caudal to the utero-vaginal junction, the corium of the folds is formed of loose connective tissue without glandular structures. In fig. 37, Plate 21, the marked increase in height, 35 μ, of the vaginal as compared with the uterine epithelium may be noted.

IV—Discussion

Before discussing the results of my own observations on the secretory phenomena of different regions of the oviduct, I have included in Table I a classification of the cell types and their probable contributions to the formation of the mature egg. This will serve also to summarize my final conclusions which are dealt with more fully in this discussion.

As might be expected, the reactions of the constituents of egg-white to different fixatives and stains, are extremely variable. The chemistry of this material has been widely investigated, but little attempt has been made, in previous papers on the histology of the fowl oviduct, to identify the components of the egg-white in the newly formed secretions lying within the crypts of a functioning oviduct, after sectioning and staining. The fact that successful histological demonstration of undistorted albumen is counter to the simultaneous preservation of the mucin secretions, can be shown to account for differences in previous descriptions of the oviducal glands. Probably the most successful investigation of the albumen region, in particular, will result from material prepared by the freezing-drying technique. The proteins of egg-white may be so little altered after freezing and dehydration *in vacuo* as to facilitate the use of reagents or criteria commonly applied in the chemical investigation of these substances. This method has not been available in the present work. The best preservation of clear albumen masses in the free lumen of the oviduct, in the gland lumina and in the cytoplasm of the gland epithelium has resulted from fixation in Zenker-formol without acetic acid. Mucin identification, of course, requires different fixation.

1—*The Infundibulum*

Apart from the mucous cells, which appear in the ciliated epithelium at the commencement of the chalaziferous region, three types of glandular structures in the infundibulum must be considered ; the glandular grooves, the chalaziferous glands

TABLE I—THE FOWL OVIDUCT

Region	Lining Epithelium		Tubular glands	
	Cell types	Function	Cell types	Function
Infundibulum—				
a. Funnel . .	Ciliated	—	Nil	—
	Non-ciliated (glandular grooves)	Fluid, distending yolk (GIERSBERG)	—	—
b. Chalaziferous region	Ciliated	—	Similar to glandular groove cells, secretion dense	Chalaza
	Mucous	Lubrication		
	Non-ciliated (glandular grooves)	As above	Scattered albumen glands (*see* below)	Ovalbumen
Albumen and Mucous Regions	Ciliated	—	Uniform, except for active and resting stages, densely packed secretion as granules or droplets	—
	Non-ciliated, goblet cells	Glucoproteins of egg-white		Ovalbumen fraction of egg-white
Isthmus	Apical ciliated	—	Uniform, with secretion always granular and viscous	Ovokeratin shell membranes, inner and outer layers
	Basal non-ciliated, bleb cells	Lubrication . . .		
Isthmo-Uterine Junction	Apical ciliated	—	Isthmal type	Shell membrane, outer layer
	Ciliated with granules (inconstant)	Shell matrix . . .	Uterine type	*See* below
	Non-ciliated basal, bleb cells	Lubrication	Type 3, with reduced heavy stained granules	Unknown
Uterus	Cells of modified epithelial pits	Mammillæ	Uniform, faintly granular .	Thin, fluid albumen transporting calcium as well
	Basal, vacuolated . . .	Cuticle		
	Apical ciliated, finely granular	Shell matrix . . .		
	Non-ciliated, bleb cells .	Lubrication . . .		
Vagina	Ciliated	—	Mucous glands	Lubrication

with dilated lumina, and the albumen glands. Apparently these have not been sharply differentiated by previous workers, and controversy concerning the function of the infundibulum centres around the glandular grooves of SURFACE as a possible source for the chalaza. GIERSBERG (1922) identifies the secretion in the glandular grooves, which I have been unable to find, as a homogeneous material not unlike blood plasma. This secretion is evidently very fluid and is only obtained when the ovum is within the infundibulum. Obviously the secretion does not resemble chalaziferous material or albumen and, in consequence, GIERSBERG assumes that it has the function of penetrating into the yolk and so expanding it in size and making it less rigid. Such changes in the consistency of the ovum within the infundibulum, as compared with its condition when first shed from the ovary, have been repeatedly confirmed.

SURFACE (1912) has stated that " at the beginning of the albumen secreting region, the pouching (of the glandular grooves) is greatly increased and the first of the so-called tubular glands are found." In the present material it has been found necessary to define the albumen region as commencing where the first albumen glands occur. These glands are identified, in the secretory phase, by their alveolar epithelium, darkly staining nuclei and restricted lumina, which are filled with typical albumen. The junction between the infundibulum and the albumen region is, therefore, strictly histological and not macroscopic in its boundaries. BRADLEY (1928) regards the progressive deepening of the glandular groove depressions as accompanied by a gradual specialization of the epithelium into albumen secreting cells, while the remaining proximal cells (typical glandular groove cells) form a duct to the rudimentary gland. It is well known that the histogenesis of the glands in the corium of the oviduct as a whole, involves the evagination of the lining epithelium, followed by the elongation and organization of the pit-like structures so formed, into closely packed tubular glands. It has been assumed, by previous workers, that the smaller tubules in the infundibulum, derived from extensions of the glandular groove epithelium into the corium, are merely an incomplete expression of this general process leading to primitive non-functioning structures. In the opinion of most investigators the appearance of true albumen secreting epithelium in the glands marks the first instance of glandular activity in the corium. But it must be emphasized that, before the true albumen glands are encountered in serial transverse sections of the cranial part of the oviduct, chalaziferous glands are quite well developed in the infundibular region. These glands contain a secretion which is entirely different from albumen or from that described by GIERSBERG, as issuing from the glandular grooves of the funnel region.

The chemical nature of the chalaza is undecided. It has been analysed, according to NEEDHAM (1931), by only one investigator in the era when the egg-white proteins were incompletely understood. The relative density of the secretion forming this structure and its staining reactions, as compared with the typical ovalbumen, seem sufficient, however, to identify it with the special secretion described in the lumina of the chalaziferous glands (*ante* p. 155).

The development of mucous cells in the ciliated epithelium of the infundibulum seems to coincide with the first definite need for special lubrication in the lower funnel and chalaziferous regions. Here, the delicate ovum has to enter a tube of small diameter, with walls which are not covered with flowing albumen even during the maximum secretory phase. TURCHINI (1924) has put forward an interesting interpretation of the richness of the vascular supply in the funnel wall as providing a means for the erection and dilation of this region through engorgement of its blood vessels. This formation of a type of erectile tissue may partly account for the remarkable process by which a large sized yolk is able to enter what is ordinarily a membranous funnel shaped opening.

2—*The Albumen Region*

SURFACE (1912), using Zenker fixation, has noted the variable staining of different areas of the tubular glands in the albumen region, and he refers to the fixative as a possible cause. BRADLEY (1928) (after fixing in Susa's formaldehyde-mercuric chloride solution) failed to find the large, deeply staining granules so characteristic of the albumenous glands and, being almost wholly concerned with establishing the mucin character of the goblet cells in the epithelium, he makes no further comment on the glands in the corium. BRAMBELL (1925), however, figures large granules after fixation with absolute alcohol and notes their wide affinity for different stains. In material fixed in mercuric chloride, for mucin staining, and in that fixed for the Feulgen reaction, the gland epithelium is poorly preserved, owing to marked flocculation of the secretion and its shrinkage in dehydration.

The question first arises of the possible unequal penetration of the Zenker-formol from the surface epithelium to the core of each fold and its effect on the staining properties of the secretory granules and the free albumen. That the differential staining effect observed is not the result of unequal fixative penetration, is supported by the constant pale hæmatoxylin staining of the free egg-white in the crypts. This represents a change from the deeply staining secretion present in the active gland lumina and in the deeper recesses of the crypts, but it is co-existent with pale secretion, fig. 7, Plate 14, and fig. 12, Plate 15, in the corium, where the gland epithelium appears to be entering the resting stage. Moreover, in sections through the albumen region, no graded intensity of staining, simulating a series of precipitation effects due to the progressive penetration of the fixative, is observable. The association of ebony black secretion spheres only with cells containing a normal unshrunken nucleus, fig. 9, Plate 14, and *bg*, fig. 10, Plate 15, and the demonstration of a series of stages in the glands typical of rest and active secretion, even to extravasation of albumen into the inter-glandular spaces, suggest a differential staining of the variable physical and chemical states of the secretion during its formation. But even more important is the fact that deeply staining albumen granules appear either in the peripheral or the central glands according to the actual phase of secretion in these glands when the material was fixed.

SURFACE (1912) describes three stages in the albumen glands typical of the following secretory phases :—

i. When the albumen region is just about to commence secretion, small dark-staining nuclei are present in the epithelium, with secretory droplets varying from large granules to those barely visible.
ii. When secretion is finished, an alveolar condition of the cytoplasm, containing fine granules, is noticed.
iii. Some hours before the onset of secretion, the formation of very large, intensely staining granules, appearing in light areas or solution zones of the cytoplasm, with the remainder finely granular, is found in the peripheral regions of the folds, while in the central region, the gland cells have no granules and stain lightly.

Beyond holding the opinion that the glands secrete a very dense albumen and that unequal staining throughout a fold may be due to variable fixation, SURFACE does not interpret these observations.

From the present work, it seems likely that the resting glands are those in which the epithelium is reorganized and provided with expanded nuclei and the secretion is stored as deeply staining spheres, fig. 10, Plate 15. The condition of the nuclei was not noticed by SURFACE. This resting phase of the albumen glands is seen first in the periphery of the folds, some hours before the passage of an egg. Later, and even during active secretion, the deeper placed glands undergo this transformation, while the peripheral tubules may have commenced or finished their secretory cycle.

The albumen, in the dilated ducts approaching an exit into the crypts, stains intensely with hæmatoxylin during the secretion of egg-white and, as this material collects in the oviduct, it retains, for a time, the same deeply staining appearance.

In this phase, when the albumen appears to be actually flowing at the time of fixation, the gland epithelium shows dark, though not opaque, purplish brown droplets in the cytoplasm. These features are typical of the maximum secretory stage, fig. 11, Plate 15, which is comparable to stage 1 in SURFACE's material.

The third type of secretion filling the gland epithelium and forming fused masses in the lumina scarcely stains at all with iron hæmatoxylin and resembles the free, mature albumen in the interfold spaces, fig. 12, Plate 15. At the end of a secretory cycle, before reorganization of the epithelial nuclei is evident, this stage is predominant and probably represents a cessation of active flowing or, at least, a late phase of mature albumen, similar to that in the oviducal lumen. In comparing this with stage 3, of SURFACE, it must be noted that the secretion is homogeneous, not finely granular, unless it becomes fragmented in sectioning. Feulgen staining shows that the nuclei of the epithelium, as they become crushed against the cell membrane, do not become hyperchromatic. The deeper staining with hæmatoxylin is due to the close compression of the chromatin of the resting nucleus in combination with the reduced nucleoplasm.

In studying the albumen region of the fowl oviduct, previous investigators have not referred sufficiently to the components of egg-white as determined by OSBORNE and CAMPBELL, and others, and to the possible source of these secretions in the oviducal wall. Two albumens, ovalbumen and conalbumen, with two glucoproteins, ovomucoid and ovomucin, form the major constituents. The proportions of the glucoproteins are stated by NEEDHAM (1931) to be approximately 22% with 75% ovalbumen and the remainder conalbumen. SURFACE (1912) suggested that fluid or thin albumen was produced by the ciliated epithelium, in which he described the unicellular glands, whilst dense albumen was elaborated in the tubular glands. BRADLEY (1928), however, has established beyond doubt the mucous activity of the goblet cells in the albumen region, these being the same cells which SURFACE called unicellular glands.

The localization of these egg-white components has not been attempted by histochemical methods, but there is general evidence leading to the assumption tnat the tubular glands of the oviduct, with the exception of those in the isthmus and vagina, secrete the albumens and the ciliated epithelium secretes the glucoproteins.

The tubular glands of the oviduct cannot be shown to consist of mixed albumen and mucin secreting cells. In the albumen region, there is no reaction of the gland epithelium to mucin staining and the secretory droplets, allowing for stages of rest, cessation, or actual secretion, all stain alike in the cells of an individual gland. Hence it seems reasonable to suppose that the glucoproteins of egg-white are secreted from the lining epithelium of the albumen region.

The lubricating and cleansing function of mucin, as displayed in typical mucous membranes, appears to be unimportant in the albumen region at least, where the nature of the egg, without its shell membranes, renders special lubrication unnecessary. Moreover, maximum secretion of mucin has been shown to coincide with the albumen extrusion and to continue after the egg has passed to more caudal levels of the oviduct. Egg-white formation continues for some time after the egg has passed through the albumen region, and there is no evidence against the contention that the residual secretion may drain down the oviducal walls to reach the egg below. This must be considered in estimating the percentage of egg-contents contributed in the isthmus and uterus before calcification of the shell.

STORMONT (1932), in his discussion on the mucous cells of the salivary glands, emphasizes that tests for mucin are at present non-specific and non-microchemical. This lack of reliable histochemical tests leaves the localization of ovomucin, in the oviducal epithelium, entirely unsolved. As will be shown subsequently, the function of certain non-ciliated cells in the isthmus and uterus is also still problematical.

After regeneration of the lining epithelium of the oviduct, it is impossible to distinguish future goblet cells from ciliated cells. The epithelial cells, as described by MOREAUX (1913) in the mammalian Fallopian tube, appear to undergo a cycle of ciliated and secretory phases. Cellular degeneration is seldom encountered in the active fowl oviduct. The lining epithelium continues unharmed throughout an egg-laying cycle, and is only reduced by appreciable shedding of its cells, when the

oviduct atrophies. Destruction of the epithelium and extravasation of blood, which BRAMBELL (1925) compares with that occurring in mammals during œstrus, though he considers it less extensive in the fowl, could not be found in the present material. The epithelium exhibits stages of secretory activity and repair which, owing to the excessive mucin secretion, in the albumen region in particular, may appear to be degenerative in character. The small number of cells present in the egg-white of a completed egg, referred to by NATHUSIUS (1868), confirms the relative stability of the epithelium during egg-formation, at least in the albumen region, where the greatest epithelial activity occurs.

3—*The Isthmus*

The conclusions of PEARL and CURTIS (1912) that the time taken by the egg in passing through the isthmus is considerably shorter than the estimates given by previous authors, are important in interpreting the functions of this region. PEARL and CURTIS obtained a mean period of 0·6 hours as compared with the earlier records of 2 to 3 hours. One of the most significant histological features of the isthmus is the discontinuity between its tubular glands and those of the albumen region. This was noted by SURFACE (1912) and taken as indicating a difference in function of the glands in question. Apart from a decrease in size, however, neither SURFACE nor BRADLEY (1928) found histological differences of functional significance between the glands of the isthmus and albumen region. BRADLEY described the existence of smaller granules in the albumen region, but these may be shown to be the result of mercuric chloride fixation. In the present material no differentiation in the stages of secretion, so characteristic of the albumen region, could be found in the isthmal glands. The secretory granules remain rounded and heavily staining throughout. They do not appear to liquefy or to fuse into masses lying in vacuoles in the cytoplasm. The rate of secretion is slow, so that the glands are never excessively dilated and the nucleus remains expanded in the active epithelium. The secretion, as it leaves the glands, is clearly viscous, being in the form of thread-like strands, formed from the coalescence of individual granules. These threads have the microscopical appearance, not of albumen, but of a keratin-like substance. Occasionally the secretion is shed from the glands as clumps of granules, which are entirely unaltered in shape by the fixative and lie against the cilia. Despite the absence of any microchemical proof, the evidence clearly suggests that this viscous secretion is that responsible for the shell membranes, as originally held by SURFACE on less conclusive grounds.

The structure of the shell membrane, outer and inner layers, fig. 33, Plate 20, and *im*, *om*, fig. 36, Plate 21, is well known from the excellent investigations of NATHUSIUS (1868). The fine homogeneous fibres, of which it is entirely composed, form a lace-like layer, densely felted in the inner, thin membrane and somewhat more loosely interwoven in the outer. In section the fibres are not cut for more than a short distance longitudinally and many are cut transversely, giving the appearance

of granules enclosed between the fibres. Though I have made no detailed investigation of the structure of the shell membranes to supplement those of NATHUSIUS, actual granules of ovokeratin may be included in the formation of the shell membrane, and if so these would be derived from secretory granules which had failed to fuse into strands before leaving the ducts of the tubular isthmal glands.

It should be noted that the relative number of apertures in the ciliated epithelium, belonging to tubular glands, is greater in the isthmus than elsewhere. This appears to support the theory that the secretion is deposited around the egg in the form of interlacing fibres from innumerable points in the isthmal wall. A melange of shell membrane secretion does not collect in the lumen before the material is organized in its fibrous form.

As shown by BRADLEY, the glandular cells of the epithelium do not give a very definite mucin staining. The blebbed character of the non-ciliated cells of the isthmus has been clearly depicted by SURFACE (1912), and such cells are labelled E in fig. 470 of his paper (an actively secreting isthmus) and also in fig. 471 ; whereas the uterine epithelium, in the same plate, has non-ciliated cells with unaltered apical margins. SURFACE is in error in stating that " there is no visible differentiation of the unicellular epithelial glands in any portion of the oviduct with the possible exception of the vagina." There is little doubt that these special non-ciliated cells produce a secretion of a mucous character, which expands the free cell margin. But, unlike the goblet cell, there is no extensive rupture of the expanded cytoplasm. The secretion appears to move slowly out of the cell, followed by the regeneration of the cytoplasm and the movement of the nucleus to its normal position. It should be noted that, in mammals, the non-ciliated type, regarded as the glandular elements of the epithelium, is usually bleb-like. Their secretory cycle and regeneration is described in detail by MOREAUX (1913) in the rabbit Fallopian tube. In the sow, SNYDER (1923) has noted that the cytoplasmic blebs of the non-ciliated cells do not take mucin stains.

The significant feature of all the isthmal glands is the reduction in the degree of secretory activity as compared with the albumen region. Viscous rather than semi-fluid secretions are revealed by fixation and staining. It is difficult to explain these histological findings in relation to the well-established fact that over 50% of the albumen is secreted after the egg leaves the albumen region and that this albumen is of a thin fluid type. Further, the rate of albumen production has been expressed by PEARL and CURTIS (1912) in a parabolic curve, with no discontinuity in the rate from the time the egg leaves the albumen region to its completion in the uterus. At least 10% of the egg-white is added while the egg remains in the isthmus. However, as indicated previously, it is not necessary to suppose that egg-white is added in amounts derived from successive levels of the oviduct and only when the egg is in contact with these particular levels of its wall. The secretion of albumen appears to take place both before and after the yolk has passed a particular point in the albumen region. The mucous region, at its caudal end, continues in activity until the shell is partly formed. In the absence of any evidence that the isthmus

secretes albumen, it may be concluded that the proportion gained in this region is added by osmosis through the new egg membranes from the adjacent uterine glands and by draining down the oviduct from the albumen region. In other words, the isthmus contributes no albumen to the passing egg.

The early observation of COSTE (1847), that shell membrane material commences to be deposited on the naked egg-white, as a thin layer, immediately the caudal part of the egg comes in contact with the isthmal wall, may be used to explain the formation of the inner thin, fine-textured layer of the shell membrane lying internal to the outer coarser layer. These layers separate readily from each other in section cutting, *im*, *om*, fig. 33, Plate 20, as well as normally to form the double-walled air chamber at the blunt end of the egg. The inner membrane, it may be suggested, is derived from the secretion of the isthmal glands which is deposited on the egg during the period when it is moving into position in the isthmus. As the egg becomes stationary for a short time in this region, increased activity of the entire isthmal glands may then account for the outer layer.

4—*The Isthmo-uterine Junction*

The condition of the tubular glands in this region requires more extensive investigation than the present material will allow. An egg is rarely obtained in actual passage through the junction, with its membranes completed, but without visible traces of calcification. An extremely short period intervenes between the entrance of the egg into the isthmus and its transference into the uterus proper. The absence of a macroscopical boundary indicating this special junctional region adds to the difficulty in selecting material. From the observations of previous workers, there is little doubt that the glands of the isthmo-uterine junction, apart from those which are obviously isthmal in type, are characterized by lesser numbers of secretory granules, which stain similarly to those in the isthmal glands, though they are not usually so large.

BRADLEY (1928) has suggested, with some hesitation, that the tubular glands of this region are mixed, in that they contain a proportion of isthmal and uterine epithelial cells in any individual gland. I have observed what might be this condition so rarely that it is impossible to accept this interpretation as conclusive. In fact glands having this mixed condition (B, fig. 4, in BRADLEY's paper) are quite atypical of the region. Instead, groups of glands which are typically isthmal, some which are entirely uterine, and a third or special type, *tl*, fig. 20, Plate 17, characterized by the presence of a limited number of dark granules in all the cells of a transverse section, more accurately represent the condition at the isthmo-uterine junction.

The secretion filling the lumina of the special junctional glands has been referred to as staining with difficulty, but it has not been observed in its passage from the glands to the oviducal lumen. No conclusive interpretation of the function of the special glands can be made, though it should be noted that they remain longest in close relation to the shell membrane at the blunt end of the egg. A separation of

the shell membrane layers readily occurs in this part of the egg, which leads to the formation of the air chamber, following cooling of the egg and the intake of air through the more porous shell covering this area, *see* RIZZIO (1899).

5—*The Uterus*

The primary function of the uterine glands in secreting the thin, fluid albumen is confirmed by most authors and need not be further discussed beyond mentioning that its addition to the egg-white must necessarily be confined to the period during which the partly calcified shell matrix is still elastic and pliable. There is general agreement also that this secretion is not usually preserved in a stainable form in the uterine lumen. In figs. 34 and 35, Plate 21, both being from sections of a uterus which had been fixed, containing an undisturbed and partly completed egg, isolated masses of albumen, *al*, are shown in the interfold spaces. Similar secretory masses in fig. 35 have obviously been fixed just as they were issuing from the ducts of the tubular glands.

FROBÖSE (1928) has made an interesting study of the arrangement of the isthmal and uterine glands, based upon wax models from serial sections. The uterine glands appear to be intensely convoluted, or plexiform structures, with alveolar off-shoots to the main body of the gland ; an interpretation which explains the relative scarcity of ducts leading to the surface of the epithelium in the uterus as compared with the condition which I have emphasized in the isthmus.

With regard to the location of calcium secretion, the staining evidence, from the use of hæmatoxylin and silver salts, is invariably negative. CAMERON (1930) has stressed the uncertain microchemistry of calcium staining, even in heavily calcified tissues. The relatively small calcium content of the uterus, during active secretion, may account for the entirely negative staining reactions of this organ. In considering the results of microincineration, several important points have arisen in connection with the work of TURCHINI (1924). Reference has been made, in an earlier part of the present paper (*ante* p. 172), to the relatively greater ash content in the ciliated epithelium, as compared with the corium, even in the resting uterus. The explanation of cell crowding and more homogeneous cytoplasm in the lining epithelium, resulting in a conspicuous white band of ash covering the uterine folds after incineration, seems adequate. The incinerated sections, used by TURCHINI, were examined by him on platinum foil, by indirect illumination. He does not record the existence of special granules in the case of the ciliated cells as shown in figs. 24 and 25, *gv*, Plate 18. These granules largely contribute to the increased ash content of the epithelium as a whole. It would appear that his evidence that the ciliated epithelium is concerned with the secretion of the egg-shell calcium is not conclusive. The application of reagents, such as iodic acid, to the tissue sections, both before and after incineration, has the objection, as GIERSBERG (1922) states, that it is impossible to differentiate between the normal calcium content of the protoplasm which, in the active uterus, is supplied by the blood stream containing an excess of this ion, and the calcium which is actually in the form of a secretion.

Apart from general cell structures, such as the nucleus and cytoplasmic membranes, a variety of cellular derivatives, such as connective tissue fibres, *cnf*, fig. 23, Plate 18, possess an appreciable ash content. Recently HORNING (1934) has shown that the keratin masses forming keratin pearls in tar tumours of rodents leave a conspicuous inorganic residue. Similarly the ovokeratin granules in the isthmal glands of the fowl, *ov*, fig. 27, Plate 19, contain a heavy ash. Owing to the abundance of these granules throughout the corium, one gains the impression, from incinerated preparations, that the inorganic content of the isthmus is actually greater than in the uterus. This is confirmed by the analyses of BUCKNER, MARTIN, and PETER (1925), who obtained an average figure of 0·44% CaO in the isthmus and 0·24% CaO in the uterus of the " heavy laying hen." Unfortunately their figures do not include the calcium content of the uterus, when it contains a partly calcified shell, in comparison with material not in full secretory activity, though their analyses indicate a reduction to 0·18% in the resting, partly atrophied organ.

With these facts in mind, the special granules in the apical ciliated cells, revealed in incinerated and stained preparations, fig. 25, Plate 18, and *gv*, figs. 28 and 29, Plate 19, cannot conclusively be regarded as the pro-secretion phases of calcium secretion in the uterus. In fact, sections from material fixed in alcohol-formalin and Zenker-formol may be placed in nitric, hydrochloric, and acetic acids, without the slightest alteration in the subsequent staining of the granules with iron hæmatoxylin. The appearance of the granules and, indeed, their reactions to acids are more consistent with the assumption that they are the shell matrix secretion. The quiescent state of the basal cells during secretion, leads to the conviction that the uterine epithelium, as a whole, does not take any part in calcification. The slight increase in cytoplasmic ash in the epithelium seems nothing more than a result of increased calcium supply from the blood stream, which permeates the entire uterine wall.

During active secretion, the gland epithelium contains cytoplasm which is neither alveolar, as in the albumen region, nor homogeneous, as in the ciliated cells. Its preservation in alcohol-formalin is poor. The lack of any definite organization in the ash of the gland cytoplasm makes the identification of an increased calcium content very difficult. The cell walls, however, are definitely thickened in an incinerated preparation, during shell secretion, as compared with the condition in the non-secreting uterus. This thickening may be due to a fixation concentration of increased cytoplasmic salts, about the cell membrane, which does not occur in the ciliated epithelium where fixation is better. Hence, with inconclusive evidence, one is unable to confirm or deny the theory of GIERSBERG (1922) that the calcium is transported with the thin albumen from the uterine glands, possibly in the form of calcium albuminate. In the uterine lumen, the calcium salts are probably condensed in amorphous form in the shell matrix and, in later stages, crystals of calcite would be deposited or formed in the peripheral part of the shell.

As the eggs found in the oviducts used in this investigation were not heavily pigmented, no attempt has been made to locate the source of shell pigments. GIERSBERG believes that the pigment is conveyed by wandering cells, which penetrate

the uterine epithelium in the final stages of calcification. It must be mentioned, however, that these pigments are well known as ooporphyrins derived from hæmoglobin. TURCHINI (1924) has recorded the appearance of granules in the uterine epithelium seen in frozen sections by oblique illumination, and he considers that they are an instance of the previously recorded association of porphyrin-like substances with tissues undergoing calcification. The facts that TURCHINI does not appear to have distinguished his porphyrin granules from the shell matrix material, which is readily stainable and insoluble, in the apical cells, and that he makes no reference in his paper to the nature of the egg-shell pigmentation, which may be present or absent in different breeds of fowls, appear to weaken his evidence that the uterine epithelium secretes the calcium salts in the presence of a porphyrin. I have examined frozen sections of uterus in oblique illumination but, in my opinion, it is impossible to detect supposed porphyrin granules sufficiently clearly under such conditions, to be certain of their chemical nature.

A discussion of the evidence associated with the secretion of the organic portions of the egg-shell is confined almost entirely to a comparison between the work of FROBÖSE (1928) and my own observations, since he alone appears to have studied sections from uteri fixed with the developing egg *in situ*.

The observations of NATHUSIUS (1868), on Passerine birds, that the shell membranes show an increase of 50% in thickness following the passage of the egg into the uterus, have not been definitely confirmed in the fowl. But granules so clearly differentiated from other types of secretion as are the ovokeratin granules of the isthmal tubules, are never encountered in the uterine glands. And, finally, the secretion of mammillæ appears to be the first phase in uterine activity, commenced immediately after the egg has entered this region. In consequence it is definitely established that the uterus takes no part in the formation of the shell membrane.

A fundamental difference appears to exist between the finer details of the uterine epithelial reactions illustrated by FROBÖSE (1928) and in the present paper. In my preparations, apart from areas of imperfect contact between the uterine epithelium and the shell membrane, which I believe to be technical in origin, the relationship between these two sets of structures is much more intimate than is shown in the plates of FROBÖSE's paper. At no stage in the secretion of the egg shell have I observed the production of outgrowths from the uterine epithelium, which he terms " Haftknopschen." FROBÖSE describes these structures as the sole points of contact with the shell membrane. Spaces almost equal to the thickness of the shell membrane, separate the developing egg-shell from the lining epithelium in his preparations.

The secretion of mammillæ from pits in the modified epithelium is not recorded by FROBÖSE. Instead, he derives these bodies partly by secretory activity of the entire uterine epithelium and possibly from the uterine gland epithelium as well. With azan staining, FROBÖSE does not record any significant differences between the secretion condensing in concentrically layered units or mammillæ and that derived

from the apical granular cells of the lining epithelium, which migrates to the shell membrane to become mobilized as a separate layer external to and around the completed mammillæ. The genesis of the mammillæ in my preparations may be followed from its outset as an extremely small granule associated with the shell membrane surface, to the larger body which finally lies half embedded in the peripheral fibres of the membrane and which projects from it externally. During this process, the larger secretion masses, which I have identified as shell matrix, have not reached the surface of the shell membrane.

In discussing the origin of the shell matrix itself, FROBÖSE refers to the process as comparable to the formation of a membrana decidua which involves the degeneration of maternal tissues, from which the keratin-like matrix might easily be derived. It must be stressed, however, that the evidence derived from portions of uterine wall, which have been removed from contact with a developing egg and fixed more rapidly than is possible with an entire specimen, is conclusive that the epithelium never degenerates to the extent of shedding its cells in any conspicuous degree. In fact, it is difficult to identify even the heavily stained areas of contact in such material. Similarly I have not been able to confirm the distinctly double layered stratification of the epithelium illustrated on Plate 3, fig. 3, of FROBÖSE's paper.

Some account must be taken also of the relative amounts of secretion contributing to the shell layers. The mammillæ, which are not so numerous in the present material, either in the partly developed shell, *ma*, fig. 32, Plate 20, or in the mature shell, *ma*, fig. 36, Plate 21, as compared with the illustrations of NATHUSIUS (1868), are relatively small bodies spaced only at intervals on the shell membrane. Small epithelial pits, such as I have described, seem sufficient source for these bodies. In contrast, the shell matrix is an even, well-developed layer of dense secretion requiring, one would imagine, much more than the activity of the epithelial cells which happen to lie in contact with the shell, to produce its volume within the time-period of shell formation. If cellular degeneration is at all significant in this process, it ought of necessity to be very extensive, but as already pointed out this is not so. The interpretation of the origin of the shell matrix secretion as from the granular apical cells of the entire unmodified uterine epithelium, fits in more closely, therefore, with these observations on its relative volume.

While it is impossible to form a definite opinion concerning the actual nature of the "Haftknopschen" and their cellular degeneration, it may be suggested tentatively that these cells may be concerned with egg-shell pigmentation. In this connection, GIERSBERG (1922) is of the opinion that the ooporphyrin is probably transported to the uterine lumen by pigment laden wandering cells, though detailed description of this process is wanting. In the present material no strongly pigmented egg shells were encountered.

The cuticle forms, in the material I have examined, the dense staining layer of the decalcified shell, which, in scattered areas, is still granular in composition. It would appear significant that the basal cells of the uterine epithelium, where contact is made with the egg surface, show vacuoles and pro-secretion in the cytoplasm which

stains very deeply. This activity in the basal cells occurs after the shell matrix is completed. The suggestion of GIERSBERG (1922), that the cuticle is derived from residual albumenous secretion collecting in the uterine lumen after the egg shell is completely calcified, is not supported by the staining reactions of this layer.

6—*Possible Homologies between the Oviducts of the Fowl and the Monotreme*

Although a chemical investigation of the egg-white in Echidna and Platypus has not been made, it has been established that a keratin is secreted by the oviduct to form the major portion of the egg-shell to which, in Platypus, a small amount of calcium is added. The following regions in the Monotreme oviduct have been clearly distinguished by HILL and HILL (1933) and their sub-division depends, as in the fowl, largely upon the grouping and structure of the tubular glands in the corium.

About two-thirds of the total length of the oviduct, comprising the " funnel and upper segment of the tube," possesses no tubular glands. In the tube proper, the only glandular cells are the non-ciliated type which are sparsely arranged cranially but eventually increase to out-number the ciliated cells. Next, a " glandular region " is described in which glands are developed in the corium, and it is emphasized that there is no sharp boundary between this region and the more cranial section of the Fallopian tube. A further gland cell (Type III) appears in the epithelium lining the lumen, while the non-ciliated cells are greatly reduced in numbers and in secretory activity.

Towards the uterus, a " junctional region " occurs, in which special dilatations of the basal ends of the glandular region contribute to a progressive thickening of the oviducal wall. A mixing of the tubal and uterine glands is referred to in examples where the epithelium consists of the tubal and uterine types of gland cells. The uterus possesses glands lined with ciliated epithelium which, in the superficial and middle parts of the tubules, shed a finely granular secretion from two cell types, in the initial stages of uterine activity. In the deeper portions of the tubules, larger secretory granules are found to lead to non-ciliated bleb formations on the epithelium during the later stages of the maturing egg-shell. These give rise to secretory masses as they are constricted from their parent cells.

The functions, ascribed by HILL to these various glandular structures, may now be tabulated for comparison with similar phenomena in the fowl.

The albumen layer of the Monotreme egg is not so well developed as in the fowl and it is interesting to note that its secretion is associated with the non-ciliated cells of the lining epithelium of the upper non-glandular region. In view of the fact that this layer has not been investigated chemically with the same precision as in avian egg-white, an important point remains to be determined, as to whether the secretion is a true albumen or a gluco-protein. If it is the latter, the secretory activity of the ciliated epithelium would be closely comparable to that in the fowl.

The glandular region is similar in function to the fowl isthmus and, though the basal layer of the shell is not in the form of ovokeratin fibres, it is derived, as in the

TABLE II—MONOTREME OVIDUCT

(*cf.* Table I on p. 178)

Region	Lining Epithelium		Tubular glands	
	Cell-types	Function	Cell-types	Function
Non-glandular .	Ciliated, type II	—	Nil	—
	Non-ciliated, finely granular, type I	Albumen coat . . .	—	—
Glandular . . .	Ciliated, type II	—	Main body of gland . .	—
	Non-ciliated, type I (less numerous)	? Small residuum of albumen coat	Homogeneous, non-ciliated with clear reticular cytoplasm	Fluid secretion forming basal layer of shell
			Dilated tubule ends	
	Non-ciliated, type III, globular secretion, late in cycle	? Small portion of calcium secretion	Ciliated cells	—
			Non-ciliated, finely granular	Outer rodlet, layer II, of shell
Junctional . . .	Similar to glandular region	—	Glands same as glandular region	*See* above.
			Glands of uterine type . .	*See* below
Uterus	Ciliated, type II	Majority non-secretory	Main body of gland	
			Ciliated, type B	—
	Non-ciliated, type III (numerous in Platypus)	Major portion of calcium	Non-ciliated, type A, finely granular	Nutritive fluid
			Basal ends of glands	
			Ciliated, lose cilia and form coarse granules, which are shed with apical cytoplasmic blebs	Layer III of shell
				Layer IV of shell (unknown origin)

fowl, from tubular glands in the corium. These glands appear to be specialized in forming the outer rodlet layer as well.

The junctional or transitional region is of interest and appears to have no extra functions in relation to these layers. Similarly in the fowl, I have not definitely detected any distinctly new activity in the isthmo-uterine junction. In the uterus, a close comparison must first be noted in relation to the activity of the tubular glands. A nutritive fluid, evidently of an albumenous nature, appears to be added to the shell contents in much the same way as the thin albumen of the hen's egg. But the subsequent final layers of the egg shell, which may be homologized with the shell matrix and cuticle, are derived from the tubular glands and not from the lining epithelium.

The evidence of HILL (1933) in identifying the calcium secretion is derived from staining reactions. However, in view of the small degree of calcium deposition in the Monotreme shell, it is not unlikely that individual epithelial cells may be differentiated as calcium glands.

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SUMMARY

1—The secretory processes, leading to the formation of the chalaza, the egg-white, shell membranes, and the shell of the fowl's egg, have been studied in detail with cytological techniques, including microinceneration.

2—In the Infundibulum of the oviduct, the secretion of the chalaza has been identified for the first time and certain suggestions are made with regard to the terminology applied to the anatomy of this region.

3—The secretion of the ovalbumen and glucoprotein fractions of egg-white has been localized in the glands and lining epithelium of the albumen region. Stages of maximum secretion, regeneration of the gland cells, and rest before a further egg-forming cycle, have been identified in these tissues.

4—The formation of the fibrous, ovokeratin shell membranes has been shown to result from the secretion of fibre-like strands of viscous material from the tubular glands in the corium of the isthmus.

5—Uterine activity appears to consist in the formation of the organic shell matrix with its mammillæ and cuticle, the secretion of thin albumen added to the egg-white, and the calcification of the shell. Microincineration, in combination with several

cytological techniques, has been used as a means of identifying the gland cells from which these various secretions are derived.

6—The technique of microincineration does not appear to provide a sufficiently critical means of localizing the source of secretory fluids containing calcium, such as those secreted from the uterine wall for the calcification of the egg shell.

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EXPLANATION OF PLATES

The figures are from untouched photomicrographs (except Plate 12) and those forming Plates 13–17 (except fig. 21) are taken from sections fixed in Zenker-formol without acetic acid and stained with Delafield's hæmatoxylin and eosin (Plate 13), or Heidenhain's iron hæmatoxylin (Plates 14–17). Fig. 21 is from material fixed in mercuric chloride and acetic acid, with iron hæmatoxylin staining.

Plate 18, and figs. 26 and 27, Plate 19, show incinerated sections in dark ground illumination. A control section of fig. 25, fixed in the same alcohol-formalin mixture, but stained with iron hæmatoxylin, is shown in fig. 28. A similar staining following Zenker-formol fixation is shown in fig. 29.

On Plate 20, Da Fano silver impregnations, counterstained with iron hæmatoxylin are represented in figs. 30 and 31. Figs. 32 and 33 and those on Plate 21 are from material fixed *in toto* with eggs *in situ*, being stained with Delafield's hæmatoxylin and eosin.

KEY TO LETTERING

a, pale albumen in lumen of gland.
a^1, pale albumen retreating from large vacuole.
ad, paler secretion.
adl, dense albumen layer within egg.
al, thin albumen secretion.
an, apical epithelial nucleus.
ar, albumen region.
bg, first appearance of black granular albumen.
bcv, basal cell vacuole.
bl, bleb formation on mucous cell.
bm, basal membrane.
bn, basal epithelial nucleus.
bv, blood capillary.
c, chalaziferous secretion.
c^1, hypertrophied extra-glandular connective tissue.
cb, ciliated cell with basal nucleus.
ccn, ciliated cell nucleus.
ce, ciliated epithelium.
ch, chalaziferous region.
cl, crypt lumen.
cle, epithelium lining modified crypt.
cn, connective tissue of corium.
cnf, connective tissue axis of fold.
cr, regenerating epithelial cytoplasm.
cs, connective tissue spaces.
cy, cytoplasmic reticulum.
cy^1, empty cytoplasmic reticulum.
d, dark brown staining secretion.
da, albumen droplets.
de, duct epithelium.
dd, aperture of duct leading to uterine glands.
e, evagination from a glandular groove.
ed, epithelial pit containing portion of mammilla
ep, modified darkly staining epithelium.
et, epithelium of tubular glands.
f, funnel.
g, mucous cell tips.
g^1, goblet cell.
ga, Golgi apparatus of apical cell.
gb, reduced Golgi apparatus in basal cell.
gn, goblet cell nucleus.
go, granular ovokeratin secretion.
gr, glandular groove.
gs, granular secretion in glands.
gv, apical cell granules.
gx, apical granules passing through cell membrane.
ij, isthmo-uterine junction.
im, inner shell membrane.
is, isthmus.
m, mucous secretion.
ma, mammilla.
mg, mucous glands just before secretion.
mr, mucous region.
ms, muscle coat.
om, outer shell membrane.
os, extruding ovokeratin entering oviducal lumen.

ov, ash of ovokeratin granules.
pl, plasma cell commencing to migrate through epithelium.
rc, rectum.
rl, residual secretion in lumen.
sc, shell cuticle.
sm, shell matrix secretion.
smx, decalcified shell matrix.
sv, secretory granules within vacuole.
ta, tubule in secretory phase.
tb, short tubule lined with typical " glandular groove " epithelium.
tc, secreting chalaziferous gland.
te, resting gland.
tg, tubular glands.
tl, gland lumen.
tm, mixed chalaziferous and albumen gland containing the two types of epithelial cells.
tn, gland epithelial nucleus.
tn^1, regenerated gland cell nucleus.
tn^2, partly reorganized gland cell nucleus.
tlu, poorly preserved uterine glands.
ul, uterine lumen.
us, uterus.
ut, uterine gland.
v, vacuole.
va, vagina.
ve, vacuolated epithelial cytoplasm.
vg, vacuolar spaces in Golgi reticulum.
x, is used to indicate special points in certain figures.
x^1, hypertrophied epithelial cell.
y, thread of ovokeratin.
z, ovokeratin fibre.
z^1, silver impregnated secretion.

Plate 12

Fig. 1—Dissection of the empty, functional oviduct of the fowl including portions of the wall in surface view. These are placed to the left of corresponding levels in the entire specimen and illustrate the arrangement of the folds in the major regions. × ¾.

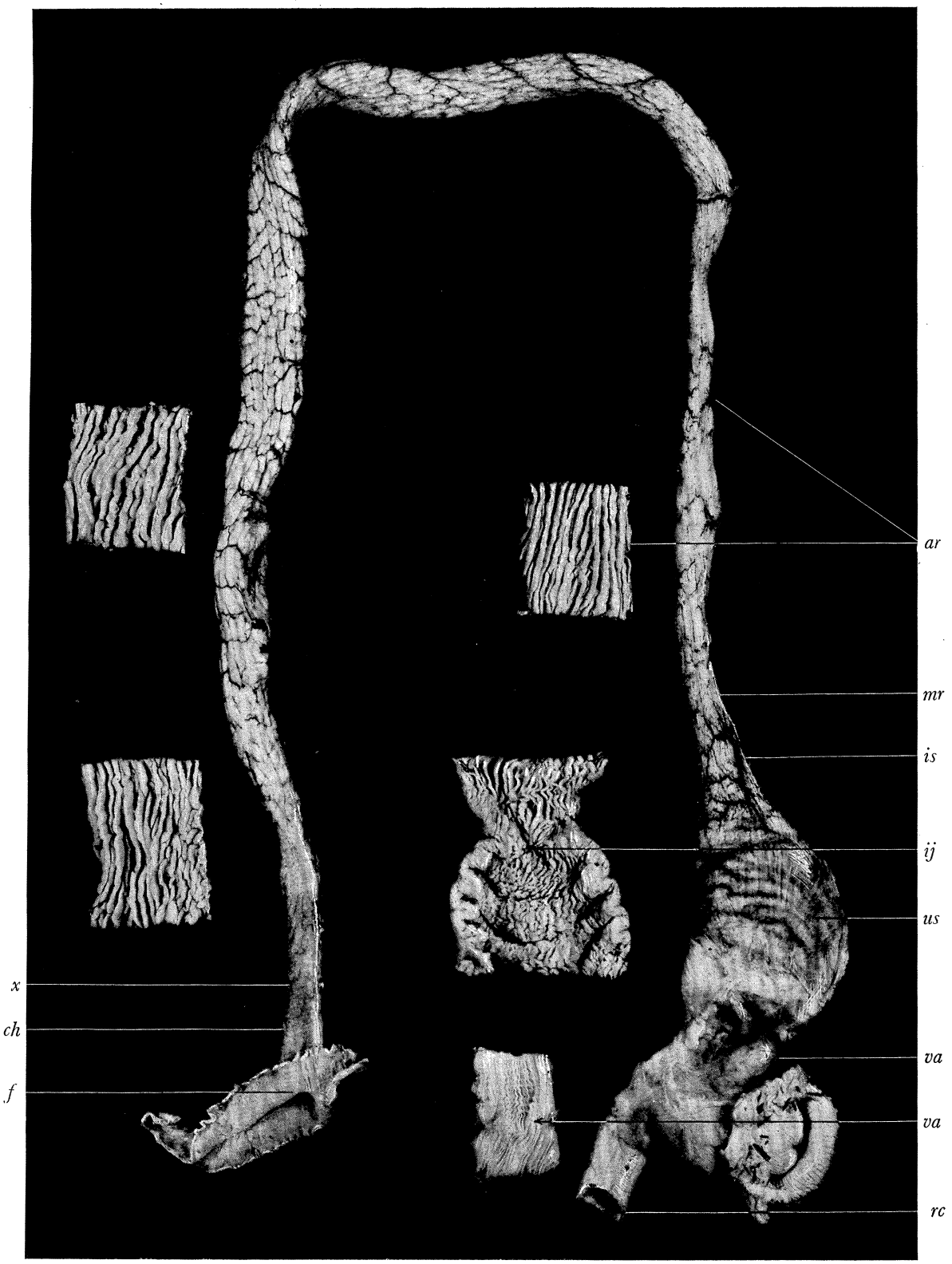

FIG. 1 × $\frac{3}{4}$

PLATE 13

FIG. 2—Fowl 29, egg in upper albumen region. Portion of the mucosa of the funnel wall showing folds in the homogeneously ciliated epithelium in which non-ciliated grooves are differentiated as the only glandular structures of this region. × 800.

FIG. 3—Fowl 32, egg in upper albumen region. Section from the cranial part of chalaziferous region, showing the tubular extensions from glandular grooves just commencing. × 250.

FIG. 4—Fowl 32. Portion of the infundibular wall in mid-chalaziferous region. Note chalaziferous glands containing heavy secretion which collects in oviducal lumen as well. × 250.

FIG. 5—Fowl 32. Section from cranial end of albumen region showing the last chalaziferous glands mixed with albumen glands. Note difference between secretion in lumen of each type. × 250.

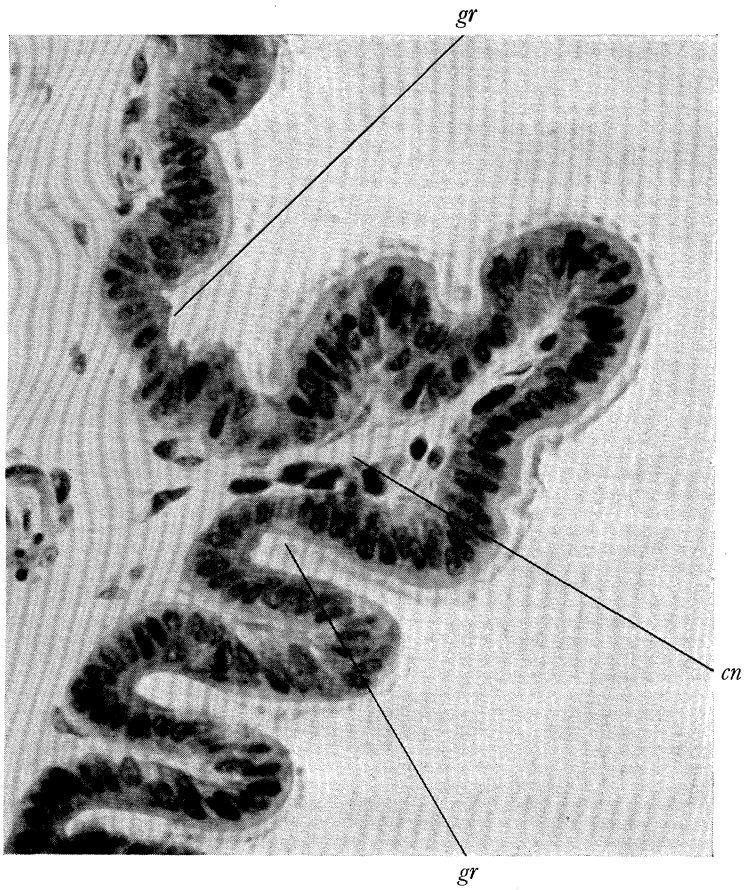

FIG. 2

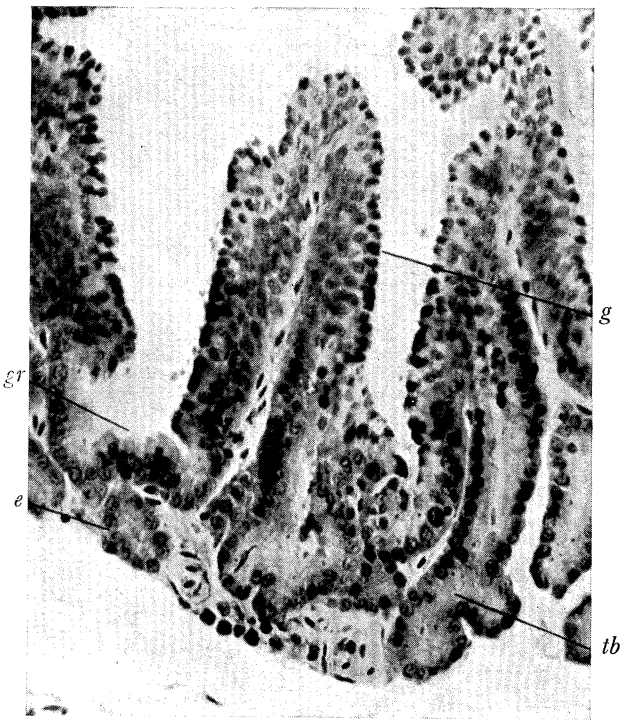

FIG. 3

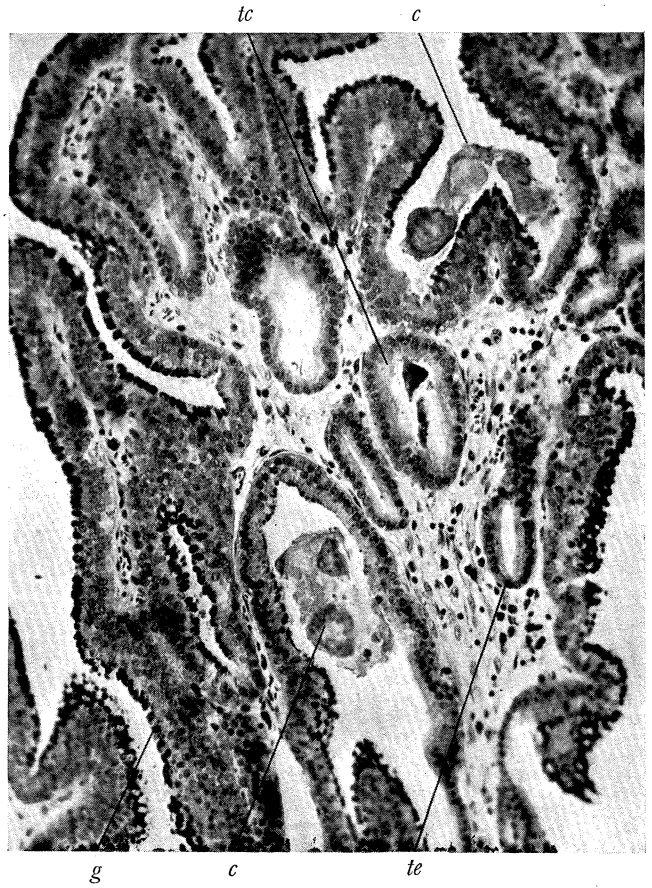

FIG. 4

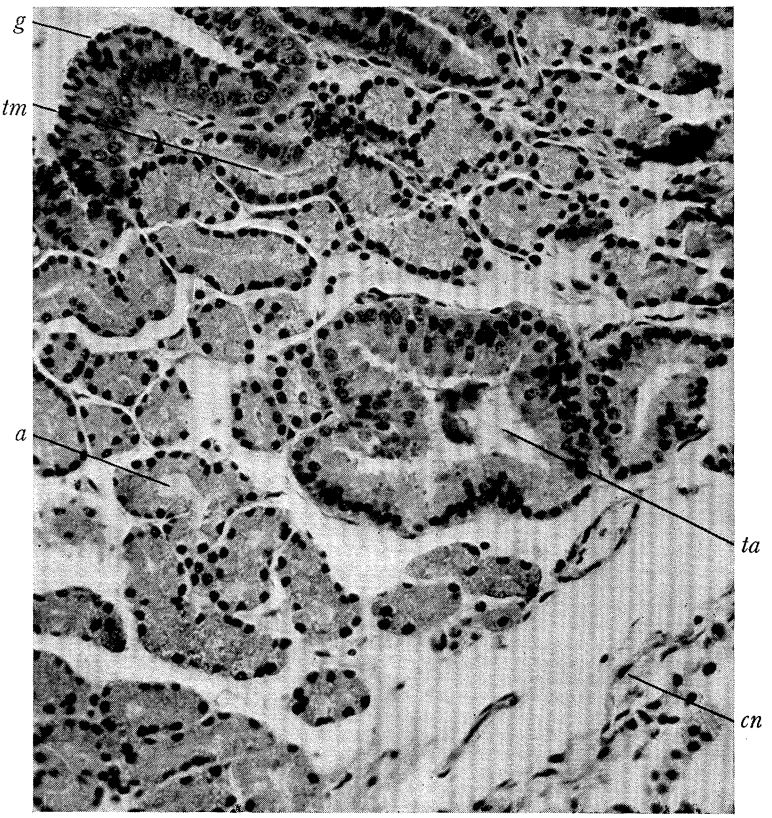

FIG. 5

PLATE 14

FIG. 6—Fowl 16, egg in uterus with partly calcified shell. Portion of ciliated epithelium and corium of albumen region, showing the final stage of maximum secretion in mucous cells, and empty albumen glands with nuclei commencing reorganization. The pale albumen has disappeared from glands, leaving a distorted, vacuolated cytoplasm. × 1900.

FIG. 7—Fowl 2, egg laid some hours previously. Similar region to fig. 6, but later stage in regeneration at a more caudal level where epithelium is higher. Note organization of mucous cell nuclei and surrounding cytoplasm. Glands beneath are filled with pale albumen and nuclei still shrunken. They are less regenerated than in fig. 6. × 1950.

FIG. 8—Further regeneration in both ciliated epithelium and albumen glands. Note basal nuclei now surrounded in homogeneous cytoplasm, regenerating in an apical direction. The ciliated cells have expanded. Gland epithelial nuclei are normal and cytoplasm is regenerating from the periphery to the gland lumen (later stage than in fig. 6) ; black granular albumen not yet appeared. × 1950.

FIG. 9—Fowl 9, egg in uterus with soft shell. Representing stage in mucous region, just before complete epithelial regeneration ; glands in typical resting phase, in which expanded nuclei and homogeneous cytoplasm are associated with black spherical secretion. Compare with figs. 10 and 15. × 1950.

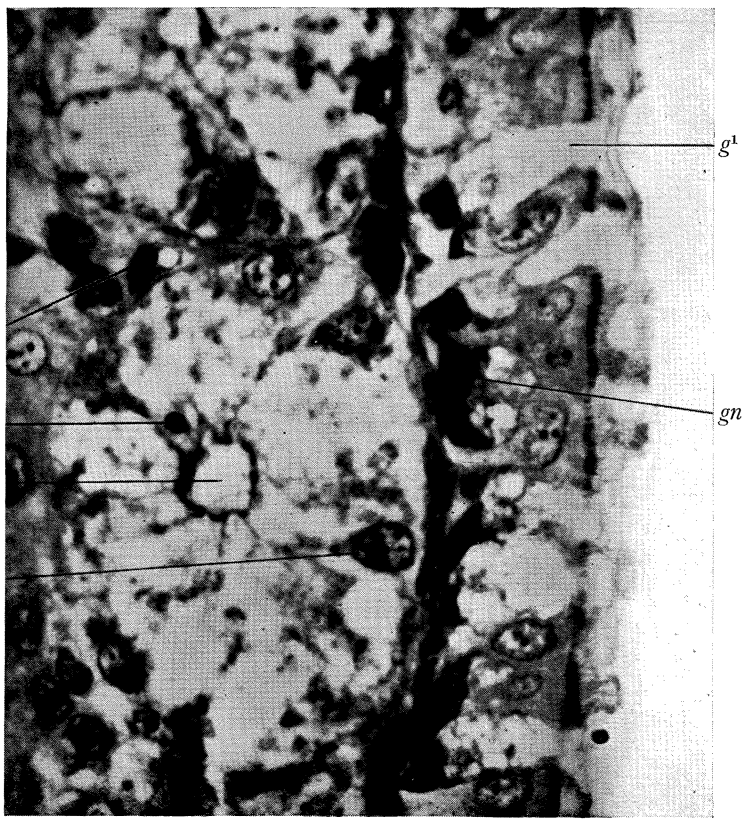

FIG. 6

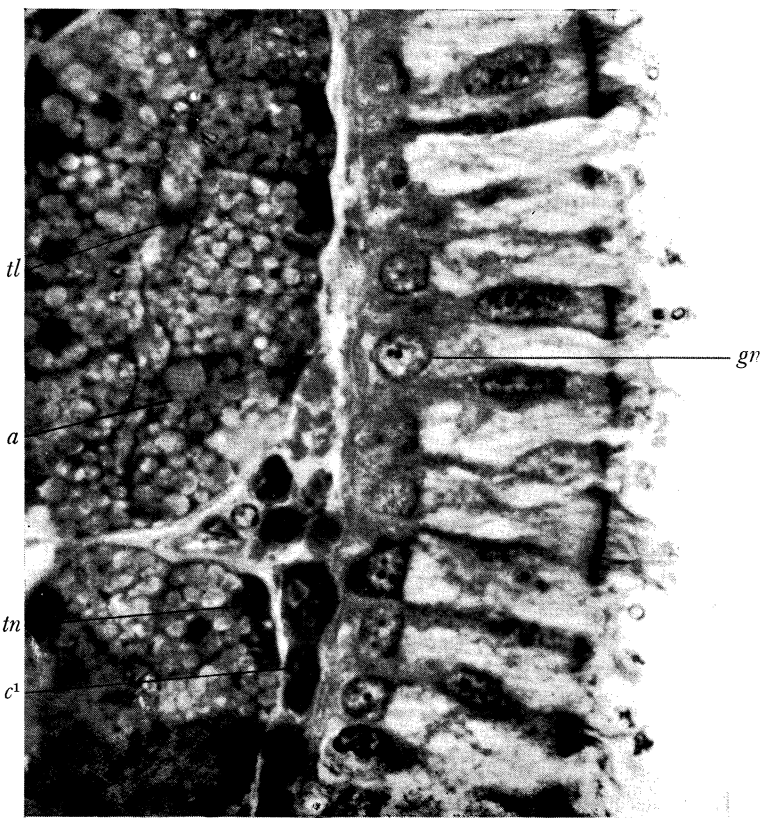

FIG. 7

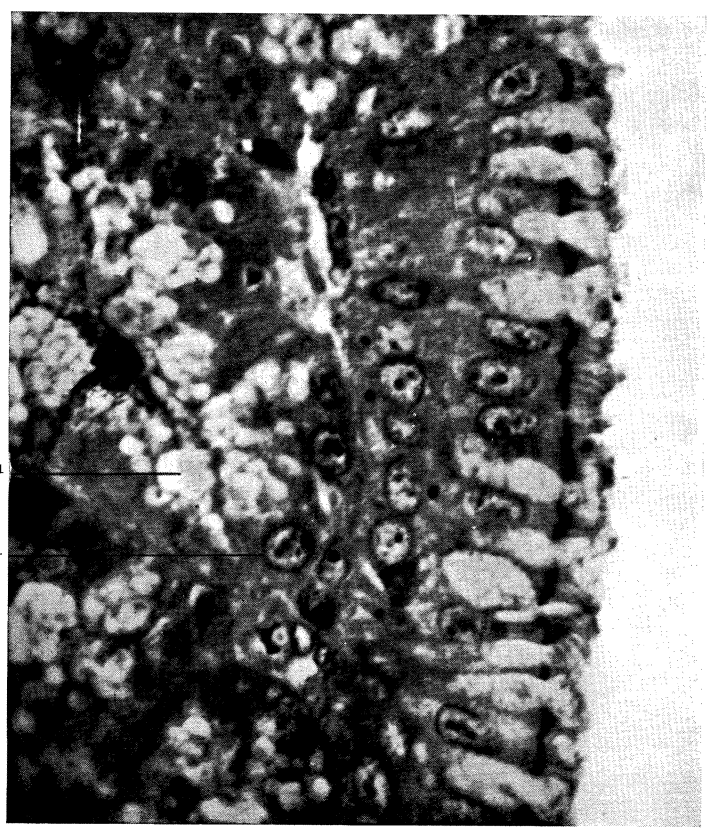

FIG. 8

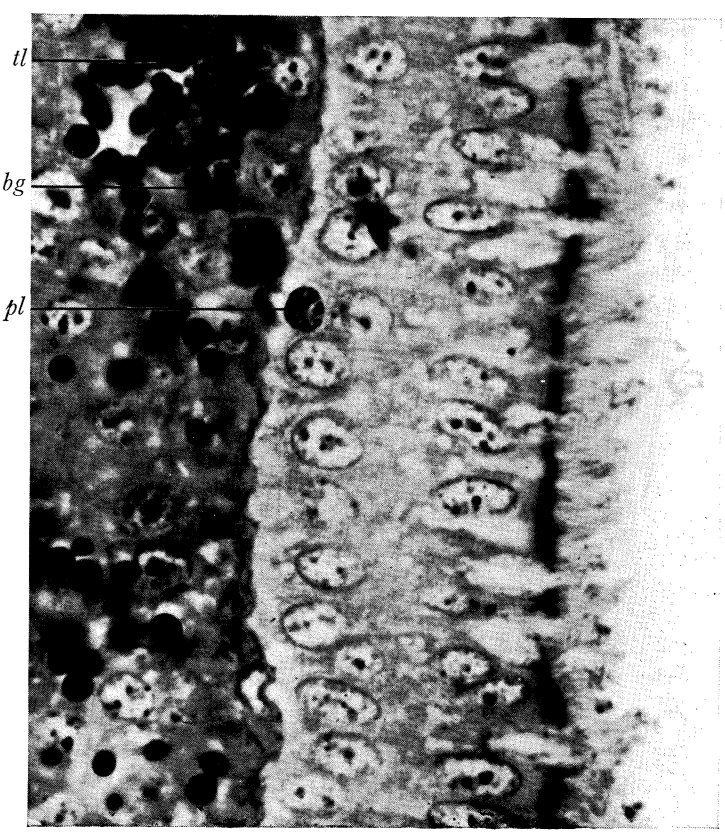

FIG. 9

PLATE 15

FIG. 10—Fowl 9. Portion of ciliated epithelium and corium from upper albumen region. Resting albumen glands appearing first on tips of folds, containing black secretion. Lumen of gland completely filled with secretion (*cf.* fig. 18). Ciliated epithelium regenerated and normally containing few mucous cells. Hence lack of differentiation into apical and basal cells. × 1500.

FIG. 11—Fowl 15, egg laid about 5 hours previously, and next yolk about to enter infundibulum. An area of heavy brown staining tubules near core of fold in albumen region. Glands separated slightly in sectioning, have maximum albumen content with secretion not opaquely stained as in fig. 10. Tubule lumina almost obscured and nuclei shrunken and opaque. Extreme reduction in connective tissue stroma shown at, *x*. × 2000.

FIG. 12—Fowl 16. Glands from albumen region, separated in sectioning. Secretion stains paler and gives areas in fold which are much less dense under low magnification than in fig. 11. Albumen has fused into larger masses, some of which are almost unstained. × 1750.

FIG. 13—Fowl 16. Glands from area of albumen region, where secretion has almost disappeared from epithelium and regeneration is commencing. Nuclei have expanded sufficiently to show chromatin masses, but not to the extent shown in fig. 8. Alveolar cytoplasm is empty and residual secretion lies in gland lumina. × 1300.

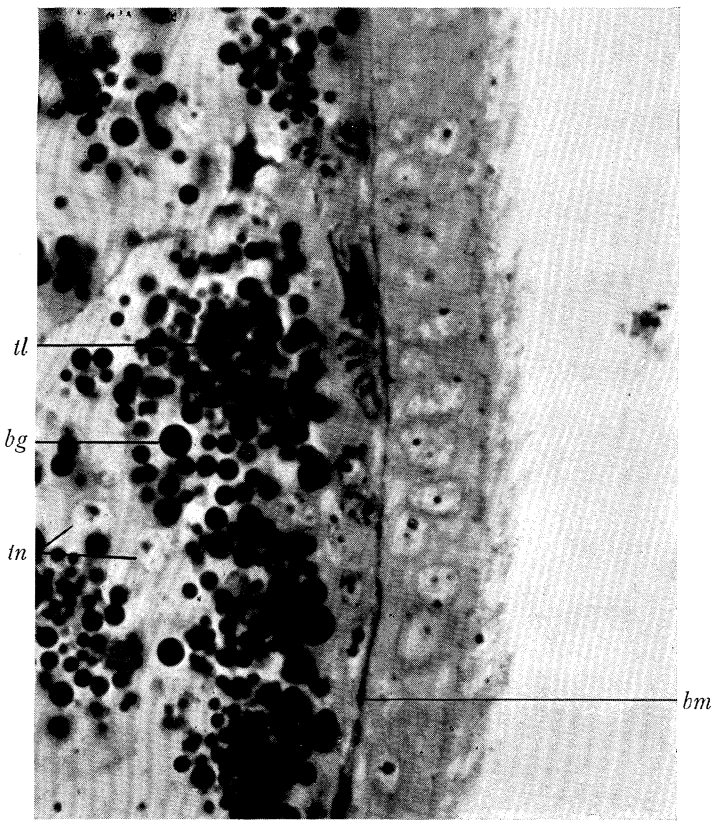

FIG. 10

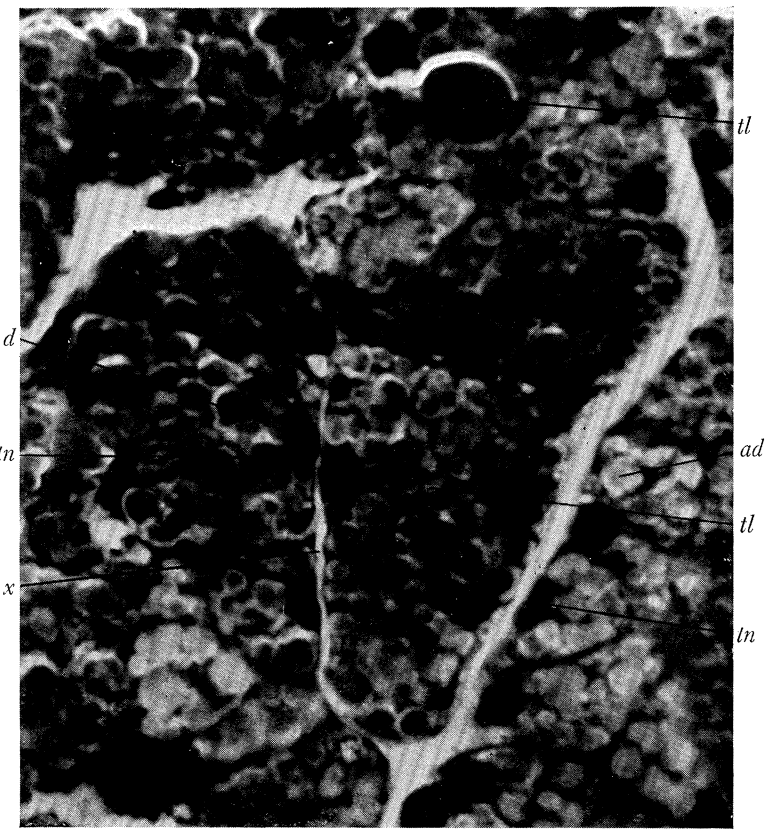

FIG. 11

FIG. 12

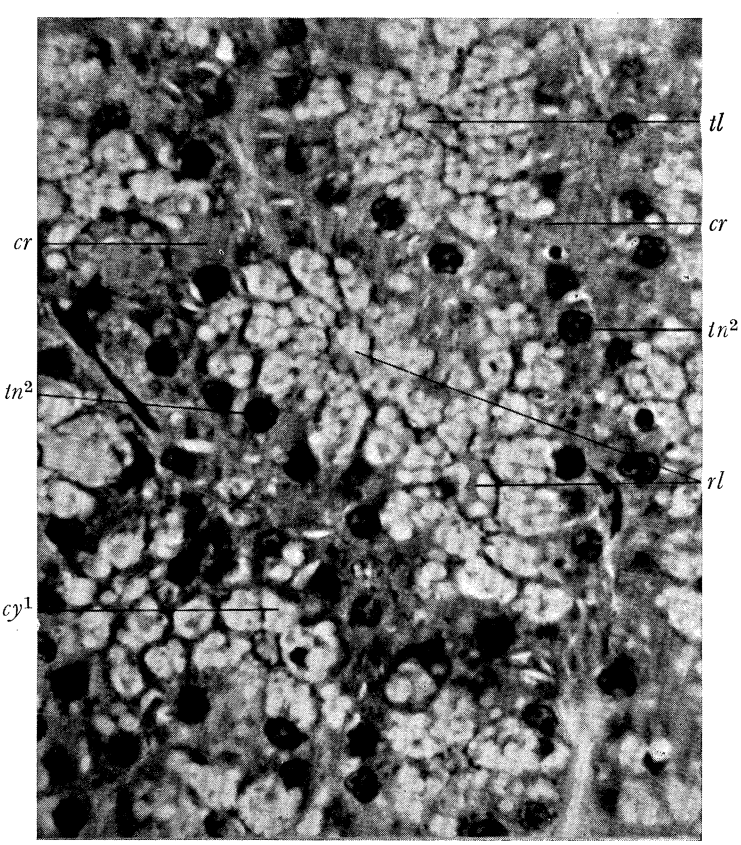

FIG. 13

Plate 16

Fig. 14—Fowl 6, with completed egg in uterus and next yolk in cranial portion of albumen region. Portion of sagittal section through opening of several albumen glands into crypt, to show residual albumen exuding in droplets. Note pale staining of secretion. × 1500.

Fig. 15—Fowl 10, egg in uterus with membranes only faintly white with calcification. Showing a duct and adjacent glands in isthmus. Note secretion is uniformly granular and heavily staining, flowing from glands as granules which coalesce into dense rod-like masses which lie in the duct like a bristle. During staining the apical end *x* of the secretion has twisted during hydration and dehydration so that it now lies above the cells of the duct epithelium. In oviducal lumen secretion remains viscous, *z*. × 1450.

Fig. 16—Fowl 10. A sagittal section through an isthmal gland duct, showing clearer detail of structure of tubular glands. As the secretion from two glands forms threads of ovokeratin, *x* and *y*, these fuse into a fibre *z*, extending into the duct. × 1900.

Fig. 17—Fowl 5, an egg in isthmus with thin shell membrane already formed. Portion of ciliated epithelium and corium of isthmus. Note glandular activity in non-ciliated epithelial cells, resulting in bleb formation. Nuclei of mucous cells not necessarily basal as in albumen region. Note vacuoles between basement membrane and basal nuclei are common during secretion. Similar vacuoles on apical aspect of the nuclei are shown in fig. 15. × 2600.

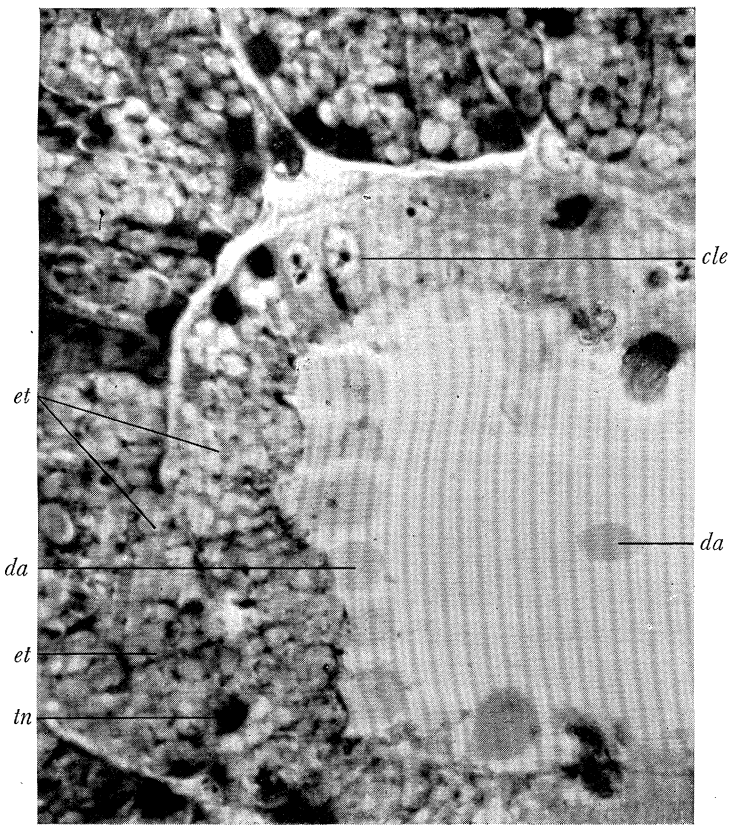

FIG. 14

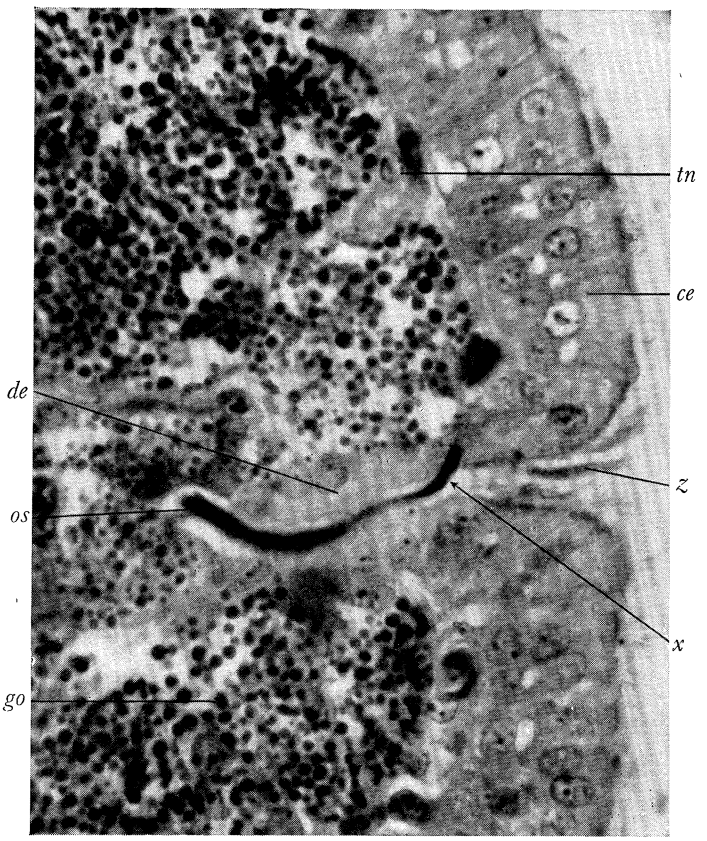

FIG. 15

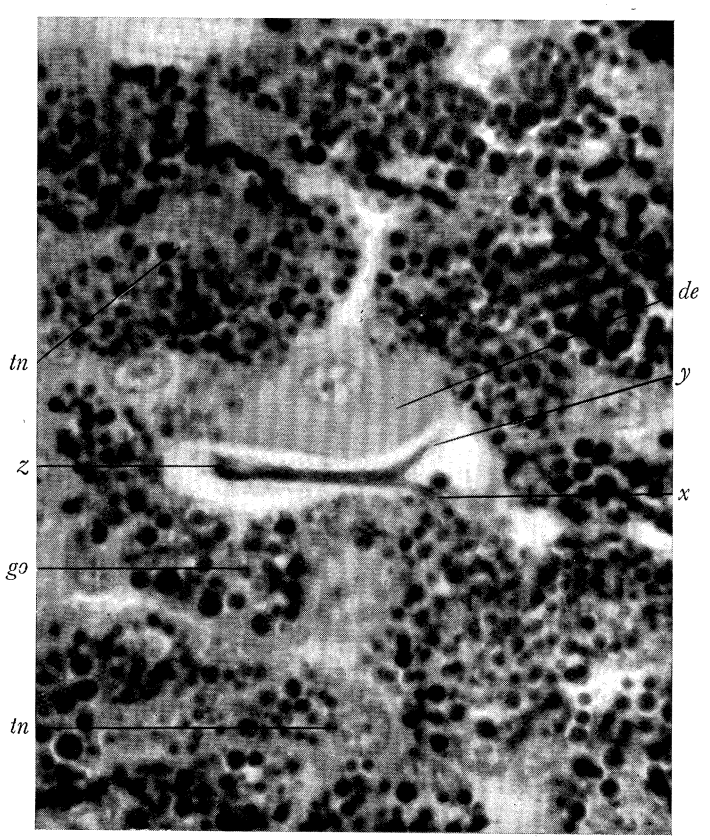

FIG. 16

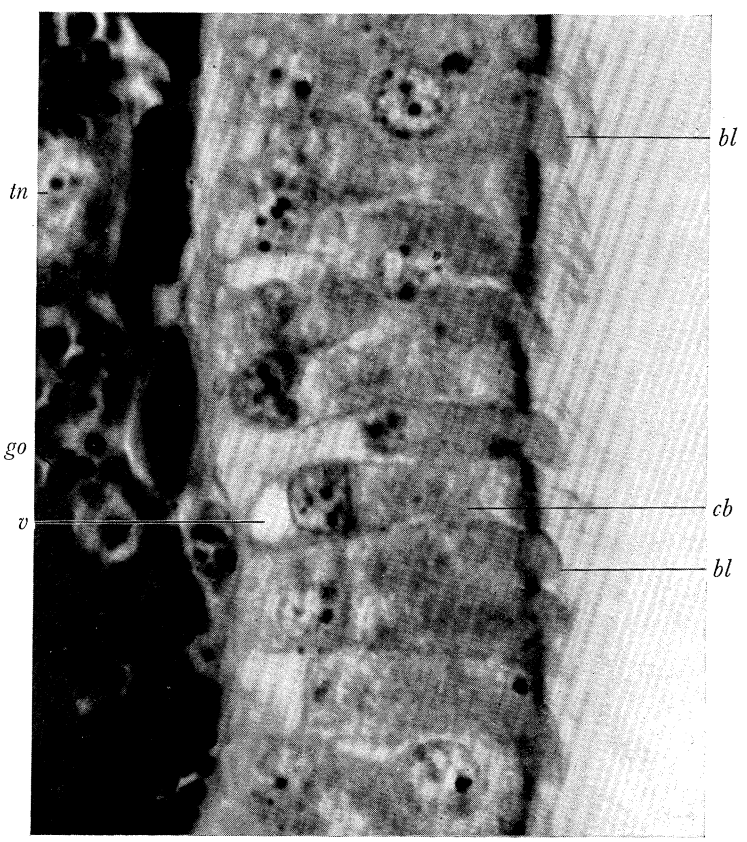

FIG. 17

Plate 17

Fig. 18—Fowl 9, uterus containing a partly calcified shell. Representing a region in the isthmus with maximum depletion of the secretion in the gland epithelium. Note particularly the lumen of the gland at *x*, where the free secretion remains unaltered as viscous droplets. × 1050.

Fig. 19—Fowl 9. An area of uterine glands during active secretion. The cytological characters of the gland epithelium are clearly shown, the preservation being better than is usually found in this region. Note the patent lumen in each gland and their general reduction in diameter (*cf.* fig. 13). The cytoplasm is only faintly granular and remains so throughout secretion and rest. × 1400.

Fig. 20—Fowl 7, an almost completed egg in uterus. Portion of the isthmo-uterine junction showing its ciliated epithelium and tubular glands of the special type. The vacuolated and sparsely granular nature of these glands is characteristic of this region. Ciliated epithelium is similar to that in isthmus and is actively secreting from its non-ciliated cells. × 1300.

Fig. 21—Fowl 8, egg laid and a fresh yolk about to enter the infundibulum. Two opposing surfaces of ciliated epithelium from the mucous region at the caudal extremity of albumen region. After fixation in mercuric chloride and acetic acid, the mucin secretion is shown migrating from the epithelium, though the oviduct is empty. Note the destruction of the gland epithelium and its stored secretion, being the result of this type of fixation. × 1400.

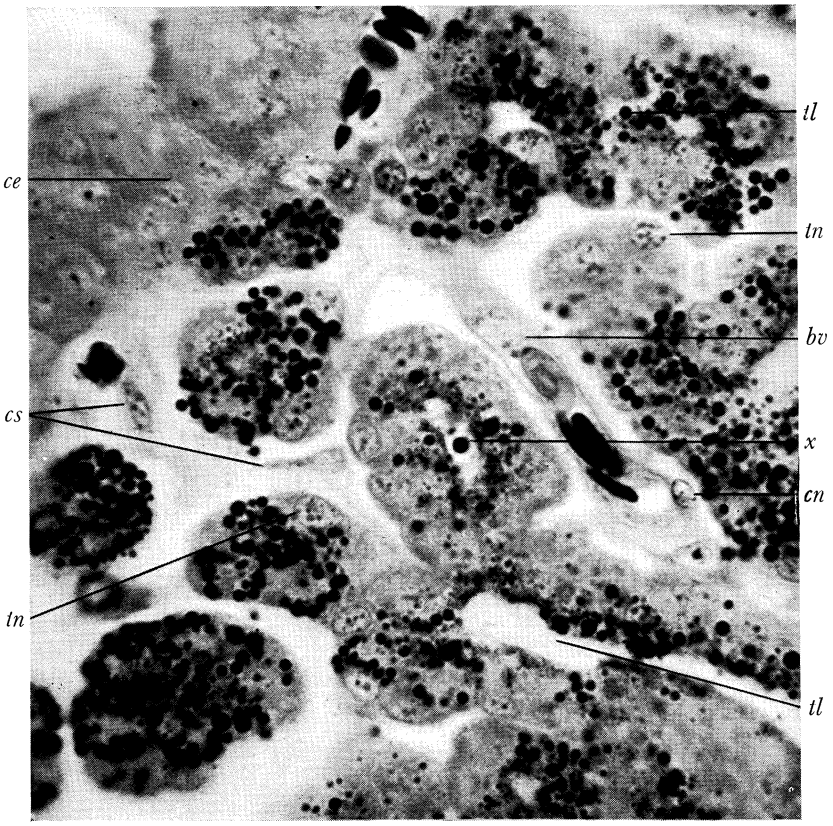

FIG. 18

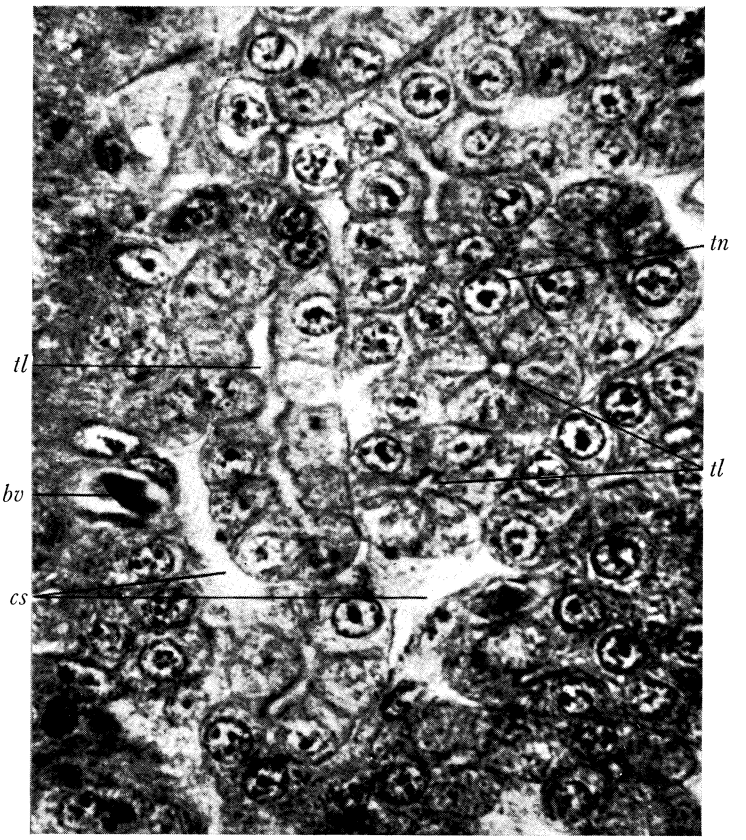

FIG. 19

FIG. 20

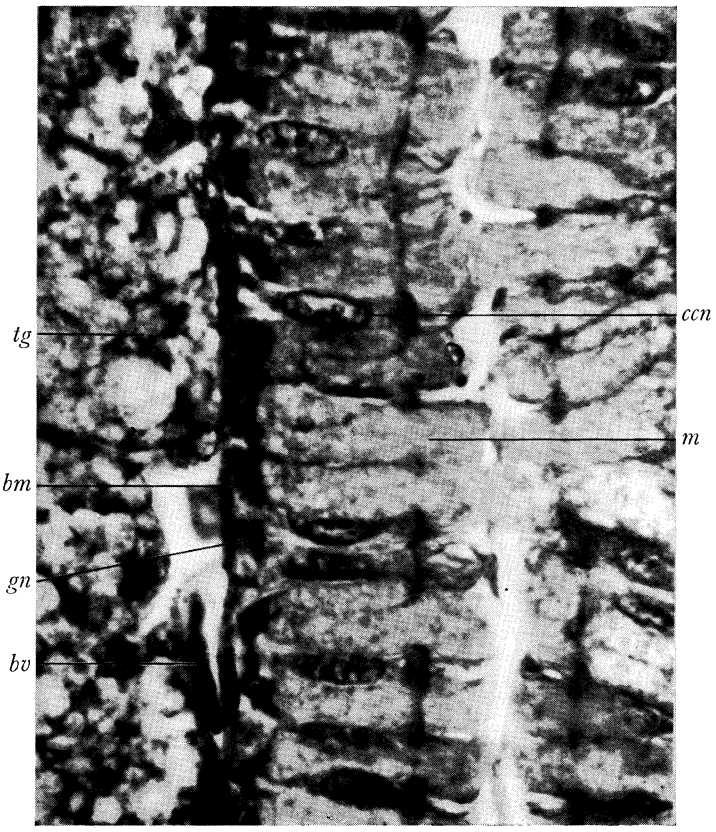

FIG. 21

PLATE 18

FIG. 22—Fowl 5, egg laid about 4 hours previously. Portion of the uterine epithelium and corium incinerated and photographed under dark ground illumination. The section, at 5 μ, shows the ash left by the ciliated apical and basal cell nuclei, together with small residues at intervals in cytoplasm. In the corium, the major portion of the ash is confined to the epithelial nuclei with the remainder consisting chiefly of the cell membranes. Under low magnification an area such as this shows an even distribution of the ash, varied only by the relative cell crowding in the ciliated epithelium. At *x* the edge of the epithelium, which is almost invisible, has been reinforced in printing. × 300.

FIG. 23—Fowl 9, with an egg in the uterus having a soft partly calcified shell. Showing an incinerated section of the uterus similar to fig. 22, but taken during active calcium secretion. Section at 5 μ shows a remarkable increase in the ash of ciliated epithelium, which, under low magnification, forms a conspicuous white band covering the folds. The increase in ash is due to a finely divided greyish deposit filling the cytoplasm, and particularly because the ciliated cell granules have left large, opaque white residues in a position apical to the nuclei. Note the alternation of these granules with the basal cells, which leave an ash confined more conspicuously to their nuclear zone near the basement membrane. The tubular glands have a slightly increased ash, particularly in their nuclei, but there is no evidence of specific secretory granules of high calcium content in their epithelium. × 175.

FIG. 24—Enlarged portion of same section as in fig. 23, showing the ciliated epithelium and uterine glands in detail. × 450.

FIG. 25—From same section as fig. 24, showing finer cytological structure of the epithelial residues. × 2500.

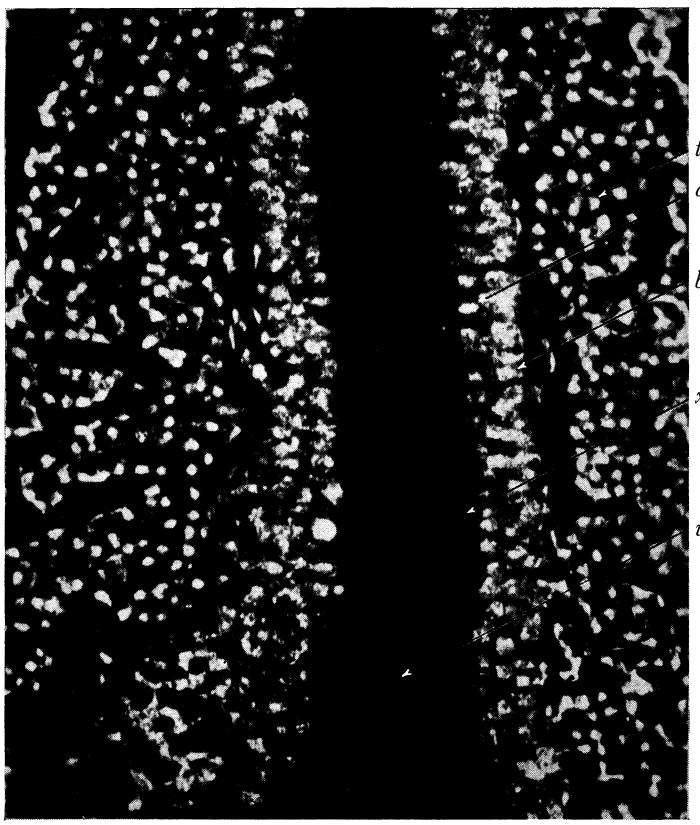

FIG. 22

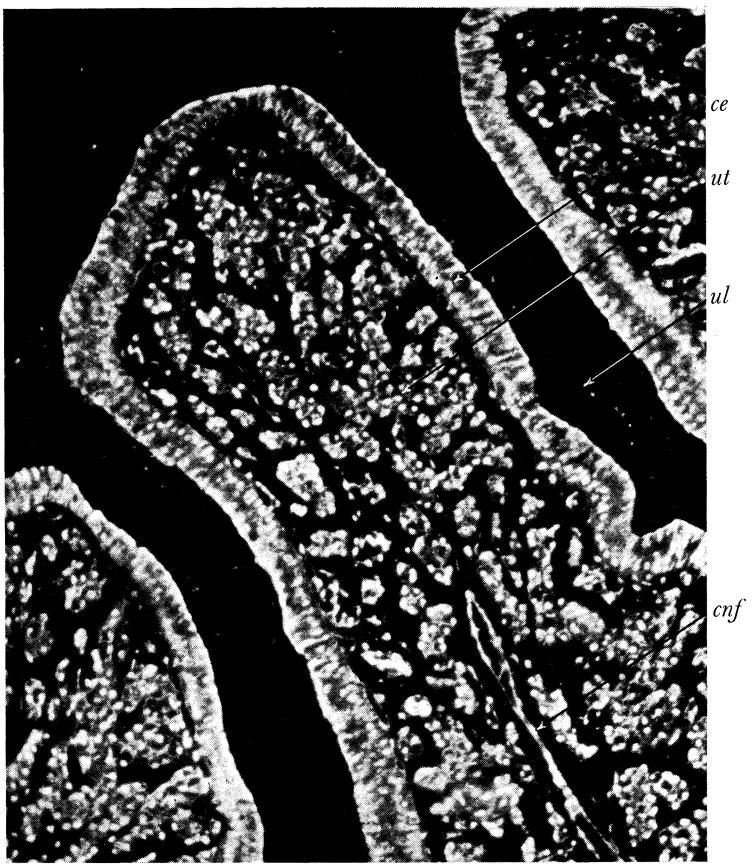

FIG. 23

FIG. 24

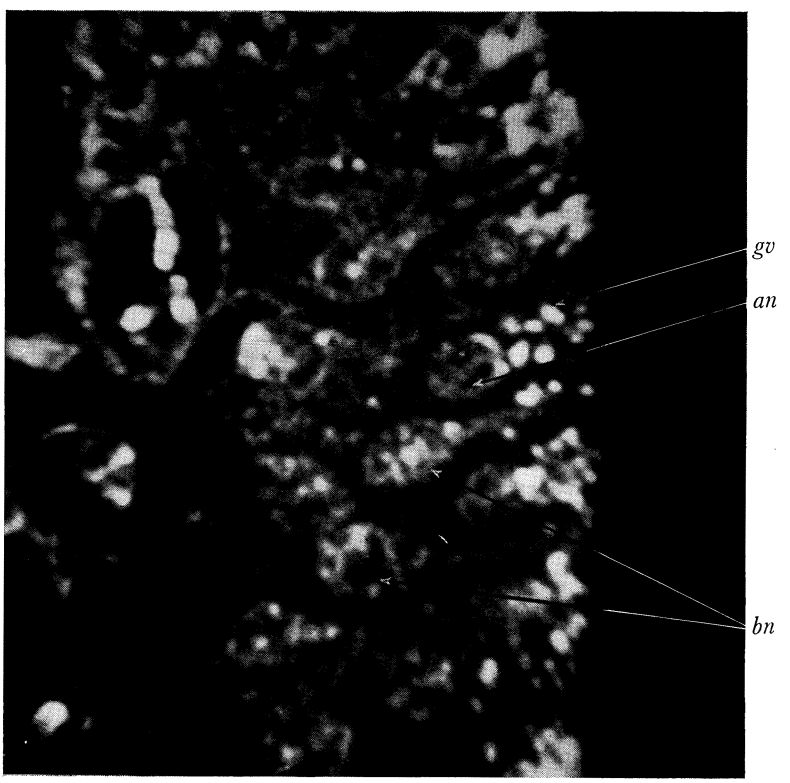

FIG. 25

Plate 19

Fig. 26—Enlarged area of uterine glands from same incinerated section as in fig. 23. × 1100.

Fig. 27—Fowl 23, with a membrane-covered egg in the uterus. Portion of isthmal wall showing the heavy inorganic content, after incineration, of the secretory granules in the tubular glands. × 500.

Fig. 28—Fowl 9. Control section of fig. 23, stained with iron hæmatoxylin. The apical cell granules, which leave a heavy inorganic residue (*see* fig. 25), are shown to extend towards the cilia, where they may be seen passing through the cuticular margin of the cells. × 2800.

Fig. 29—Fowl 10, with an egg in the uterus having membranes only faintly whitened with shell material. A portion of the uterine epithelium similar to fig. 28, but fixed in Zenker-formol. The granules in the apical cells have the same staining properties and position as those in the previous figure. × 2800.

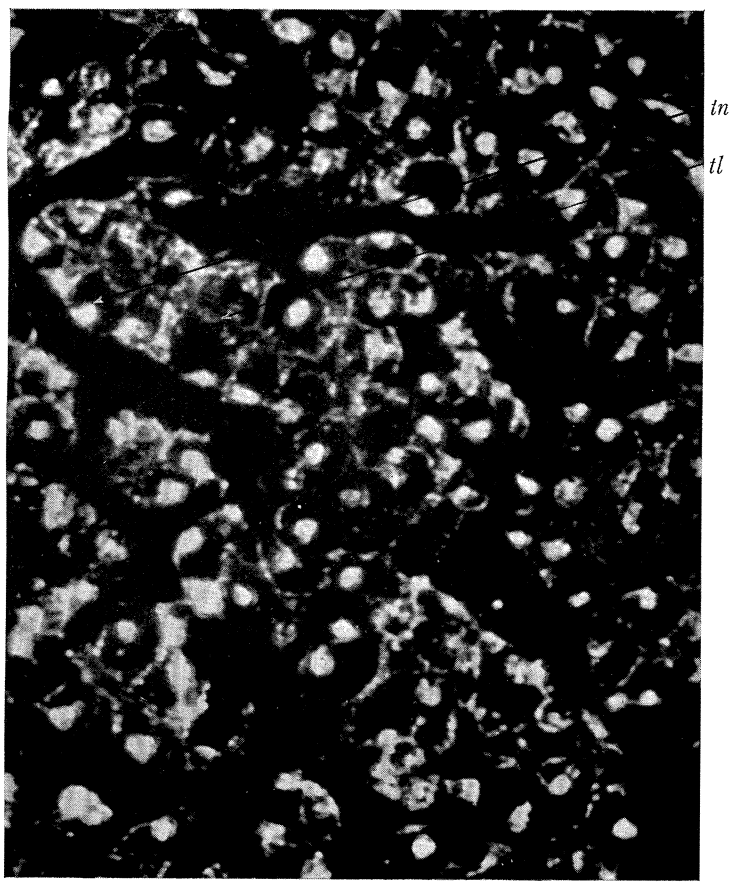

FIG. 26

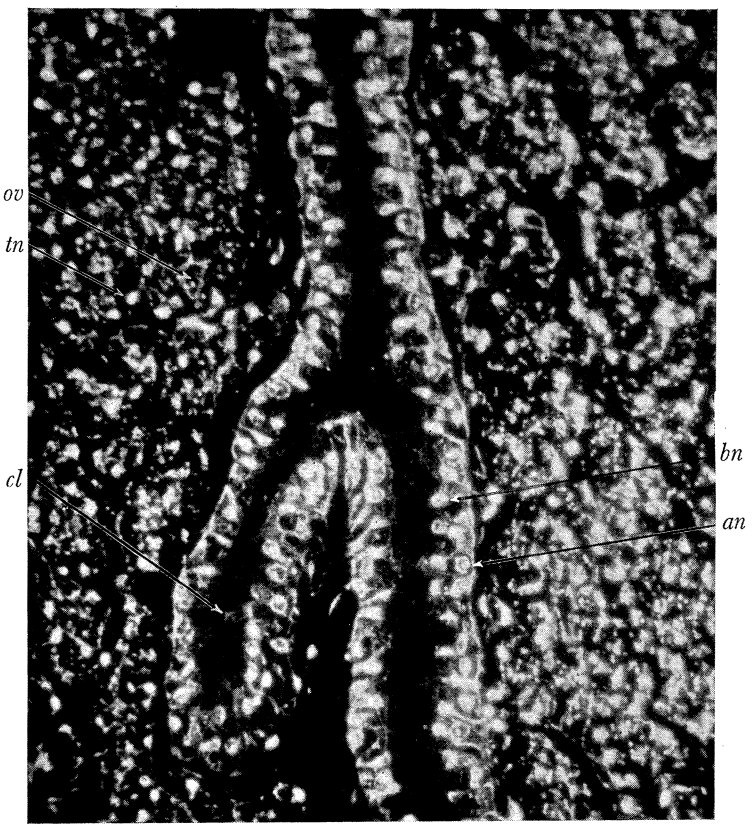

FIG. 27

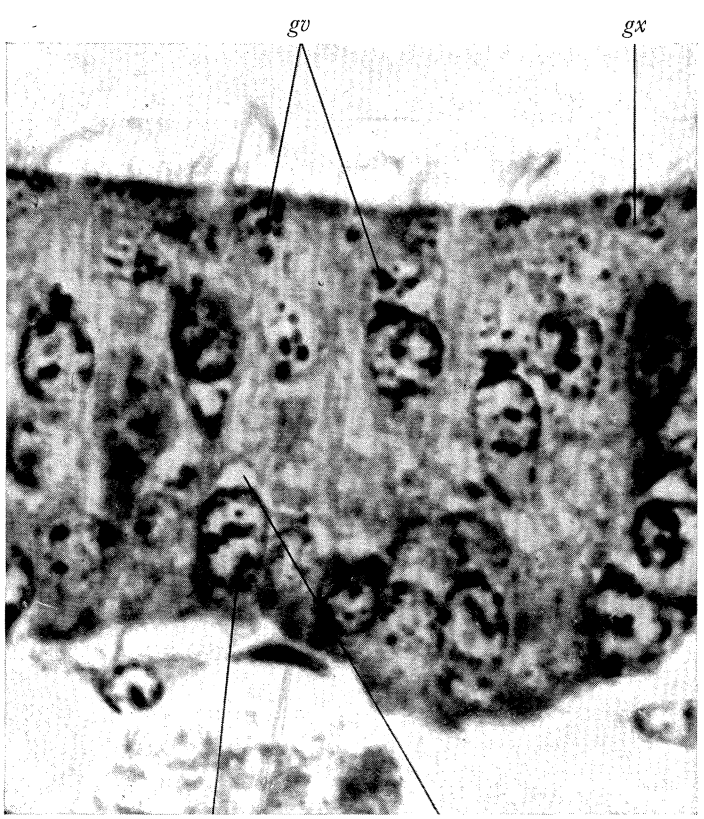

FIG. 28

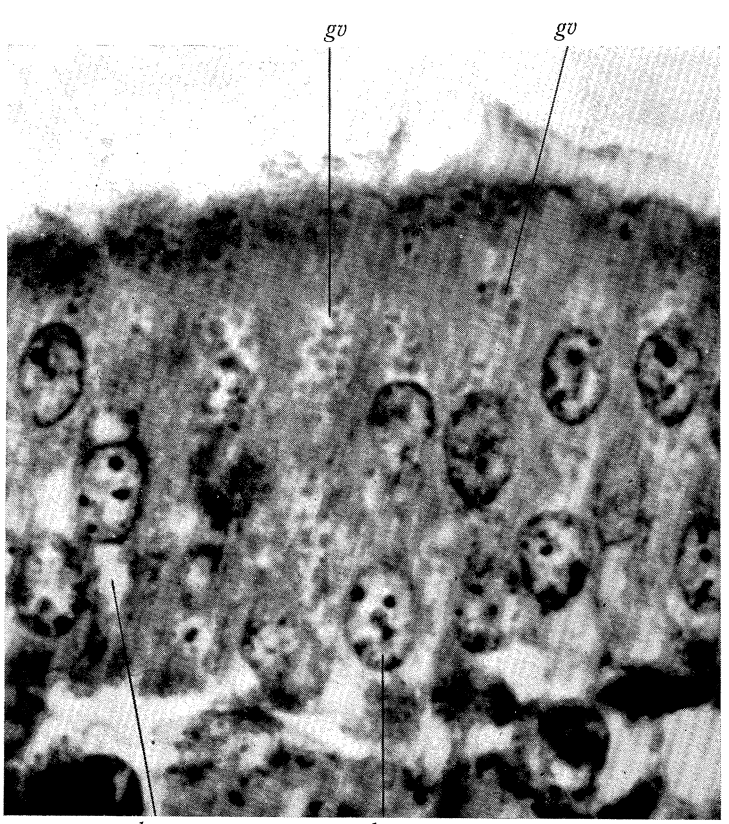

FIG. 29

Plate 20

Fig. 30—Fowl 10. Portion of a silver impregnation of the uterine epithelium. Note the Golgi reticulum does not extend towards the apical cell margins and that it is reduced in size in the basal cells. × 3000.

Fig. 31—Fowl 2, egg laid several hours previously. Showing the Golgi apparatus in the uterine epithelium. In " A " the aggregation of granules, stained with iron hæmatoxylin, is shown in a vacuolated area between the Golgi reticulum and the nucleus. In " B " clear spaces in the Golgi reticulum, which possibly represent secretory material formed within its meshes, are illustrated. × 3000.

Fig. 32—Fowl 33, egg with completed shell membranes just migrated into the uterus. Showing a section through portion of the uterine muscle coat, the folds of the mucosa lying in contact with the shell membranes. Note the intimate contact between the epithelium covering the flattened surface of the fold at *x*, and between it and the shell membrane the mammillæ of the shell matrix are clearly shown. × 55.

Fig. 33—Fowl 33.—An adjacent area to that illustrated in fig. 32. The layers of the shell have become separated in sectioning and the uterine epithelium has been brought into contact with its surface in the final print. A mass of special secretion, *sm*, staining faintly blue with Delafield's hæmatoxylin lies between the epithelium and the mammillæ. × 265.

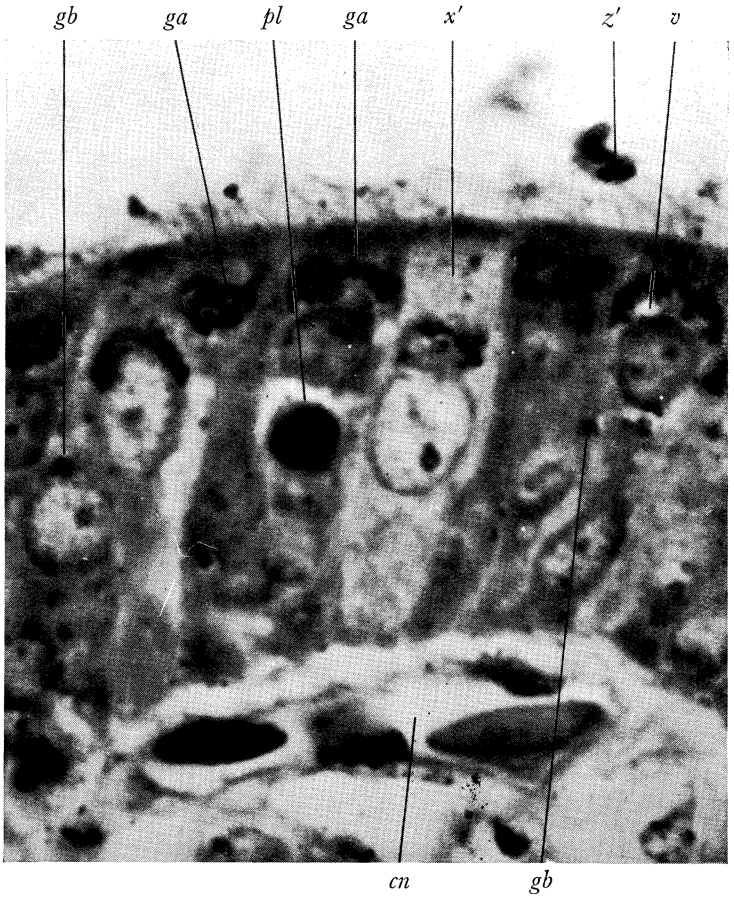

FIG. 30

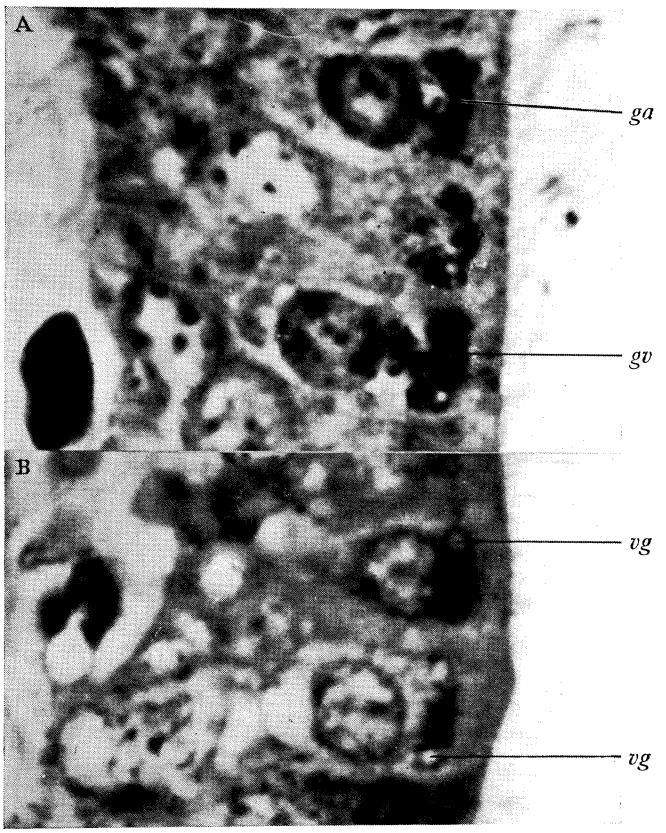

FIG. 31

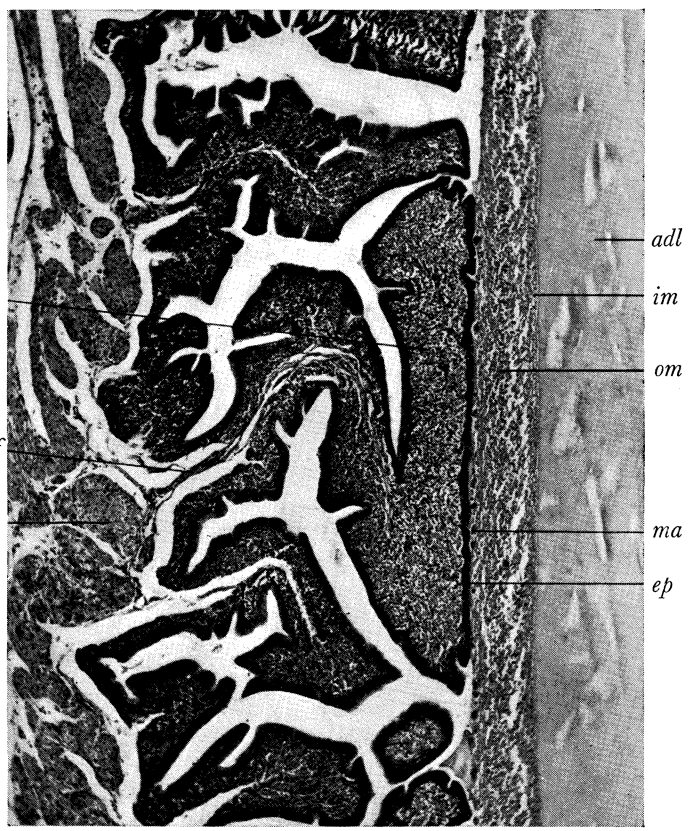

FIG. 32

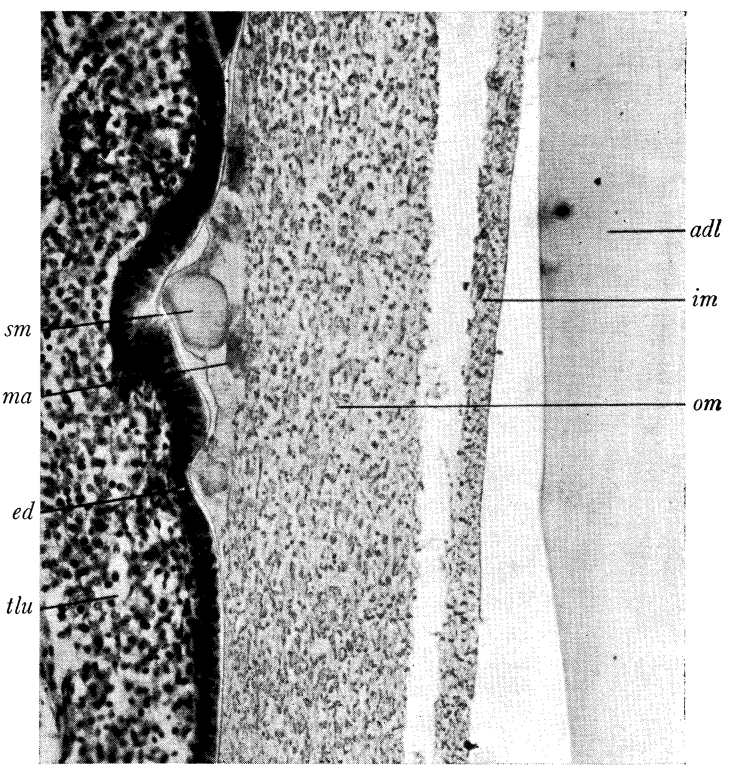

FIG. 33

PLATE 21

FIG. 34—Fowl 33. Showing portions of two adjacent uterine folds some distance from the egg region. A patch of modified epithelium, *ep*, is shown in a phase of excessive secretion. × 400.

FIG. 35—Fowl 33. A mass of shell matrix secretion lying between uterine folds, which in fig. 33 is demonstrated as lying in contact with the developing shell, is contrasted in texture and staining reactions with albumen issuing from a tubular gland. × 400.

FIG. 36—Fowl 34. Portion of a section taken from a uterus containing a completely calcified shell fixed *in situ* and subsequently decalcified. The thickness of the shell matrix and the sparseness of the mammillæ are well illustrated and the condition of the uterine epithelium should be contrasted with that in fig. 32. Note the well-defined cuticular layer on the shell surface which is granular in composition in fig. 37, from the same specimen. The basal cell vacuoles in the epithelium are characteristic of this final secretion stage. × 375.

FIG. 37—Fowl 34. Portion of the uterine wall in contact with the shell surface, of which only portions of the matrix and cuticle are illustrated, where folds characteristic of the vagina occur. The scattered tubular mucous glands are seen in the corium and the thickness of the vaginal epithelium should be noted with its absence of glandular phenomena. × 230.

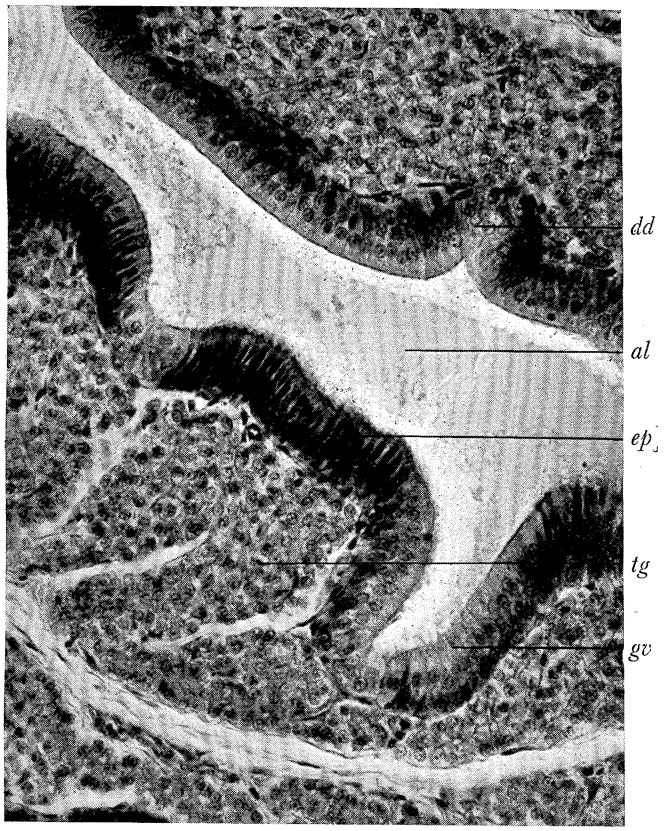

FIG. 34

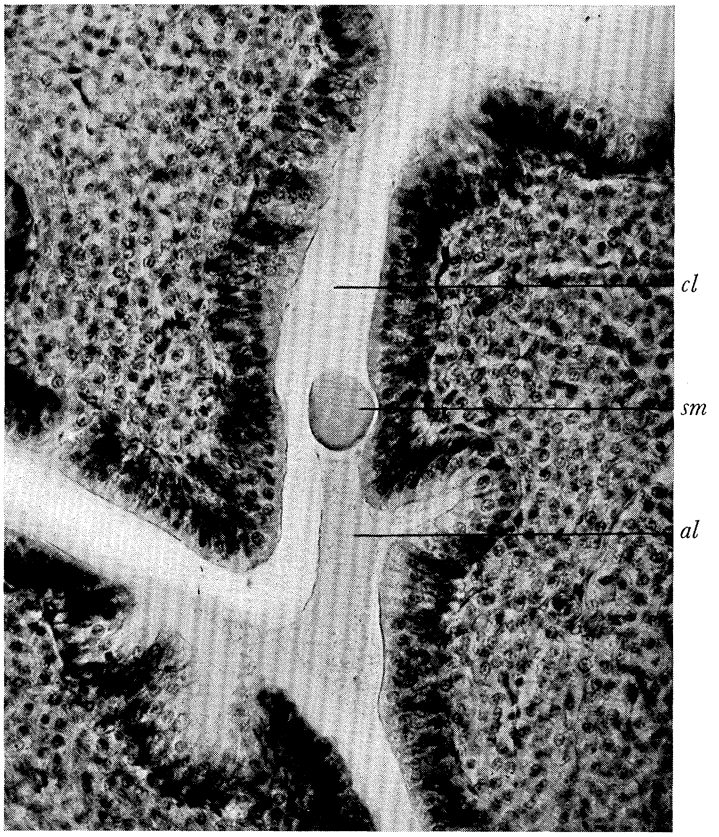

FIG. 35

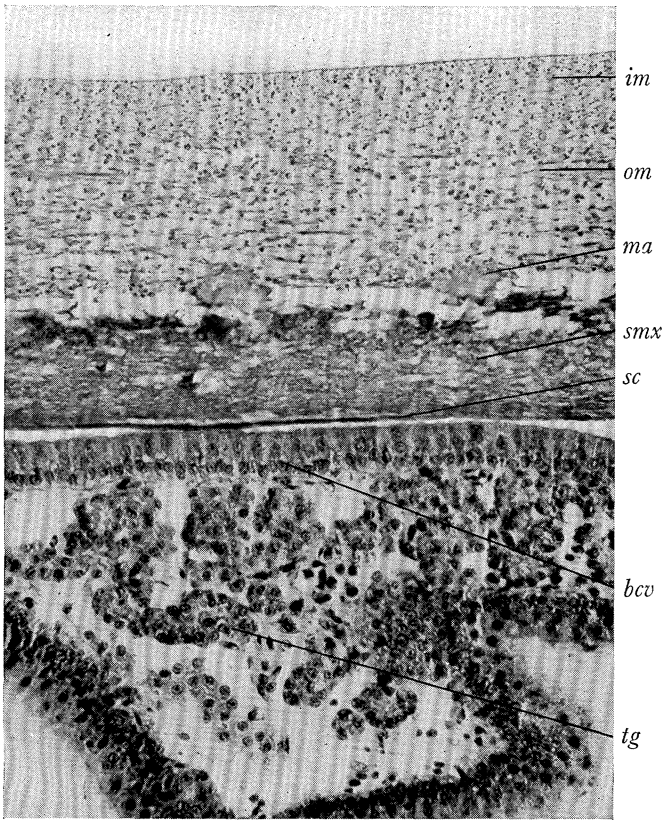

FIG. 36

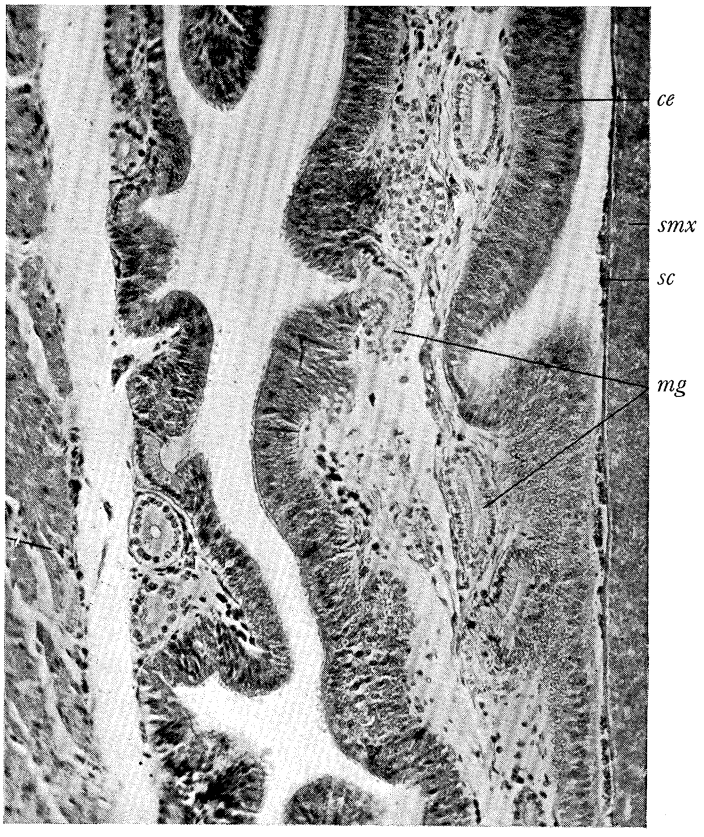

FIG. 37

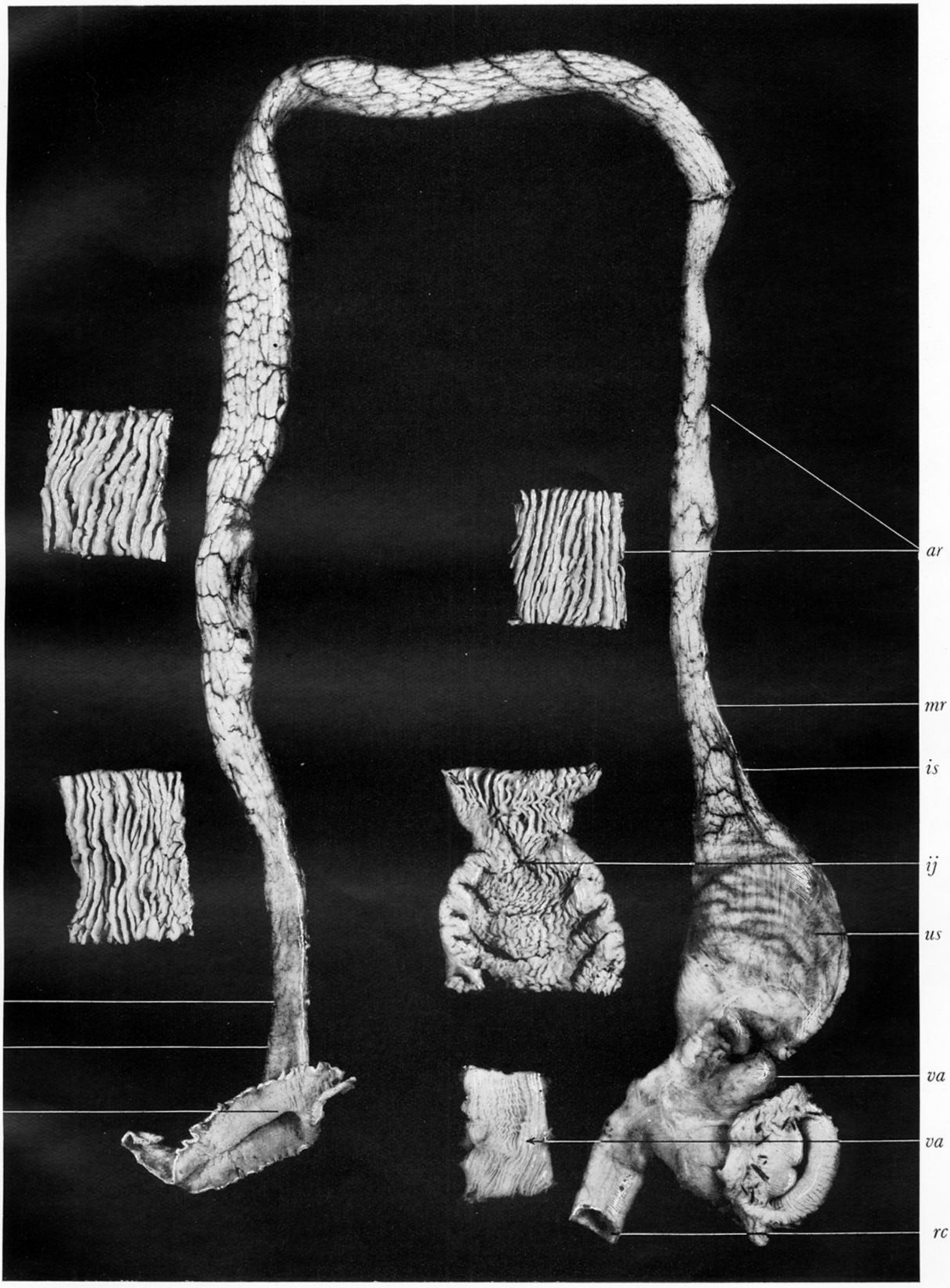

FIG. 1 × ¾

PLATE 12

FIG. 1—Dissection of the empty, functional oviduct of the fowl including portions of the wall in surface view. These are placed to the left of corresponding levels in the entire specimen and illustrate the arrangement of the folds in the major regions. × ¾.

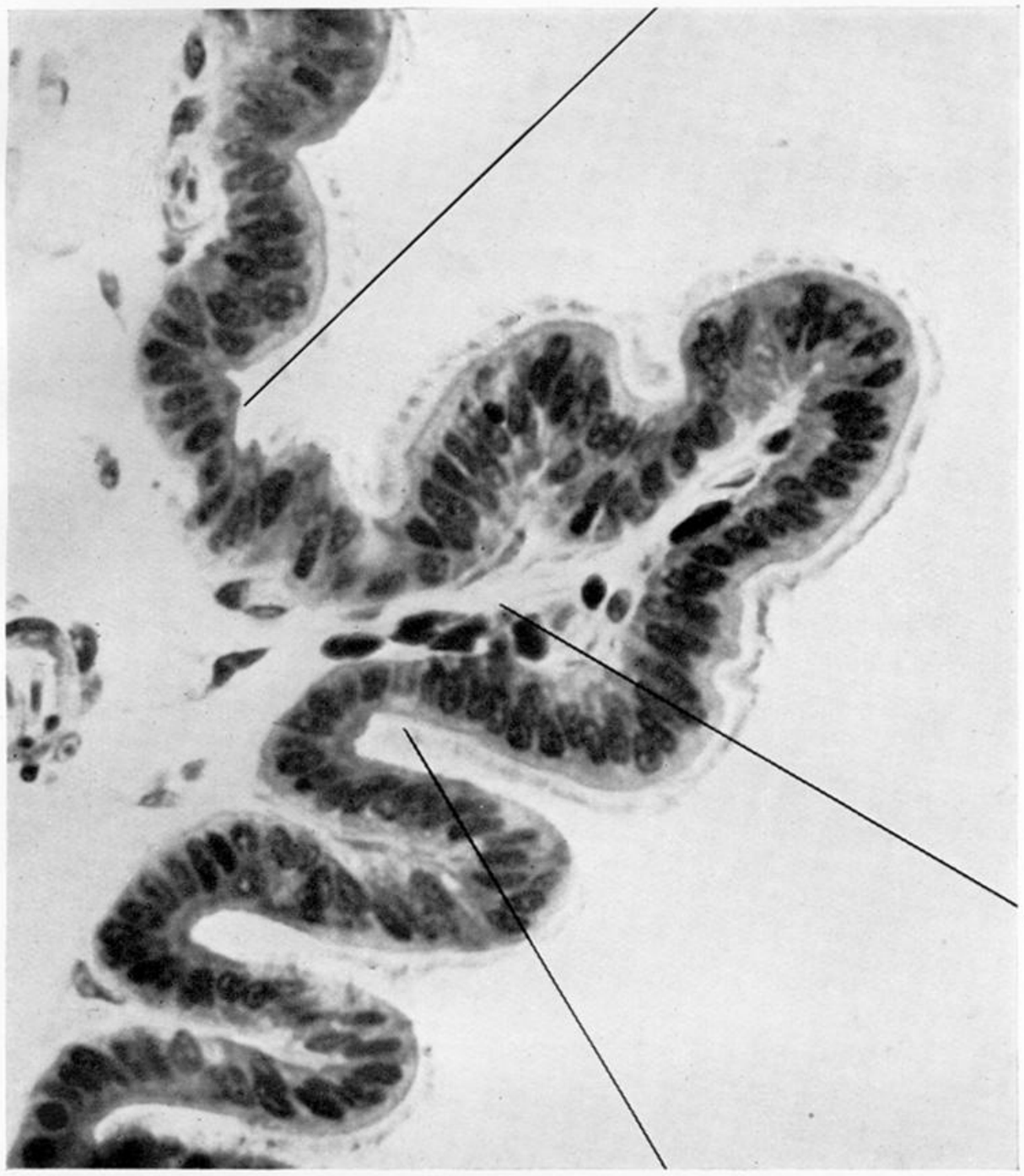

FIG. 2

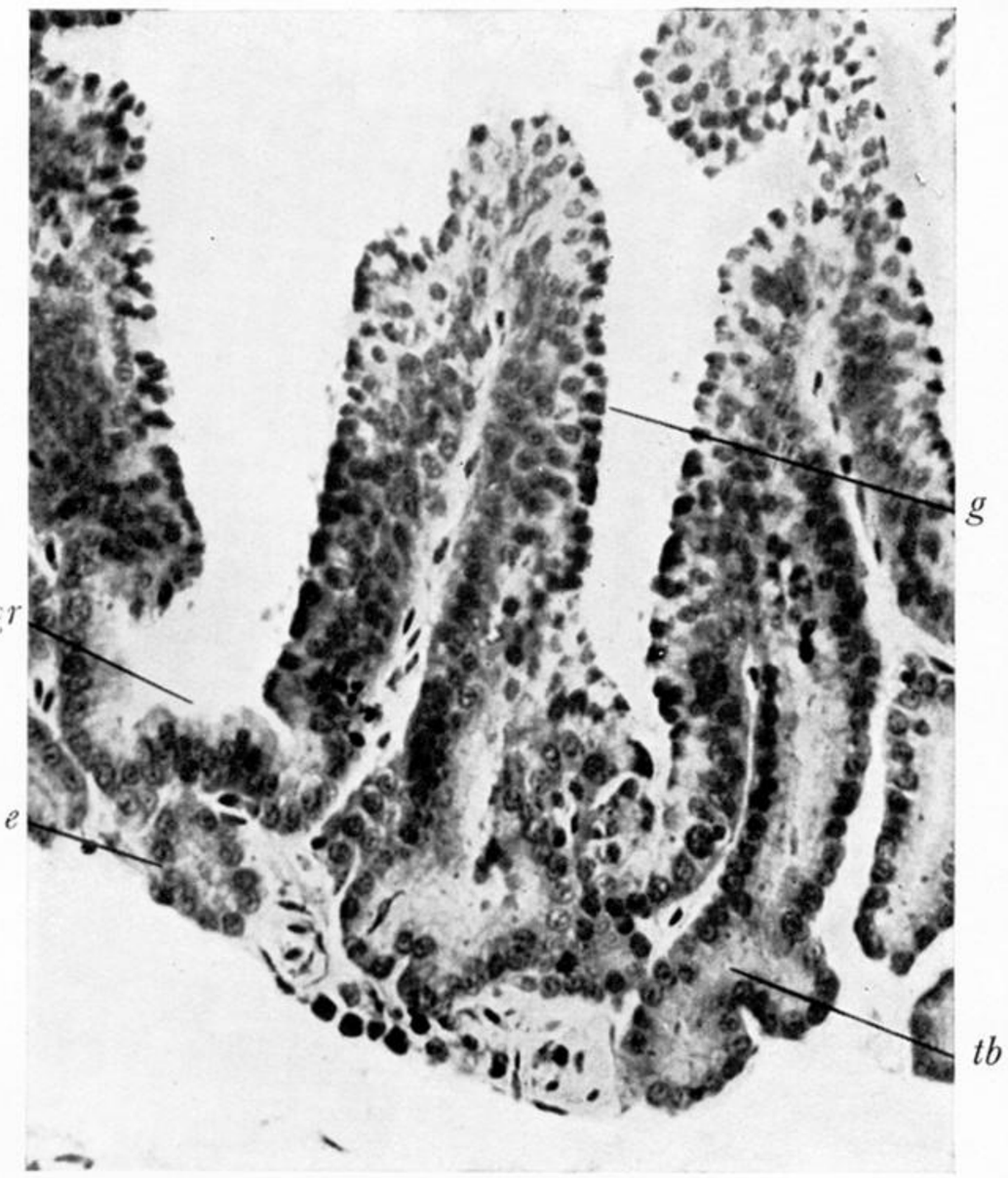

FIG. 3

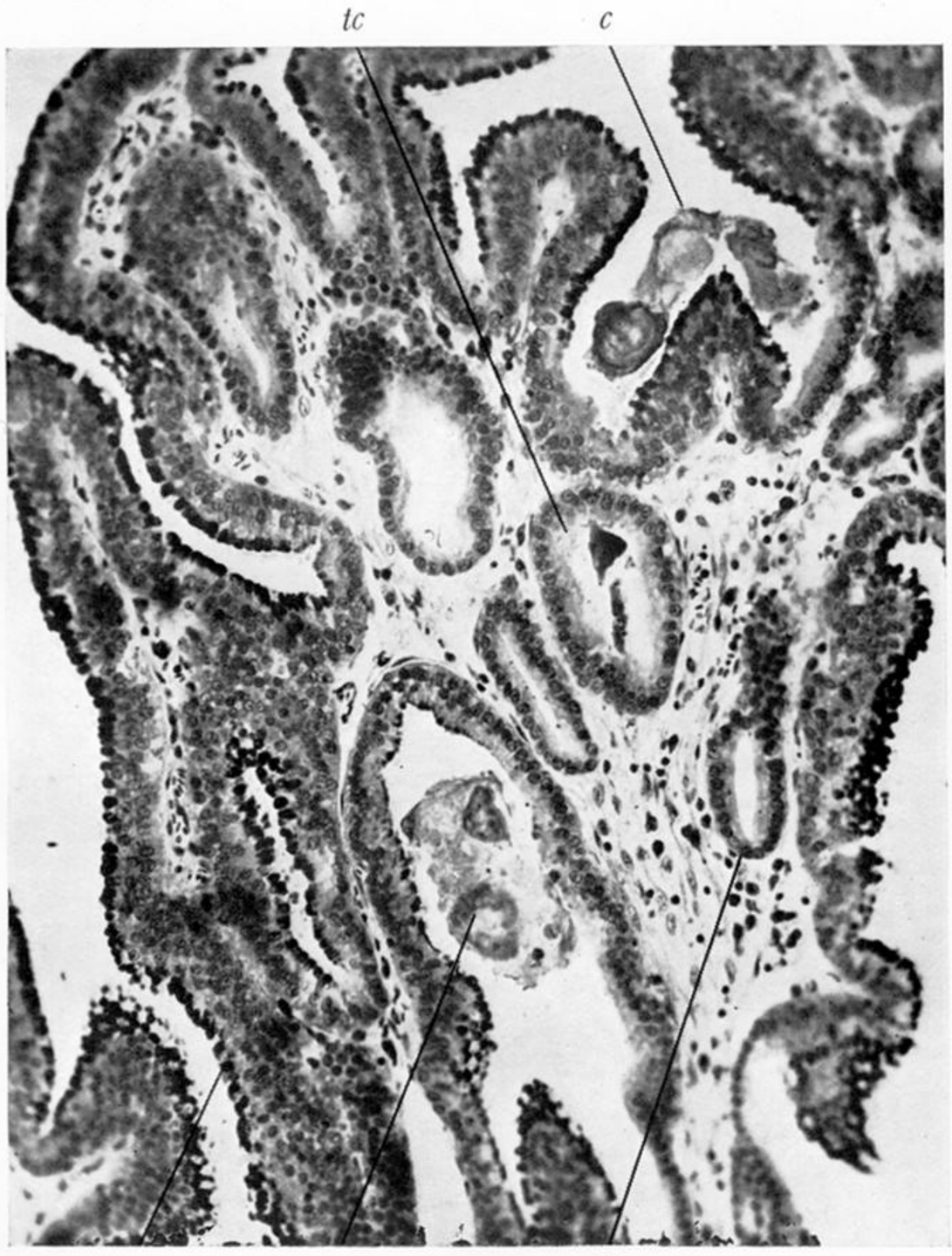

FIG. 4

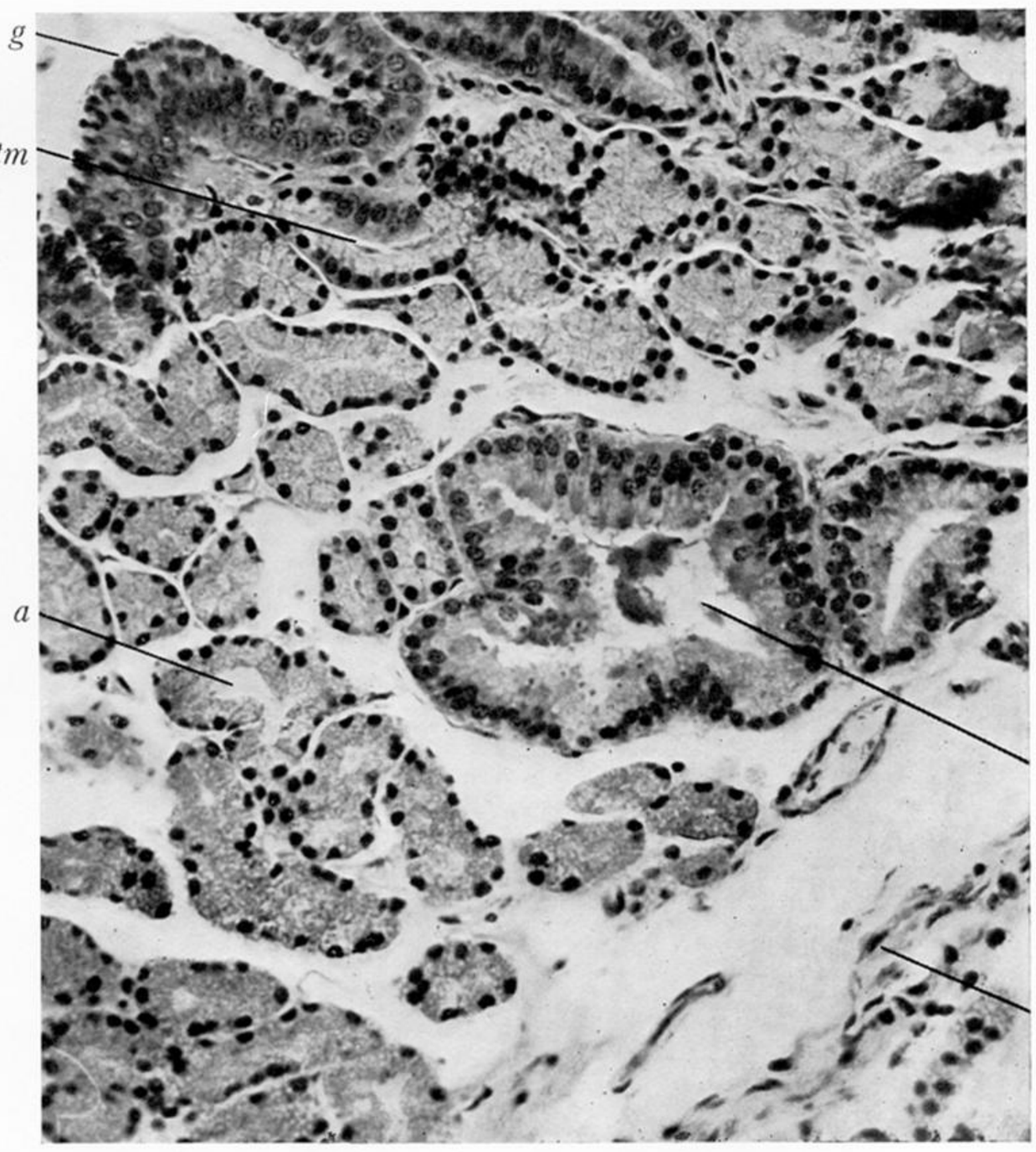

FIG. 5

PLATE 13

FIG. 2—Fowl 29, egg in upper albumen region. Portion of the mucosa of the funnel wall showing folds in the homogeneously ciliated epithelium in which non-ciliated grooves are differentiated as the only glandular structures of this region. × 800.

FIG. 3—Fowl 32, egg in upper albumen region. Section from the cranial part of chalaziferous region, showing the tubular extensions from glandular grooves just commencing. × 250.

FIG. 4—Fowl 32. Portion of the infundibular wall in mid-chalaziferous region. Note chalaziferous glands containing heavy secretion which collects in oviducal lumen as well. × 250.

FIG. 5—Fowl 32. Section from cranial end of albumen region showing the last chalaziferous glands mixed with albumen glands. Note difference between secretion in lumen of each type. × 250.

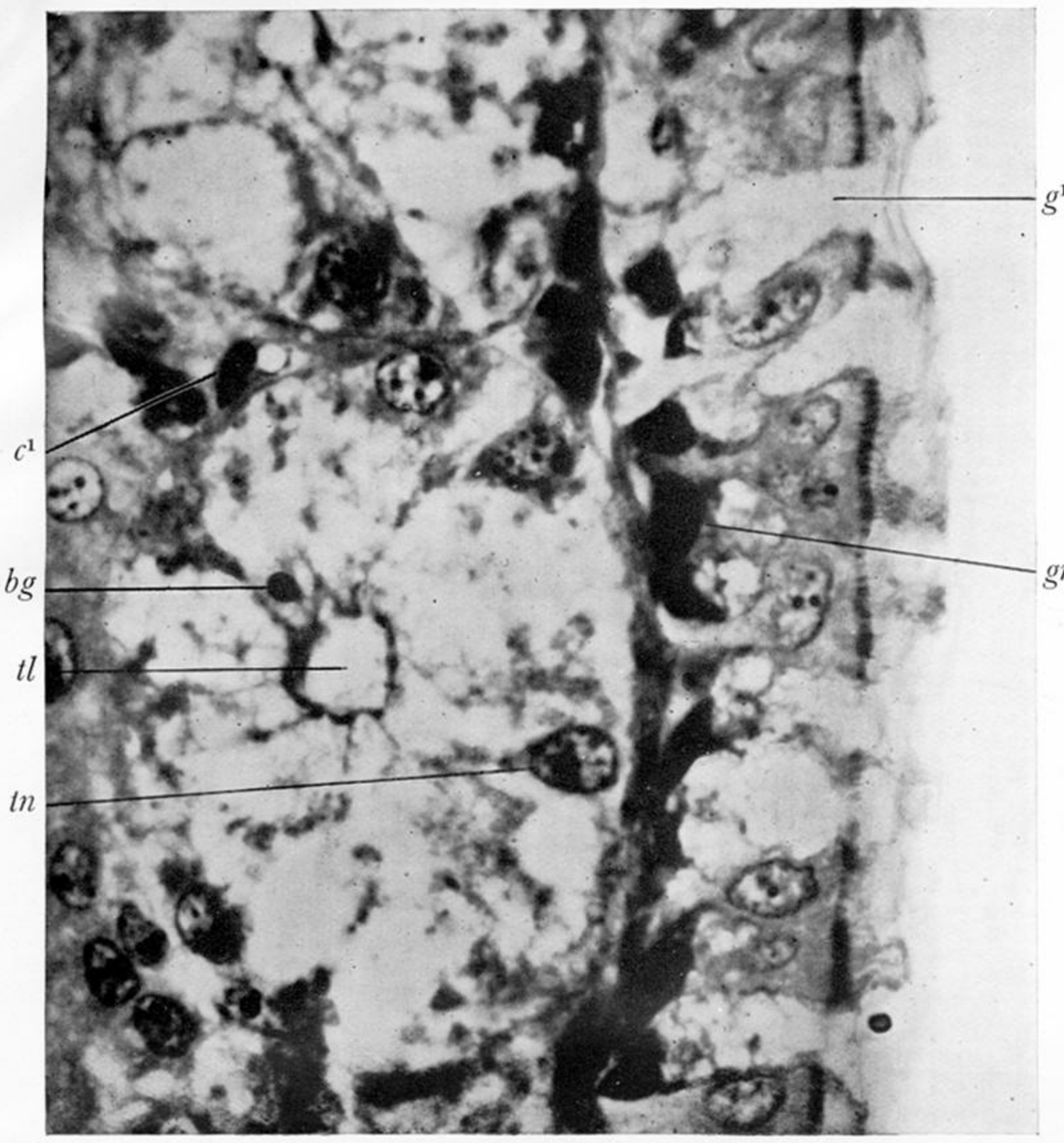

FIG. 6

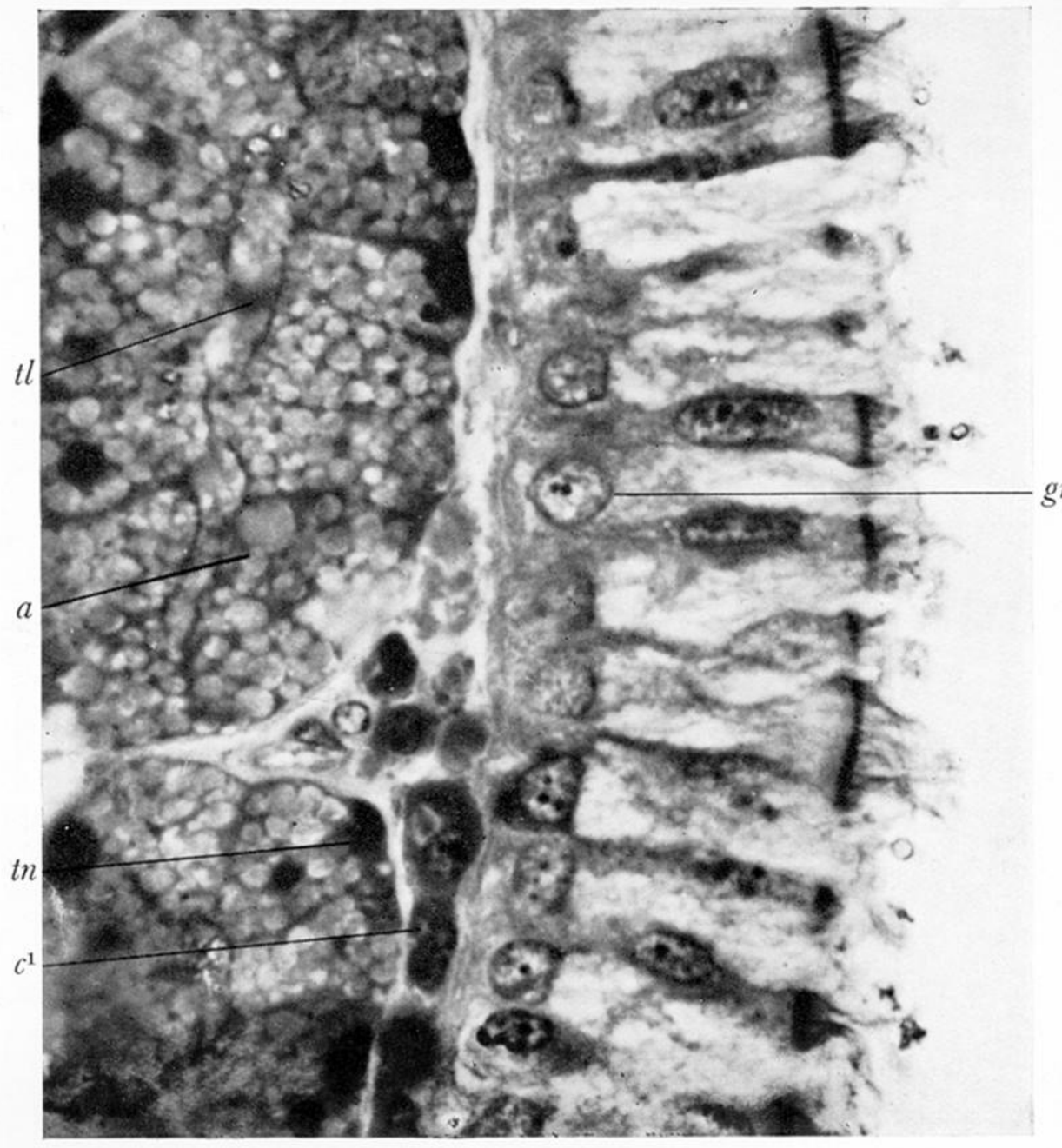

FIG. 7

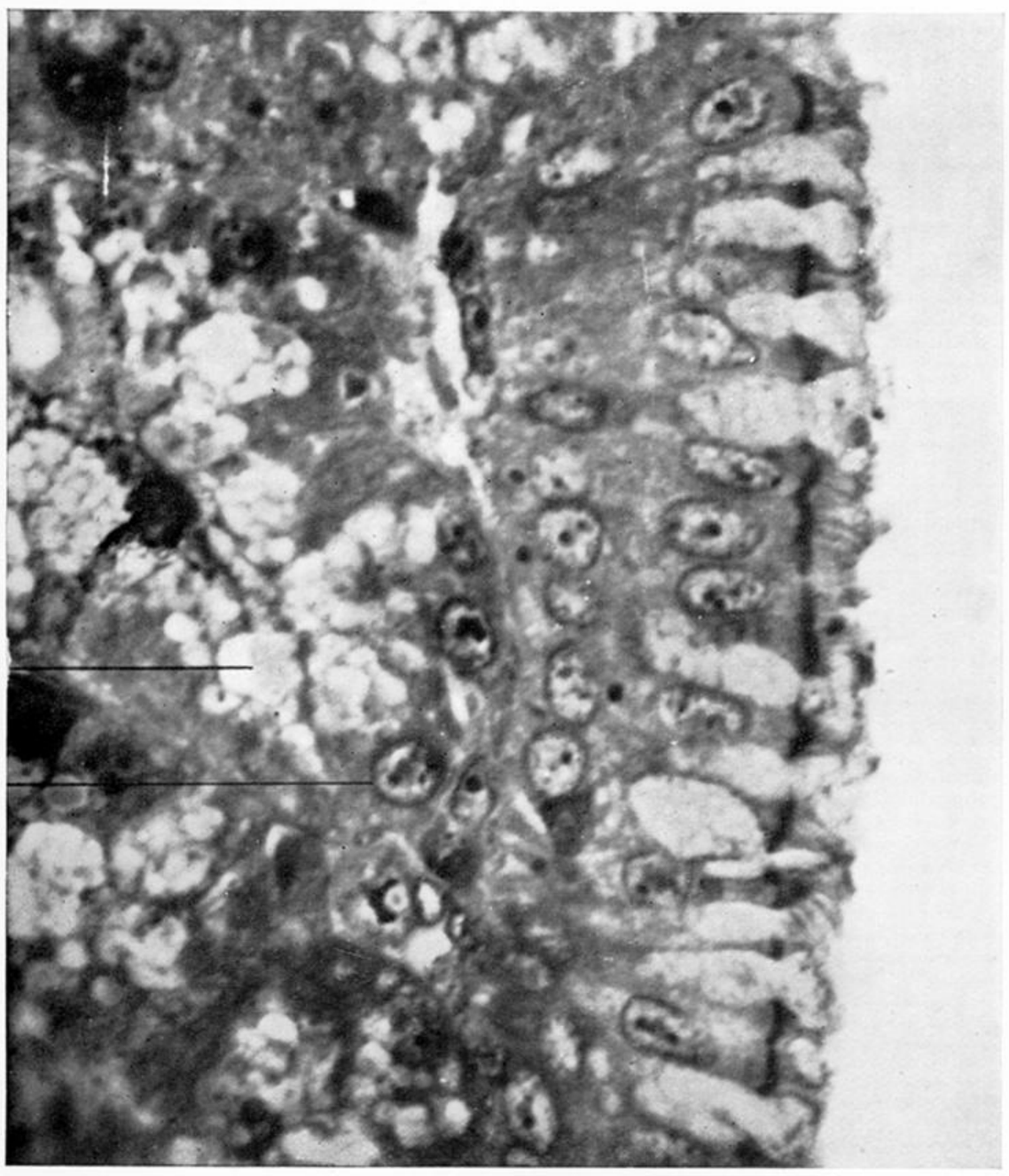

FIG. 8

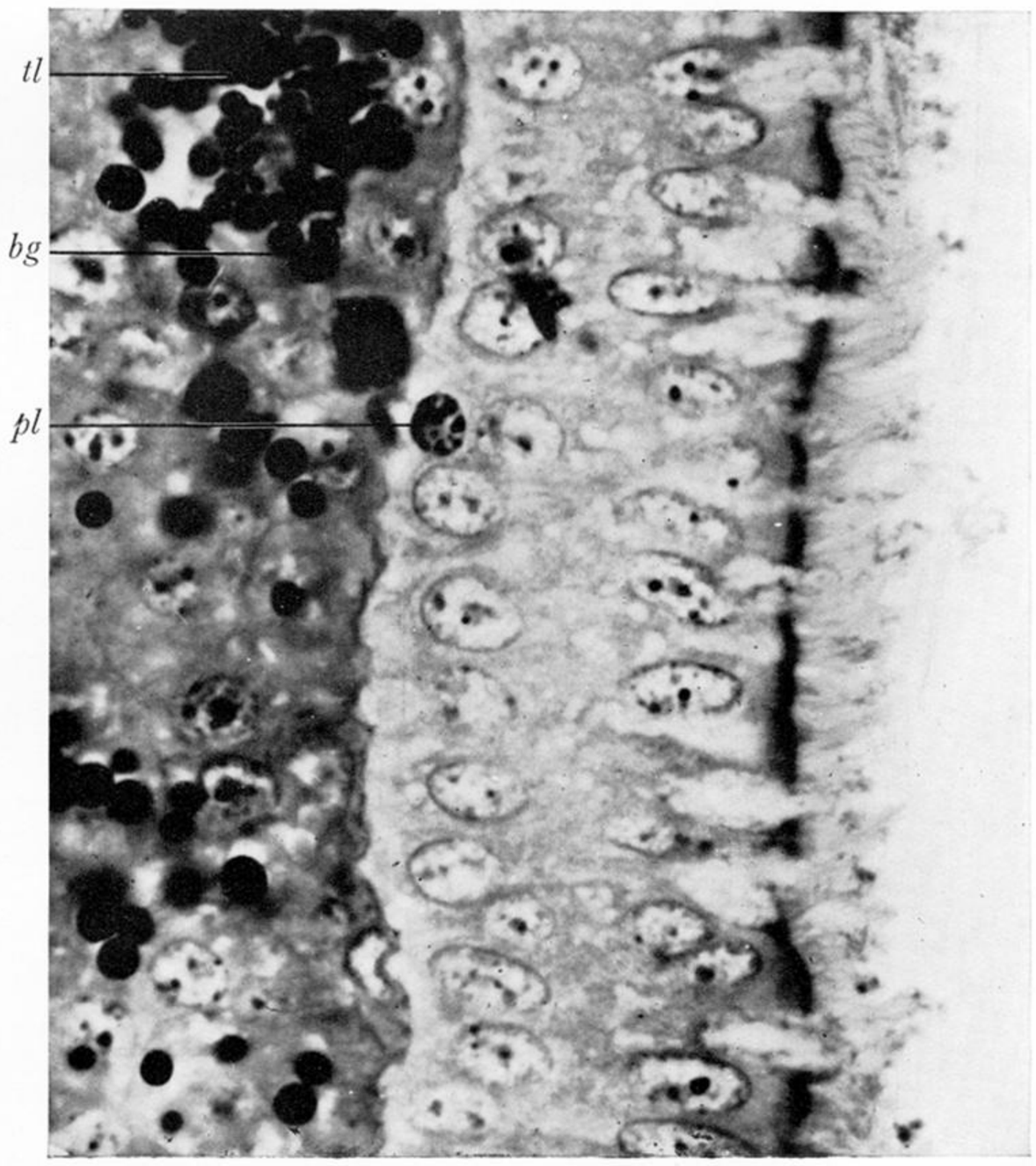

FIG. 9

PLATE 14

FIG. 6—Fowl 16, egg in uterus with partly calcified shell. Portion of ciliated epithelium and corium of albumen region, showing the final stage of maximum secretion in mucous cells, and empty albumen glands with nuclei commencing reorganization. The pale albumen has disappeared from glands, leaving a distorted, vacuolated cytoplasm. × 1900.

FIG. 7—Fowl 2, egg laid some hours previously. Similar region to fig. 6, but later stage in regeneration at a more caudal level where epithelium is higher. Note organization of mucous cell nuclei and surrounding cytoplasm. Glands beneath are filled with pale albumen and nuclei still shrunken. They are less regenerated than in fig. 6. × 1950.

FIG. 8—Further regeneration in both ciliated epithelium and albumen glands. Note basal nuclei now surrounded in homogeneous cytoplasm, regenerating in an apical direction. The ciliated cells have expanded. Gland epithelial nuclei are normal and cytoplasm is regenerating from the periphery to the gland lumen (later stage than in fig. 6) ; black granular albumen not yet appeared. × 1950.

FIG. 9—Fowl 9, egg in uterus with soft shell. Representing stage in mucous region, just before complete epithelial regeneration ; glands in typical resting phase, in which expanded nuclei and homogeneous cytoplasm are associated with black spherical secretion. Compare with figs. 10 and 15. × 1950.

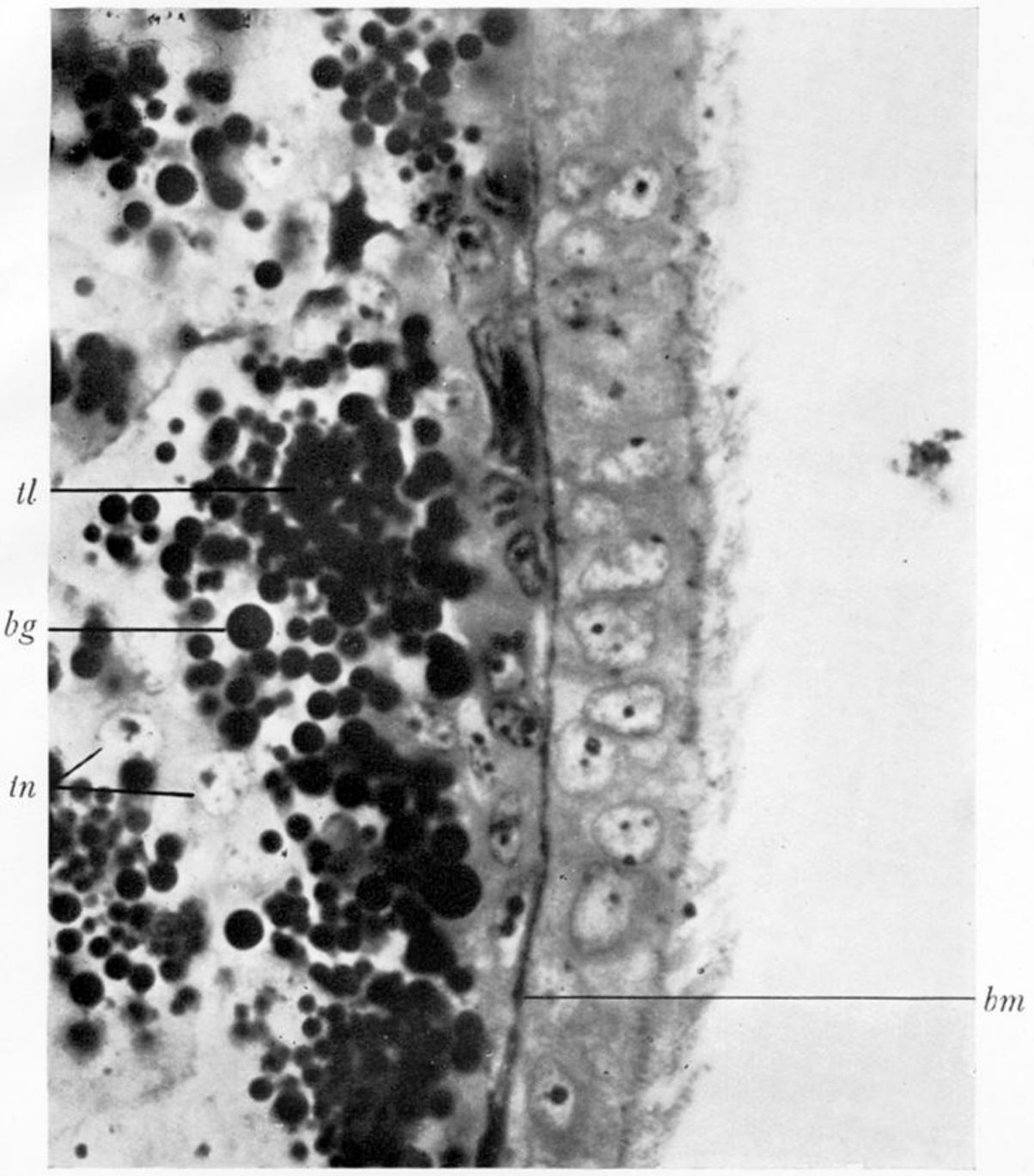

FIG. 10

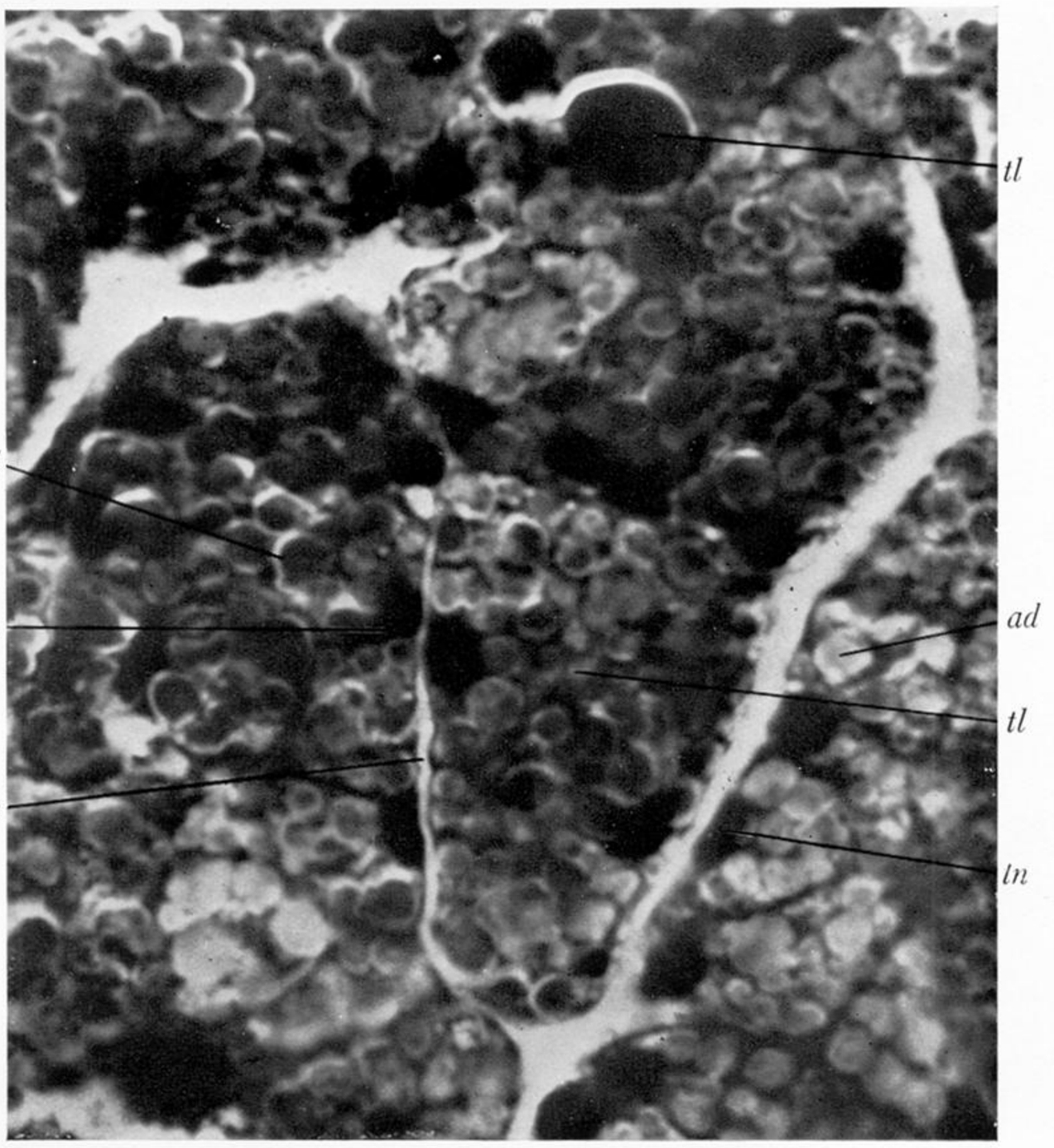

FIG. 11

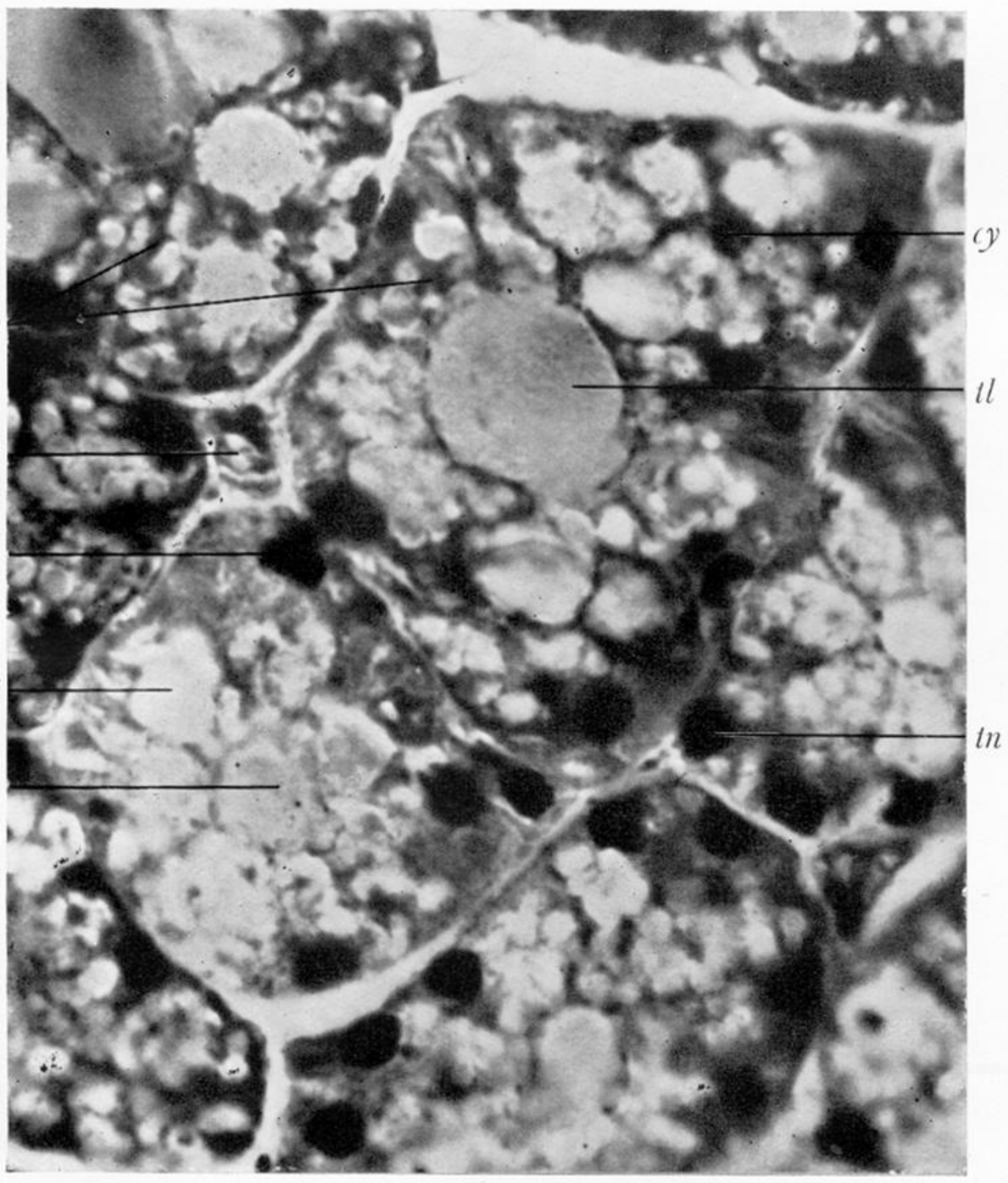

FIG. 12

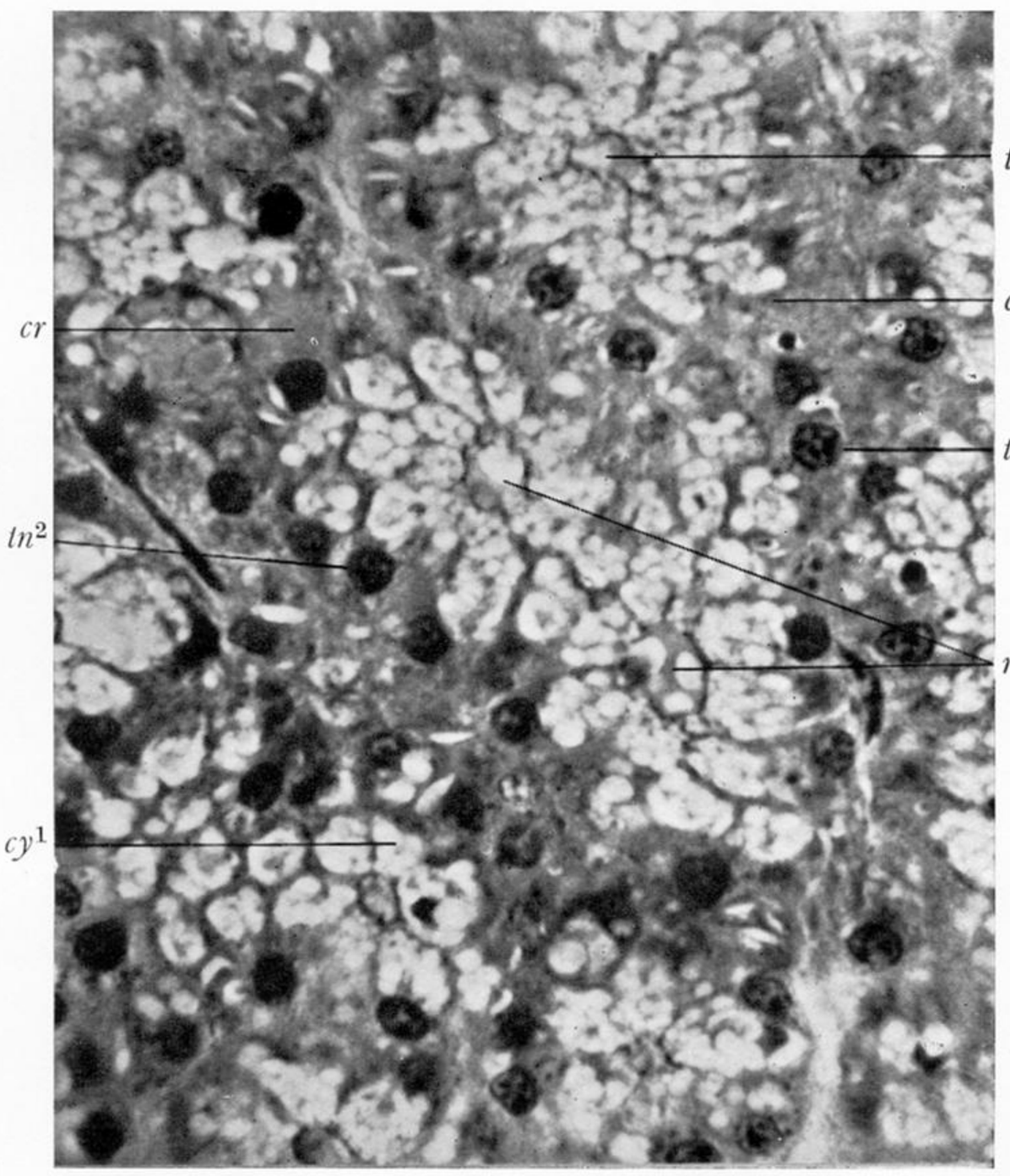

FIG. 13

PLATE 15

FIG. 10—Fowl 9. Portion of ciliated epithelium and corium from upper albumen region. Resting albumen glands appearing first on tips of folds, containing black secretion. Lumen of gland completely filled with secretion (*cf.* fig. 18). Ciliated epithelium regenerated and normally containing few mucous cells. Hence lack of differentiation into apical and basal cells. × 1500.

FIG. 11—Fowl 15, egg laid about 5 hours previously, and next yolk about to enter infundibulum. An area of heavy brown staining tubules near core of fold in albumen region. Glands separated slightly in sectioning, have maximum albumen content with secretion not opaquely stained as in fig. 10. Tubule lumina almost obscured and nuclei shrunken and opaque. Extreme reduction in connective tissue stroma shown at, *x*. × 2000.

FIG. 12—Fowl 16. Glands from albumen region, separated in sectioning. Secretion stains paler and gives areas in fold which are much less dense under low magnification than in fig. 11. Albumen has fused into larger masses, some of which are almost unstained. × 1750.

FIG. 13—Fowl 16. Glands from area of albumen region, where secretion has almost disappeared from epithelium and regeneration is commencing. Nuclei have expanded sufficiently to show chromatin masses, but not to the extent shown in fig. 8. Alveolar cytoplasm is empty and residual secretion lies in gland lumina. × 1300.

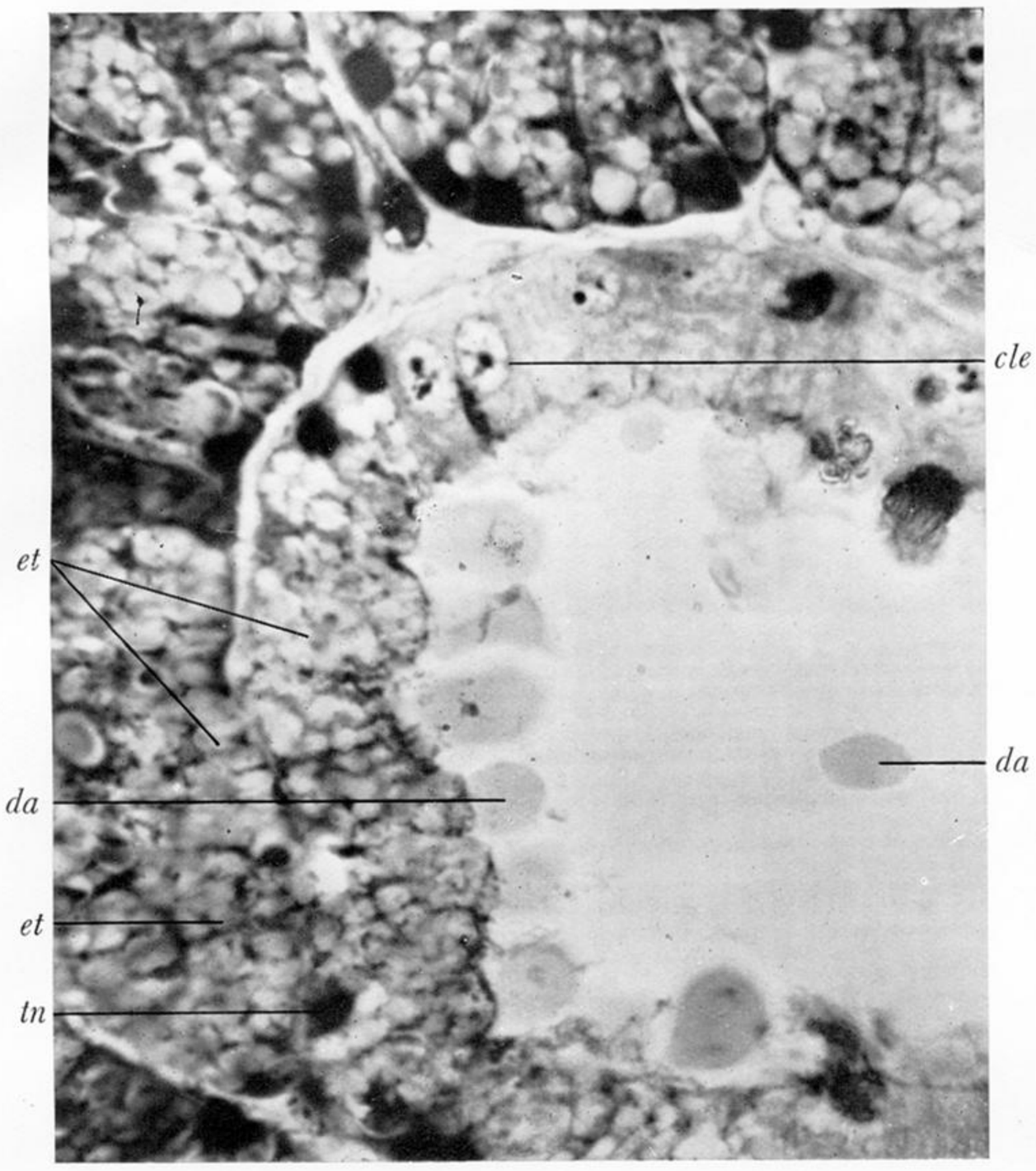

FIG. 14

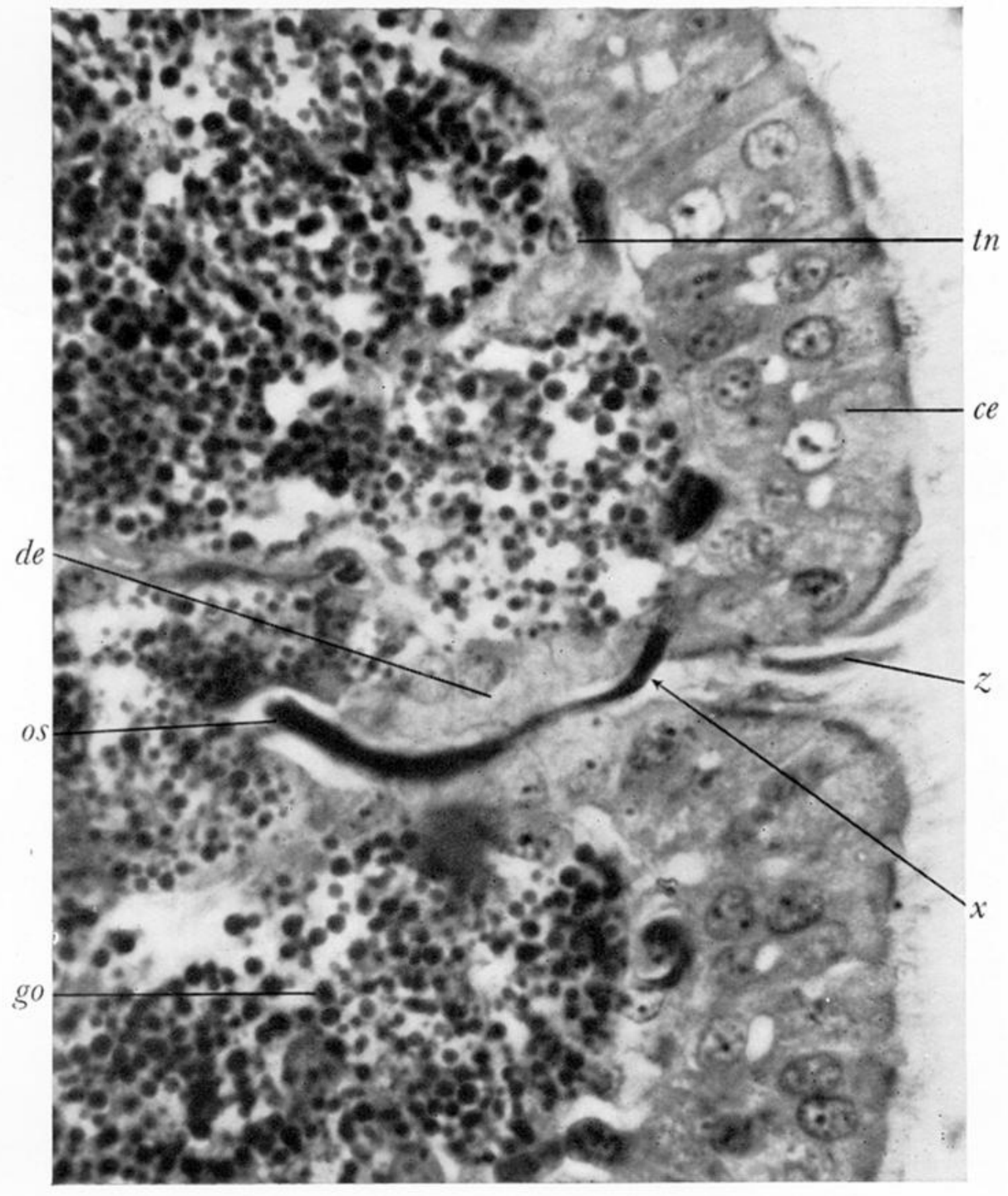

FIG. 15

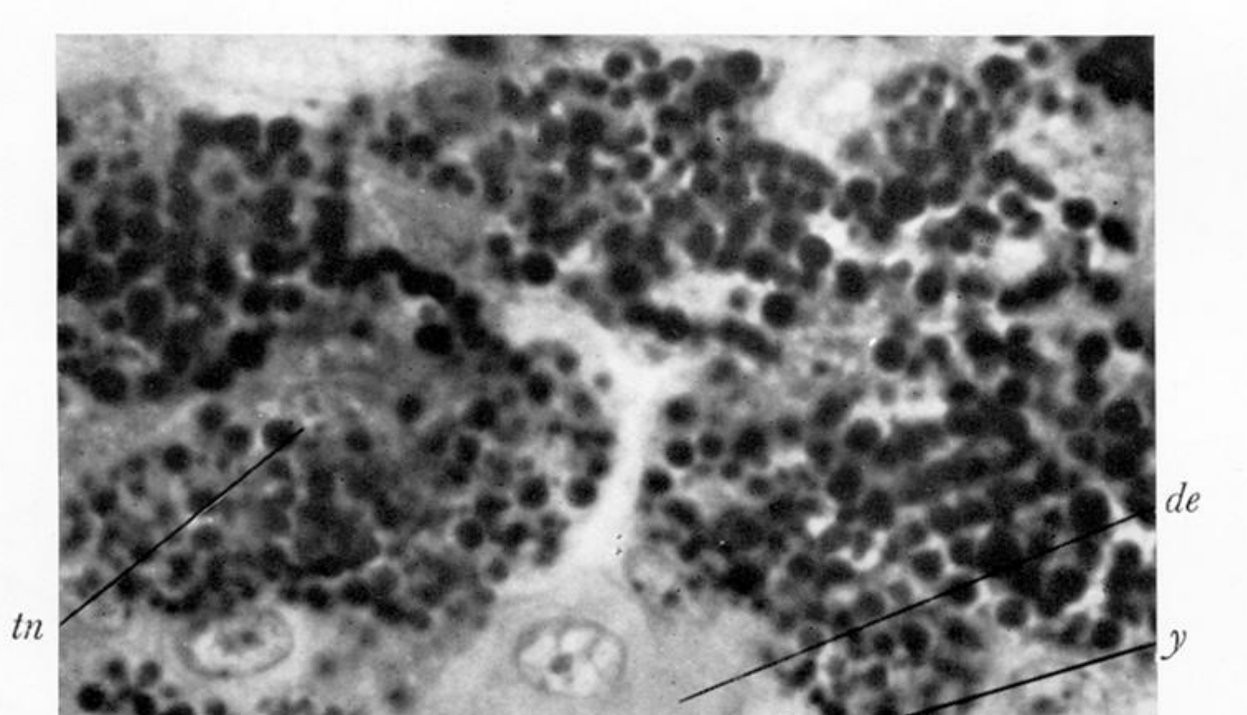

FIG. 16

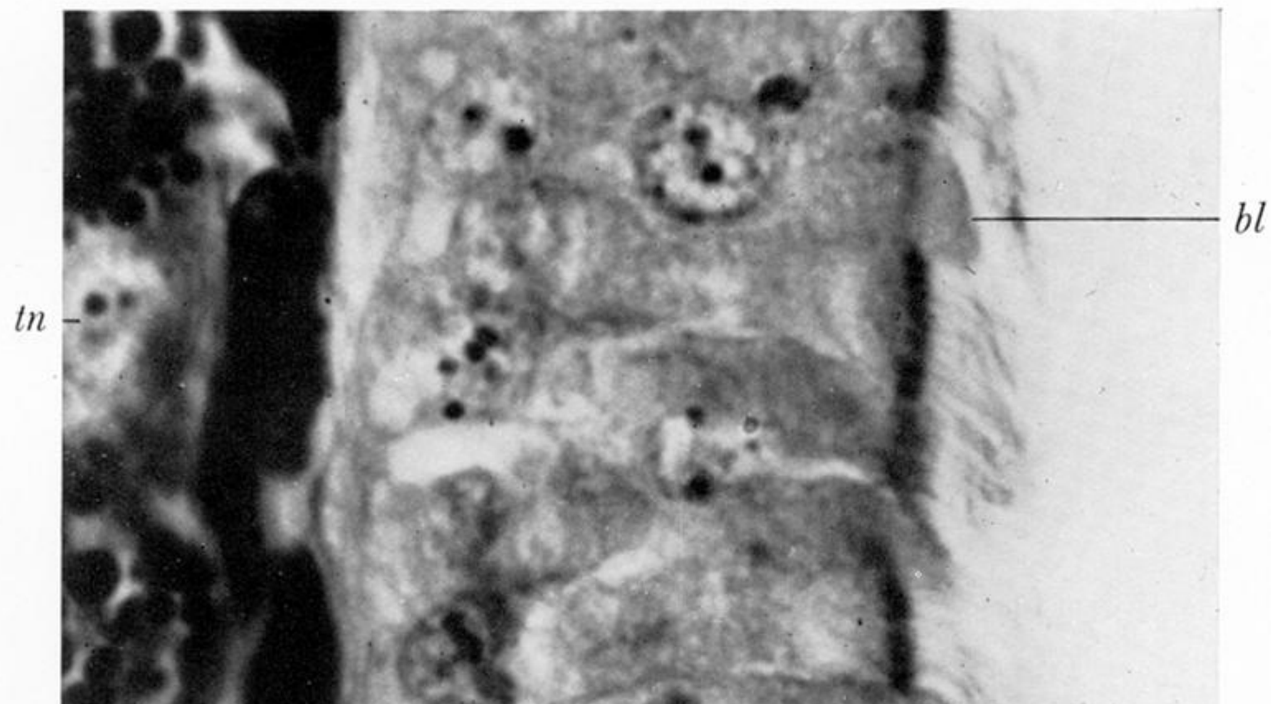

FIG. 17

PLATE 16

FIG. 14—Fowl 6, with completed egg in uterus and next yolk in cranial portion of albumen region. Portion of sagittal section through opening of several albumen glands into crypt, to show residual albumen exuding in droplets. Note pale staining of secretion. × 1500.

FIG. 15—Fowl 10, egg in uterus with membranes only faintly white with calcification. Showing a duct and adjacent glands in isthmus. Note secretion is uniformly granular and heavily staining, flowing from glands as granules which coalesce into dense rod-like masses which lie in the duct like a bristle. During staining the apical end *x* of the secretion has twisted during hydration and dehydration so that it now lies above the cells of the duct epithelium. In oviducal lumen secretion remains viscous, *z*. × 1450.

FIG. 16—Fowl 10. A sagittal section through an isthmal gland duct, showing clearer detail of structure of tubular glands. As the secretion from two glands forms threads of ovokeratin, *x* and *y*, these fuse into a fibre *z*, extending into the duct. × 1900.

FIG. 17—Fowl 5, an egg in isthmus with thin shell membrane already formed. Portion of ciliated epithelium and corium of isthmus. Note glandular activity in non-ciliated epithelial cells, resulting in bleb formation. Nuclei of mucous cells not necessarily basal as in albumen region. Note vacuoles between basement membrane and basal nuclei are common during secretion. Similar vacuoles on apical aspect of the nuclei are shown in fig. 15. × 2600.

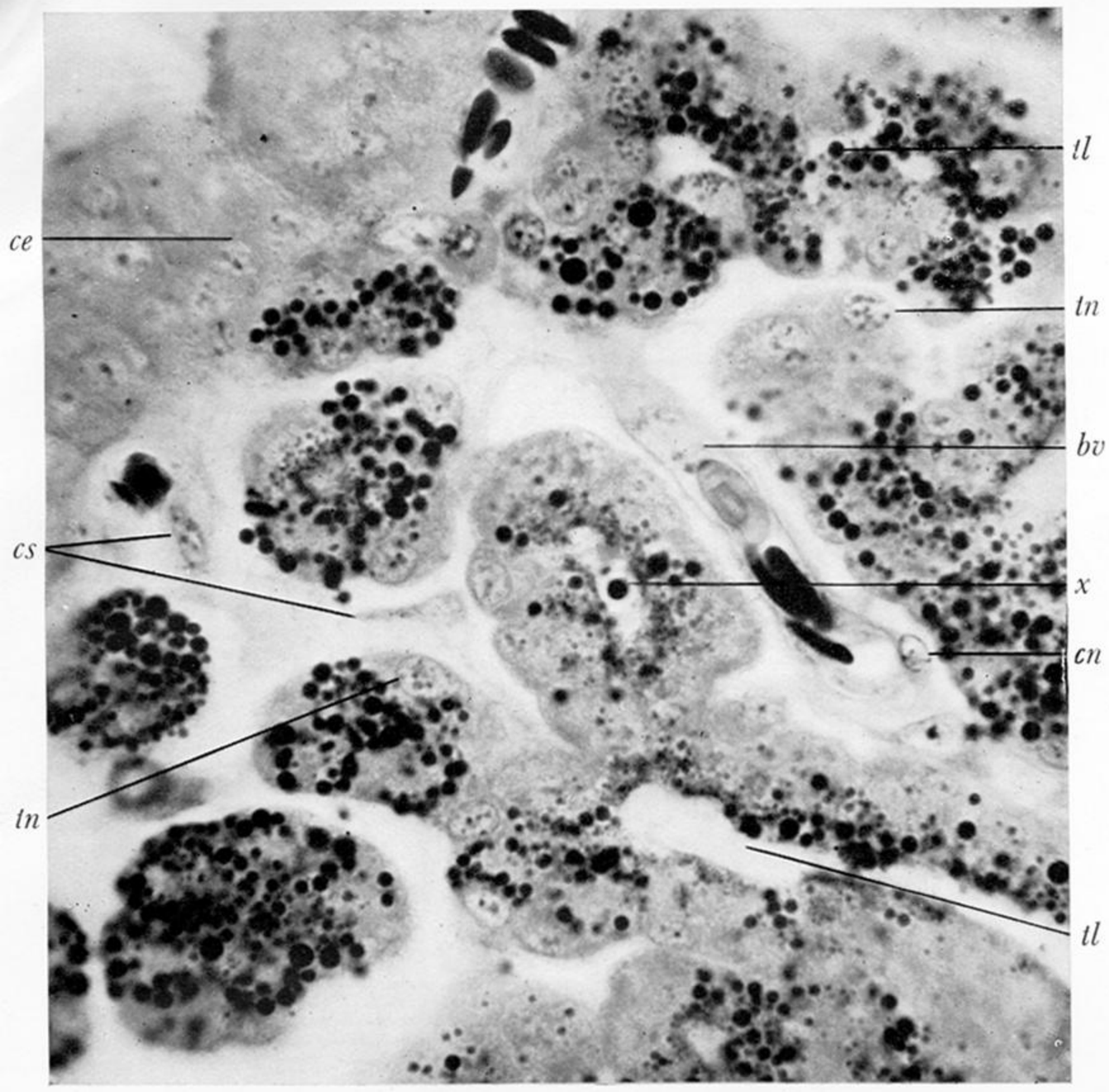

FIG. 18

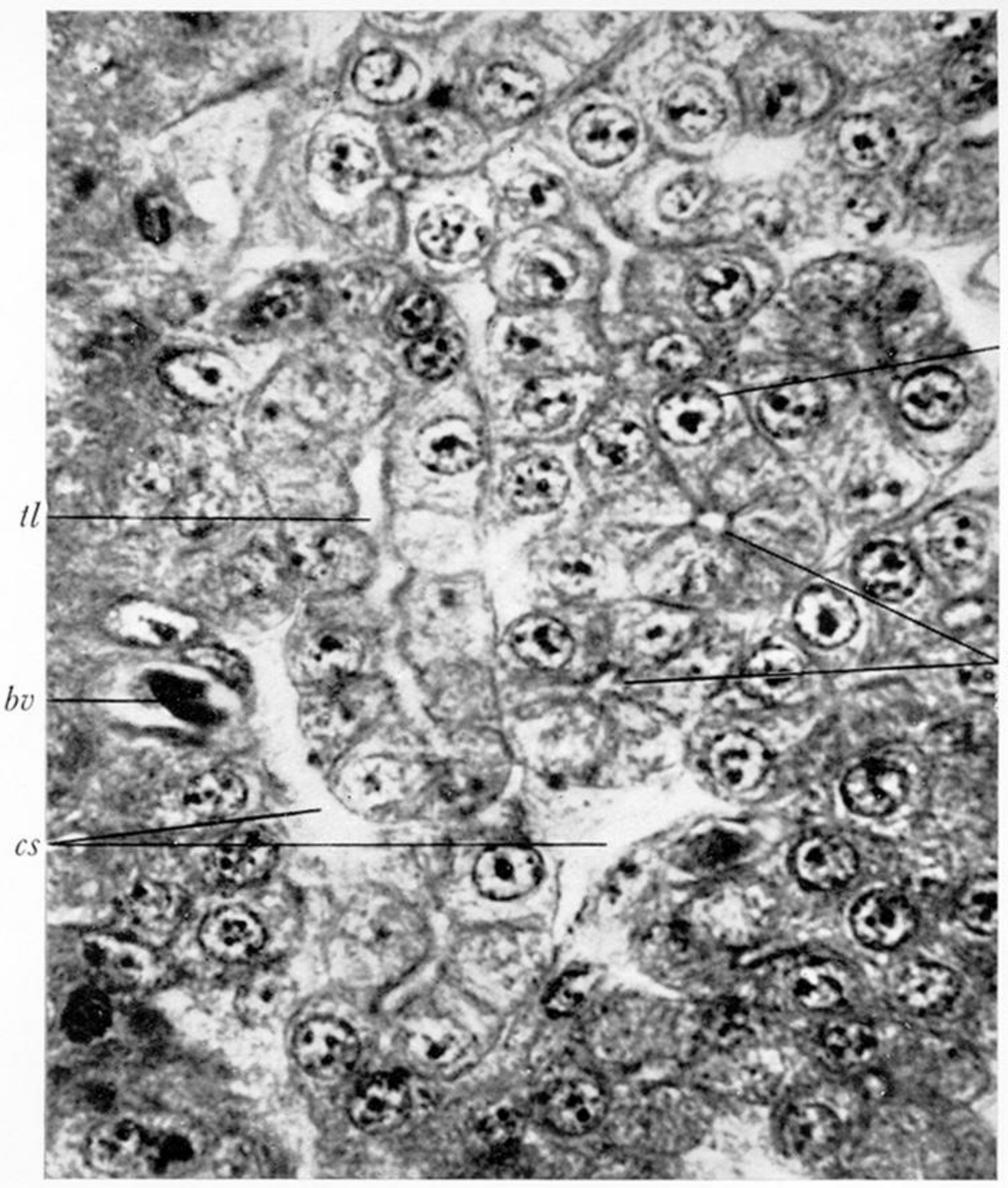

FIG. 19

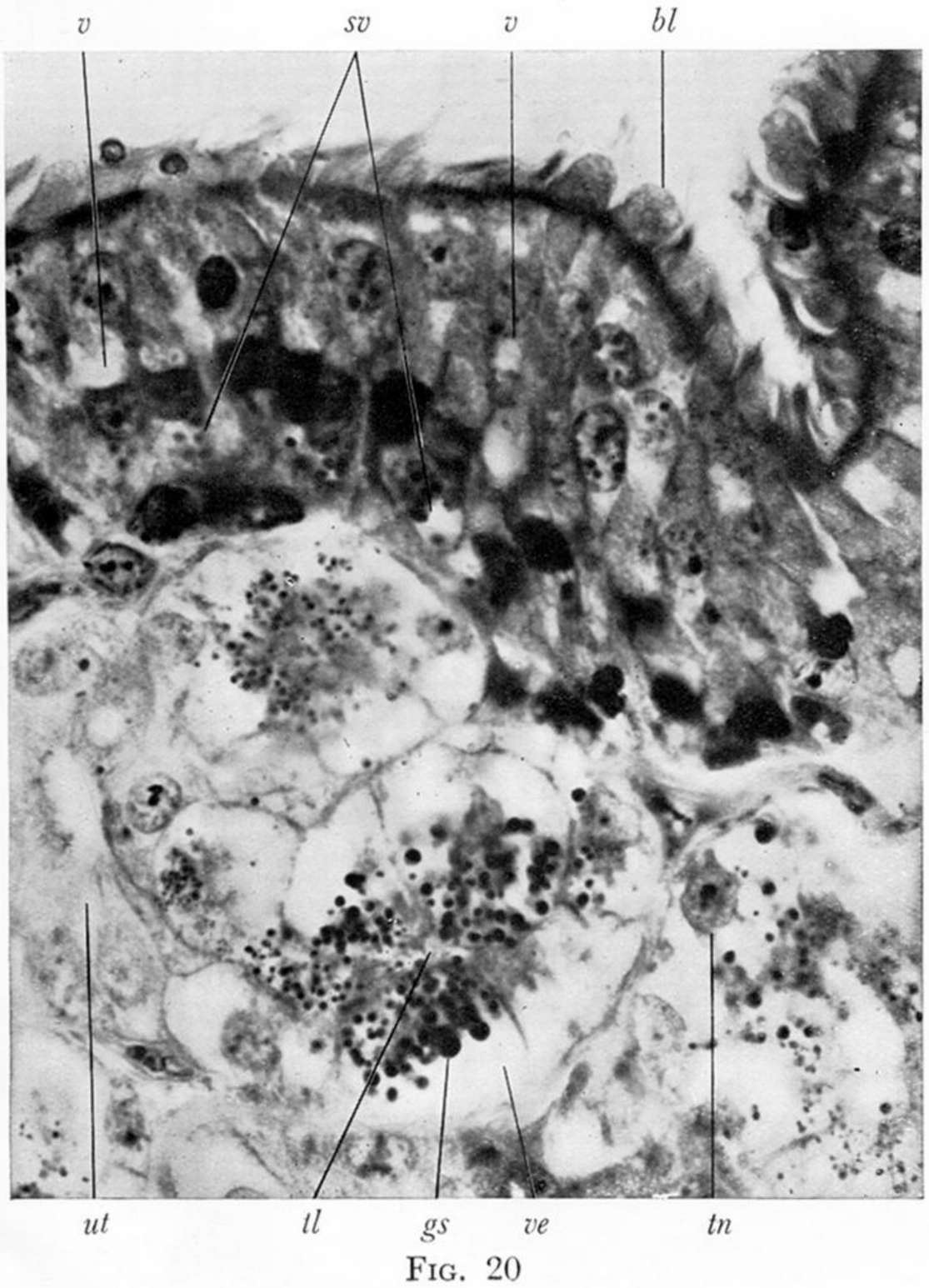

FIG. 20

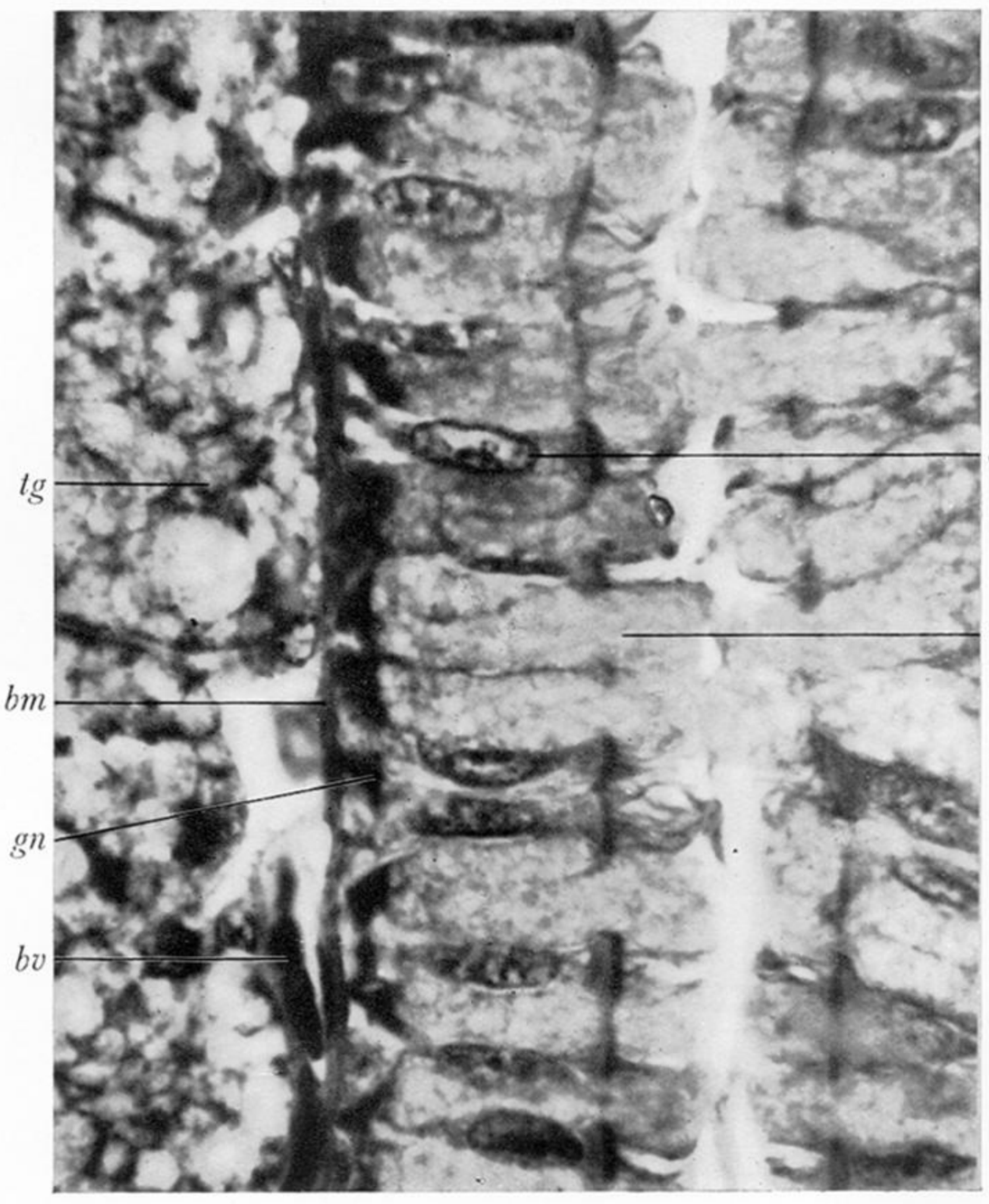

FIG. 21

PLATE 17

FIG. 18—Fowl 9, uterus containing a partly calcified shell. Representing a region in the isthmus with maximum depletion of the secretion in the gland epithelium. Note particularly the lumen of the gland at *x*, where the free secretion remains unaltered as viscous droplets. × 1050.

FIG. 19—Fowl 9. An area of uterine glands during active secretion. The cytological characters of the gland epithelium are clearly shown, the preservation being better than is usually found in this region. Note the patent lumen in each gland and their general reduction in diameter (*cf.* fig. 13). The cytoplasm is only faintly granular and remains so throughout secretion and rest. × 1400.

FIG. 20—Fowl 7, an almost completed egg in uterus. Portion of the isthmo-uterine junction showing its ciliated epithelium and tubular glands of the special type. The vacuolated and sparsely granular nature of these glands is characteristic of this region. Ciliated epithelium is similar to that in isthmus and is actively secreting from its non-ciliated cells. × 1300.

FIG. 21—Fowl 8, egg laid and a fresh yolk about to enter the infundibulum. Two opposing surfaces of ciliated epithelium from the mucous region at the caudal extremity of albumen region. After fixation in mercuric chloride and acetic acid, the mucin secretion is shown migrating from the epithelium, though the oviduct is empty. Note the destruction of the gland epithelium and its stored secretion, being the result of this type of fixation. × 1400.

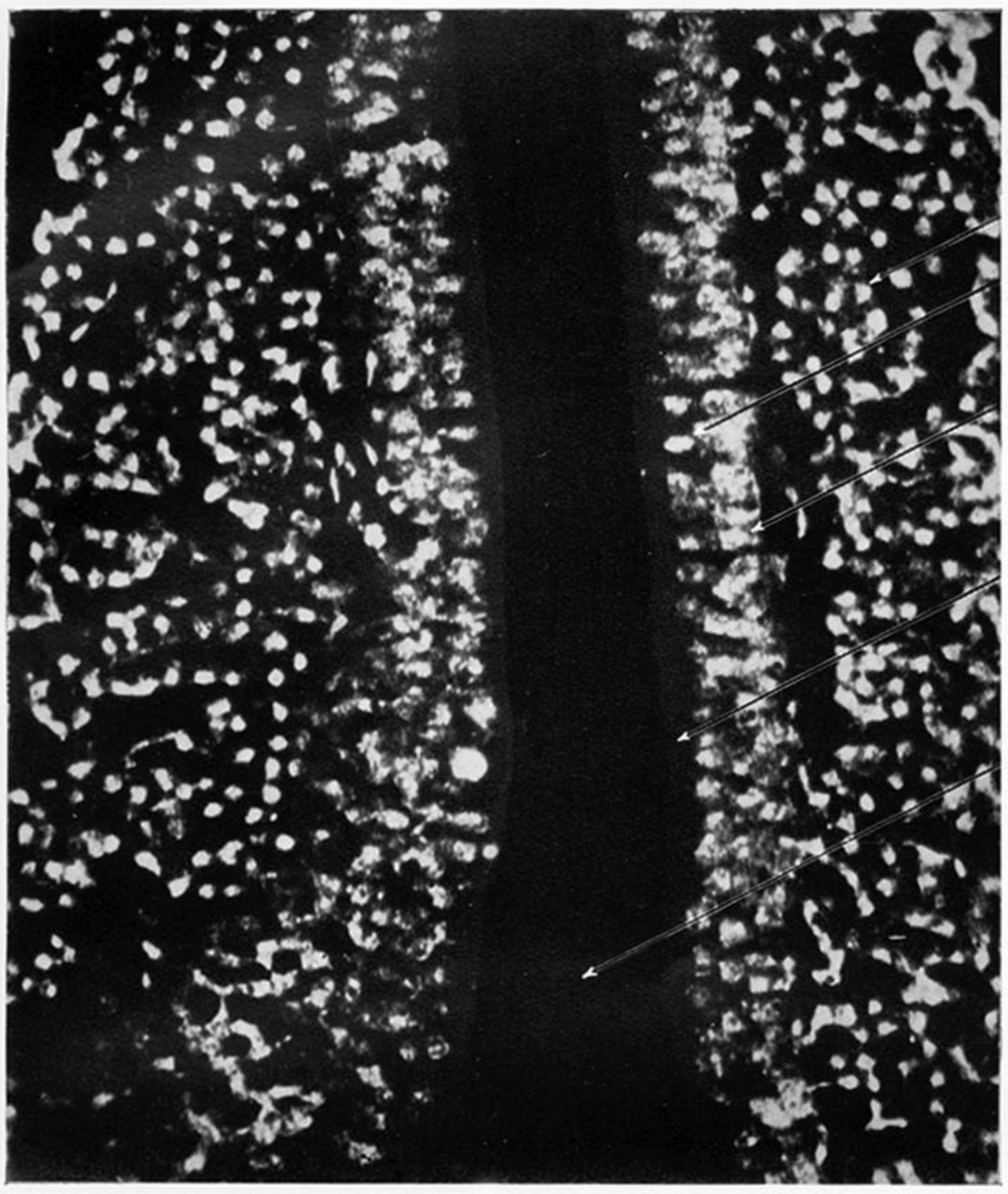

FIG. 22

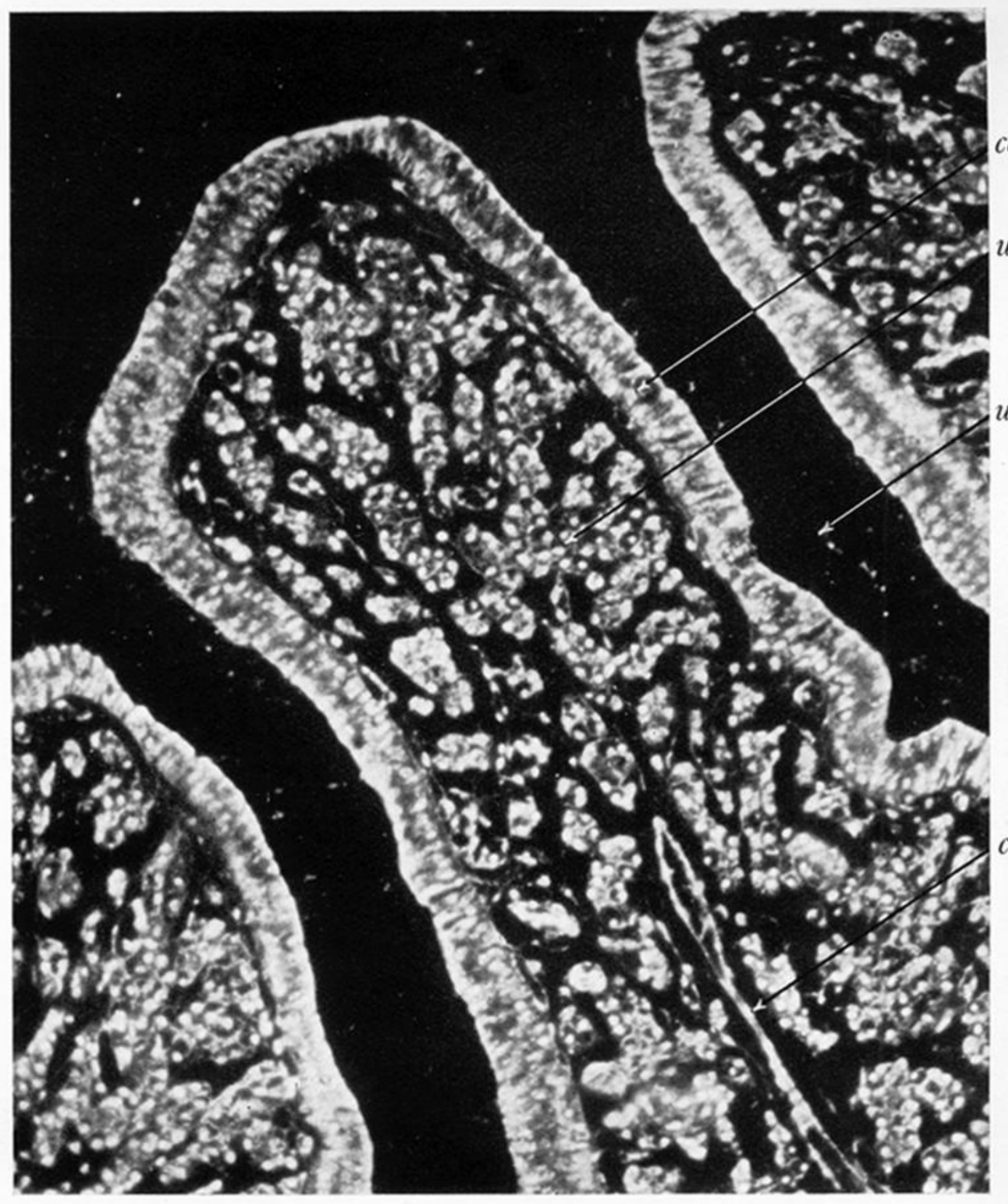

FIG. 23

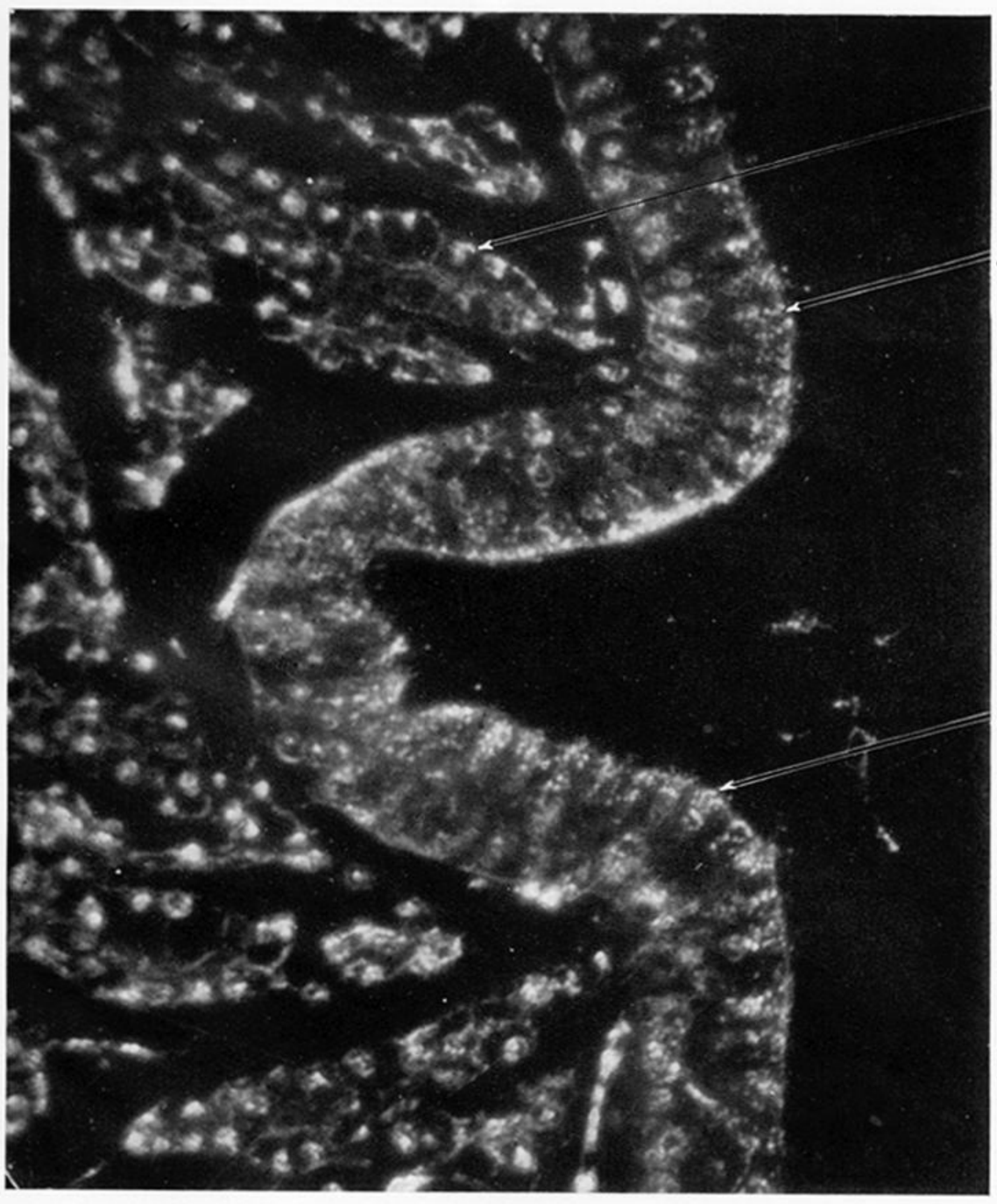

FIG. 24

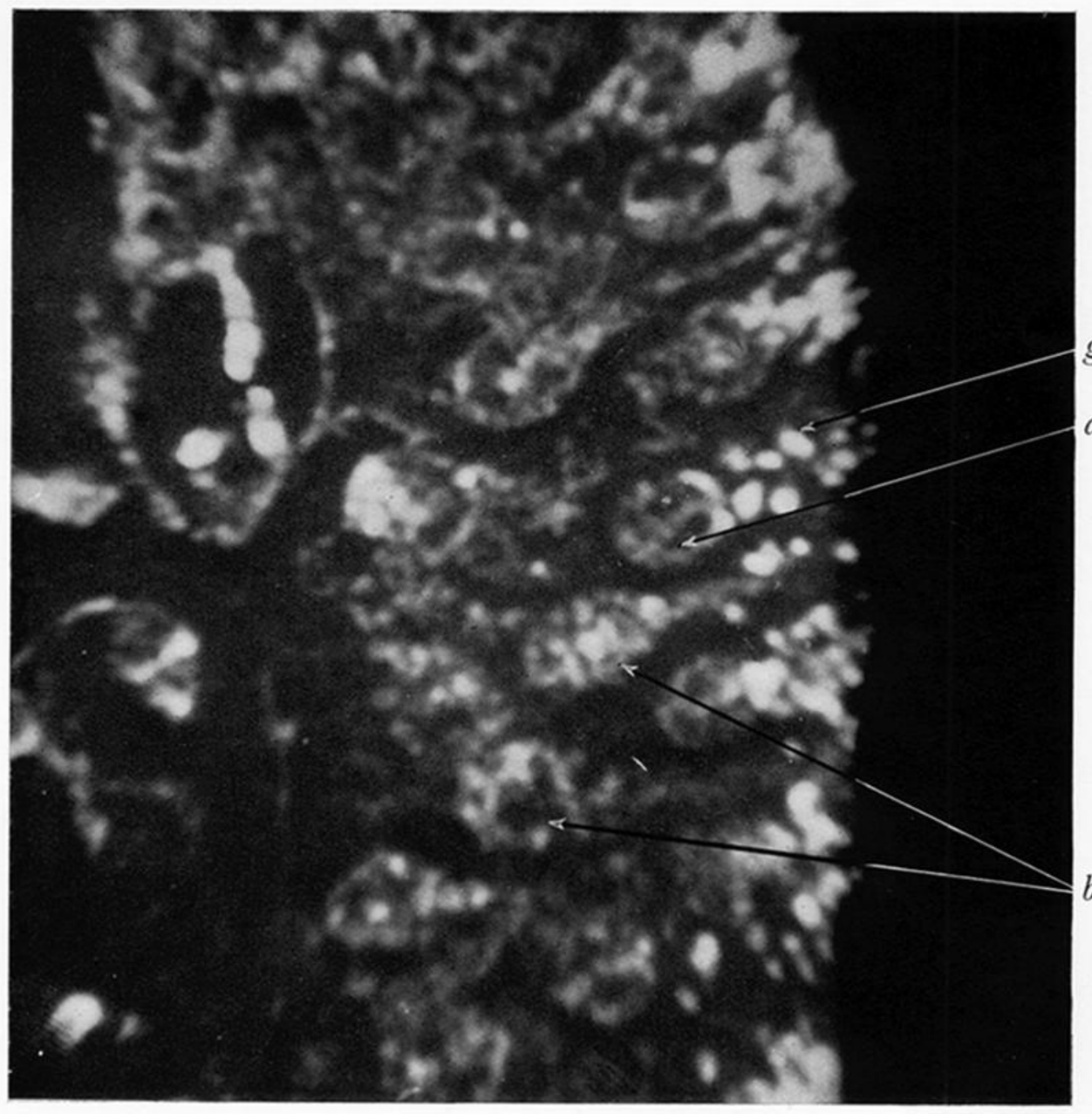

FIG. 25

PLATE 18

FIG. 22—Fowl 5, egg laid about 4 hours previously. Portion of the uterine epithelium and corium incinerated and photographed under dark ground illumination. The section, at 5 μ, shows the ash left by the ciliated apical and basal cell nuclei, together with small residues at intervals in cytoplasm. In the corium, the major portion of the ash is confined to the epithelial nuclei with the remainder consisting chiefly of the cell membranes. Under low magnification an area such as this shows an even distribution of the ash, varied only by the relative cell crowding in the ciliated epithelium. At *x* the edge of the epithelium, which is almost invisible, has been reinforced in printing. × 300.

FIG. 23—Fowl 9, with an egg in the uterus having a soft partly calcified shell. Showing an incinerated section of the uterus similar to fig. 22, but taken during active calcium secretion. Section at 5 μ shows a remarkable increase in the ash of ciliated epithelium, which, under low magnification, forms a conspicuous white band covering the folds. The increase in ash is due to a finely divided greyish deposit filling the cytoplasm, and particularly because the ciliated cell granules have left large, opaque white residues in a position apical to the nuclei. Note the alternation of these granules with the basal cells, which leave an ash confined more conspicuously to their nuclear zone near the basement membrane. The tubular glands have a slightly increased ash, particularly in their nuclei, but there is no evidence of specific secretory granules of high calcium content in their epithelium. × 175.

FIG. 24—Enlarged portion of same section as in fig. 23, showing the ciliated epithelium and uterine glands in detail. × 450.

FIG. 25—From same section as fig. 24, showing finer cytological structure of the epithelial residues. × 2500.

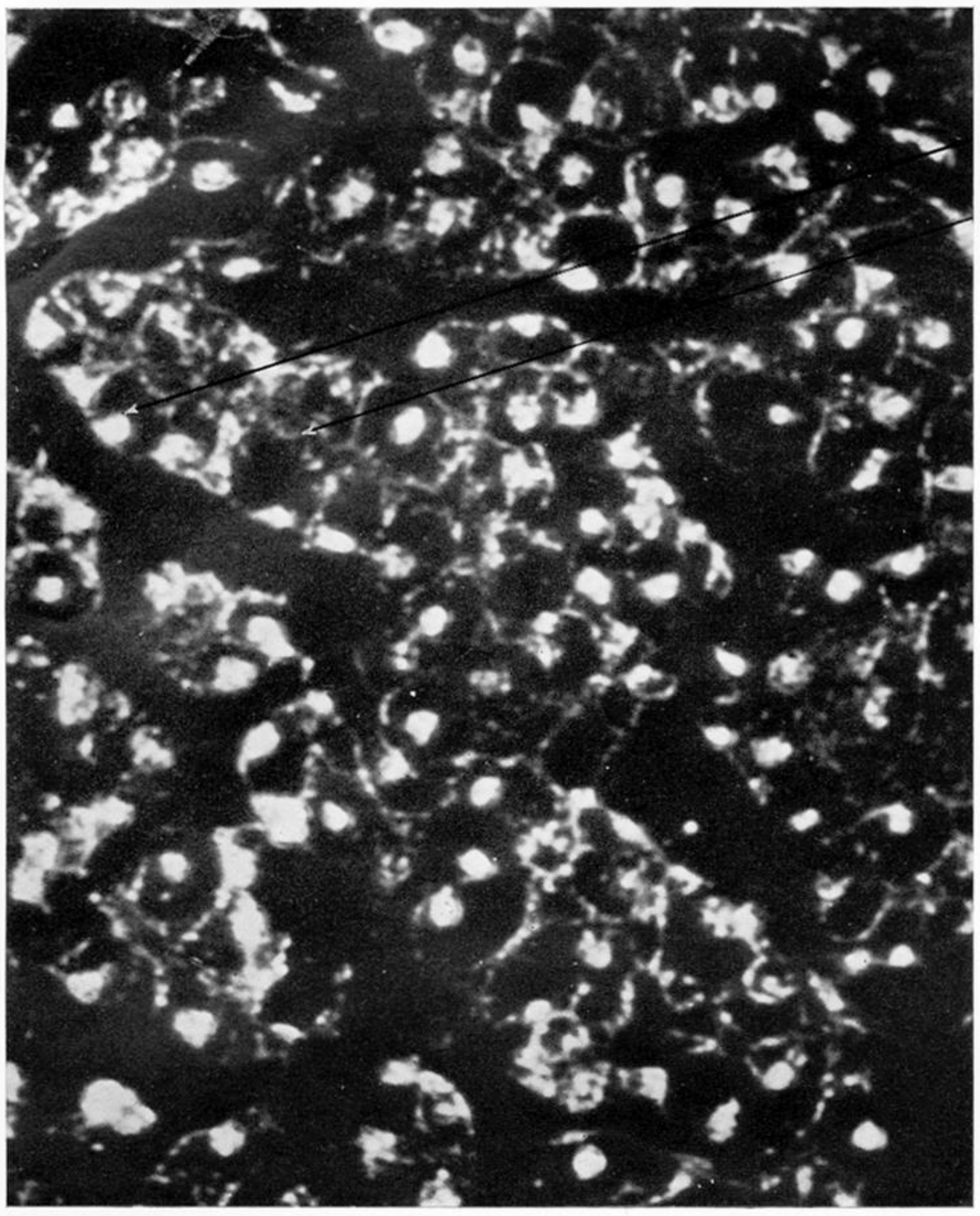

FIG. 26

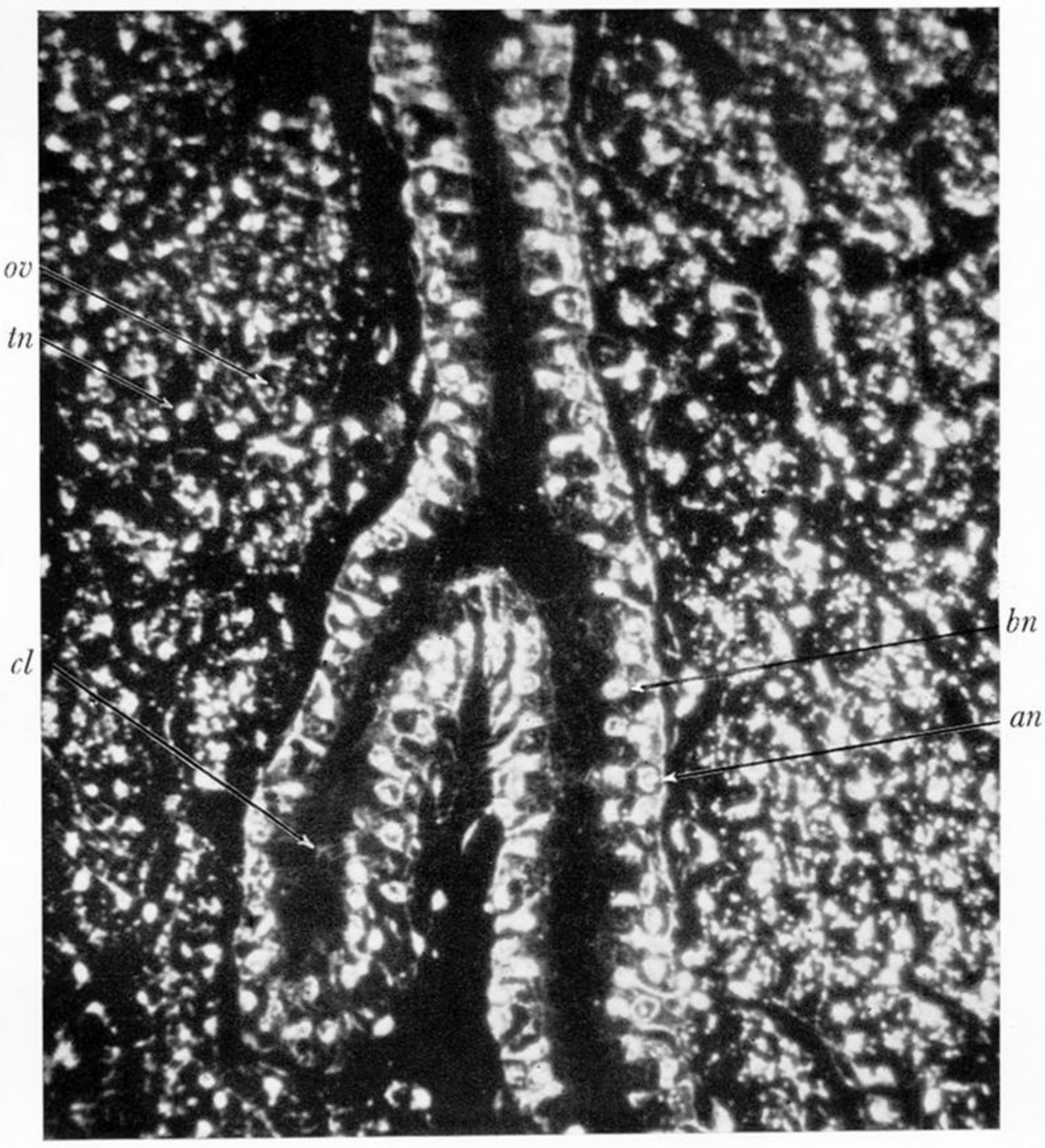

FIG. 27

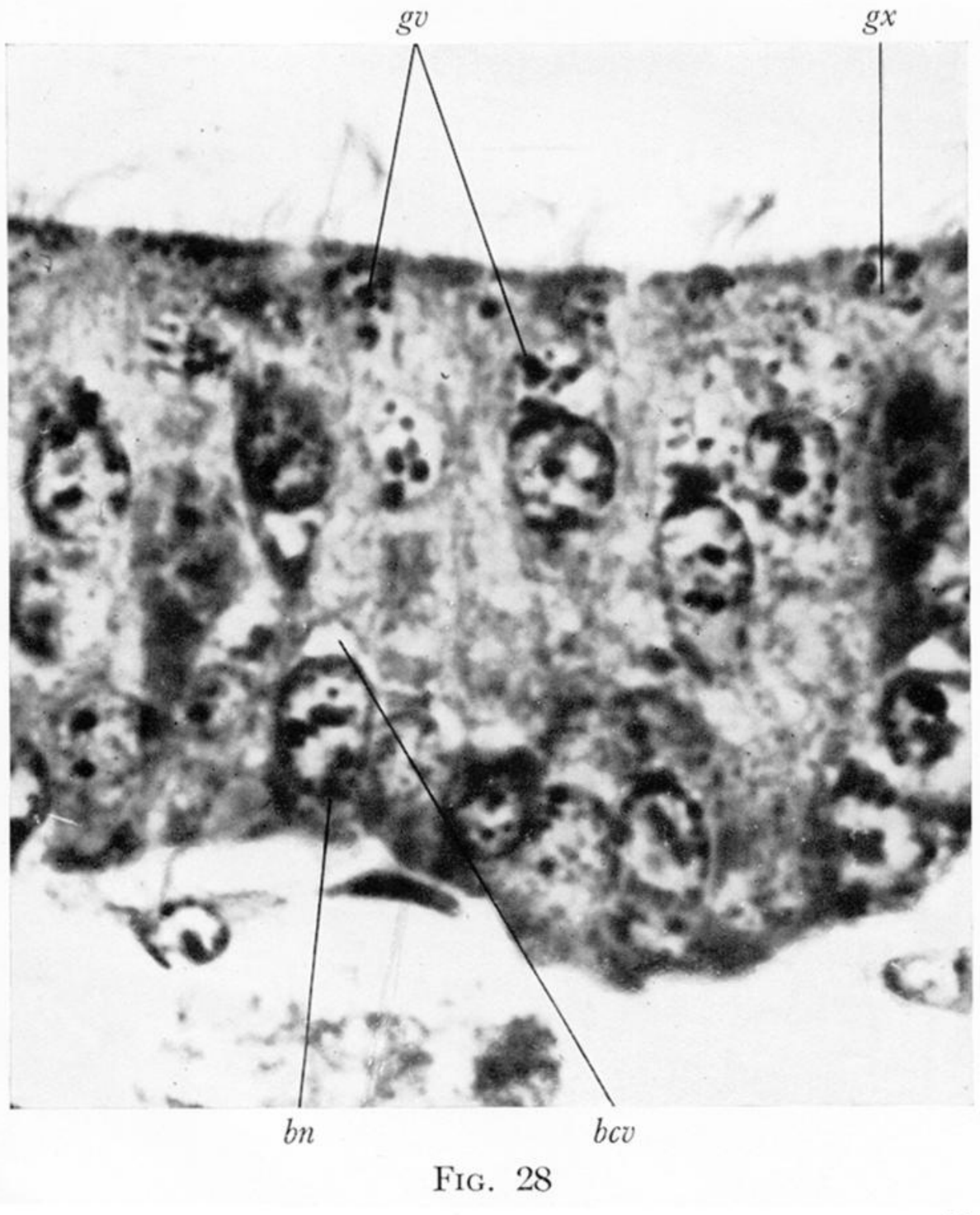

FIG. 28

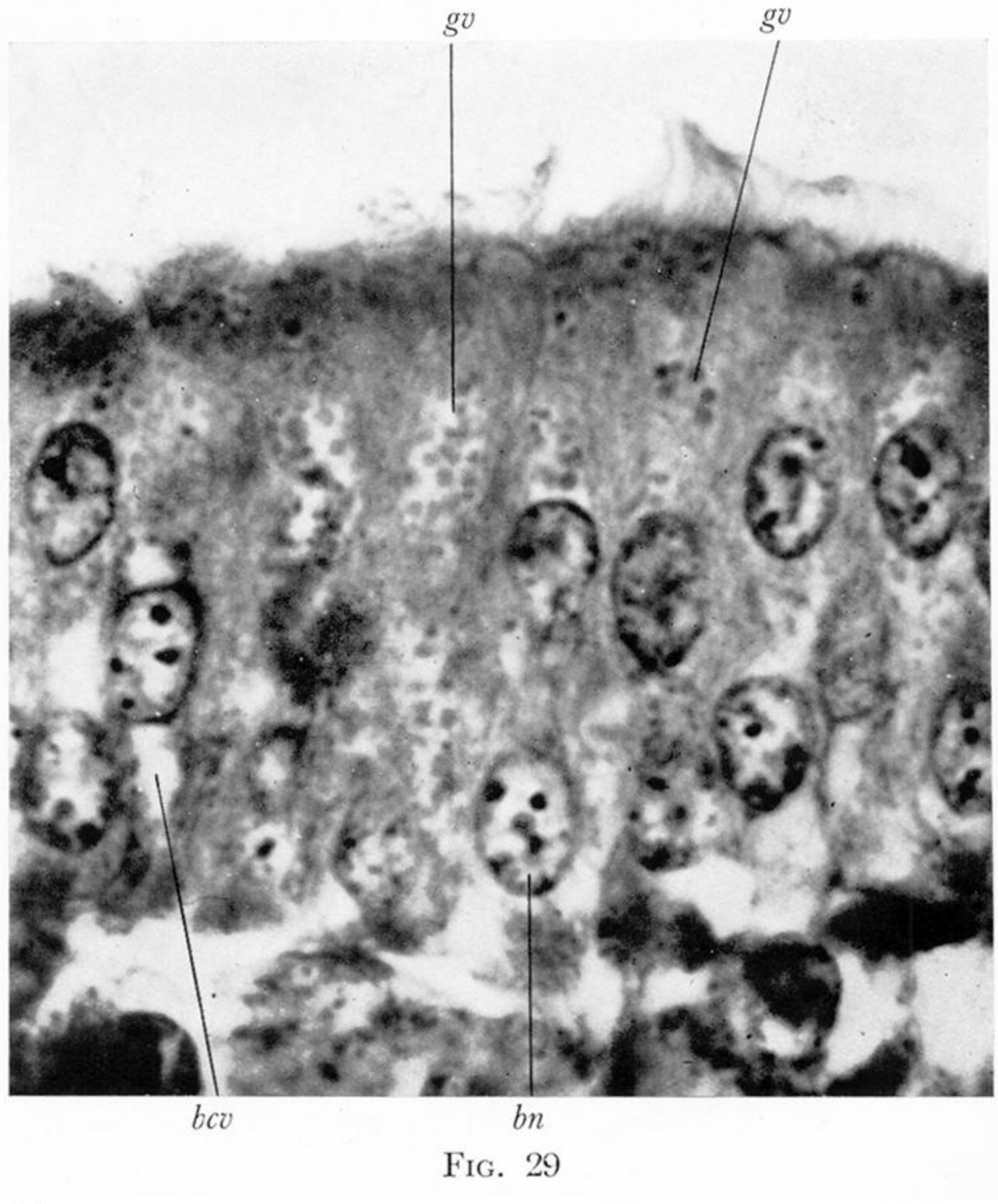

FIG. 29

PLATE 19

FIG. 26—Enlarged area of uterine glands from same incinerated section as in fig. 23. × 1100.

FIG. 27—Fowl 23, with a membrane-covered egg in the uterus. Portion of isthmal wall showing the heavy inorganic content, after incineration, of the secretory granules in the tubular glands. × 500.

FIG. 28—Fowl 9. Control section of fig. 23, stained with iron hæmatoxylin. The apical cell granules, which leave a heavy inorganic residue (*see* fig. 25), are shown to extend towards the cilia, where they may be seen passing through the cuticular margin of the cells. × 2800.

FIG. 29—Fowl 10, with an egg in the uterus having membranes only faintly whitened with shell material. A portion of the uterine epithelium similar to fig. 28, but fixed in Zenker-formol. The granules in the apical cells have the same staining properties and position as those in the previous figure. × 2800.

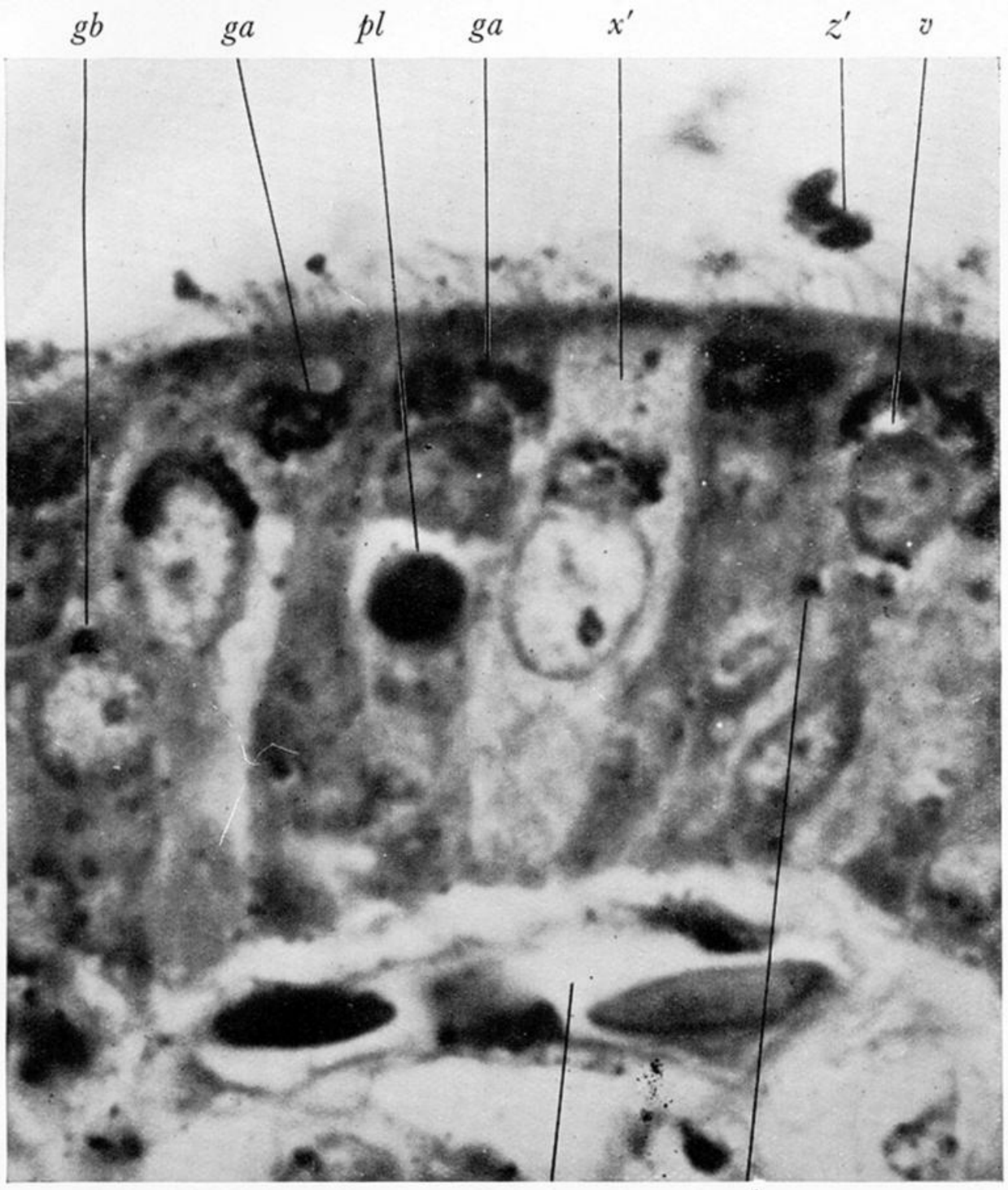

FIG. 30

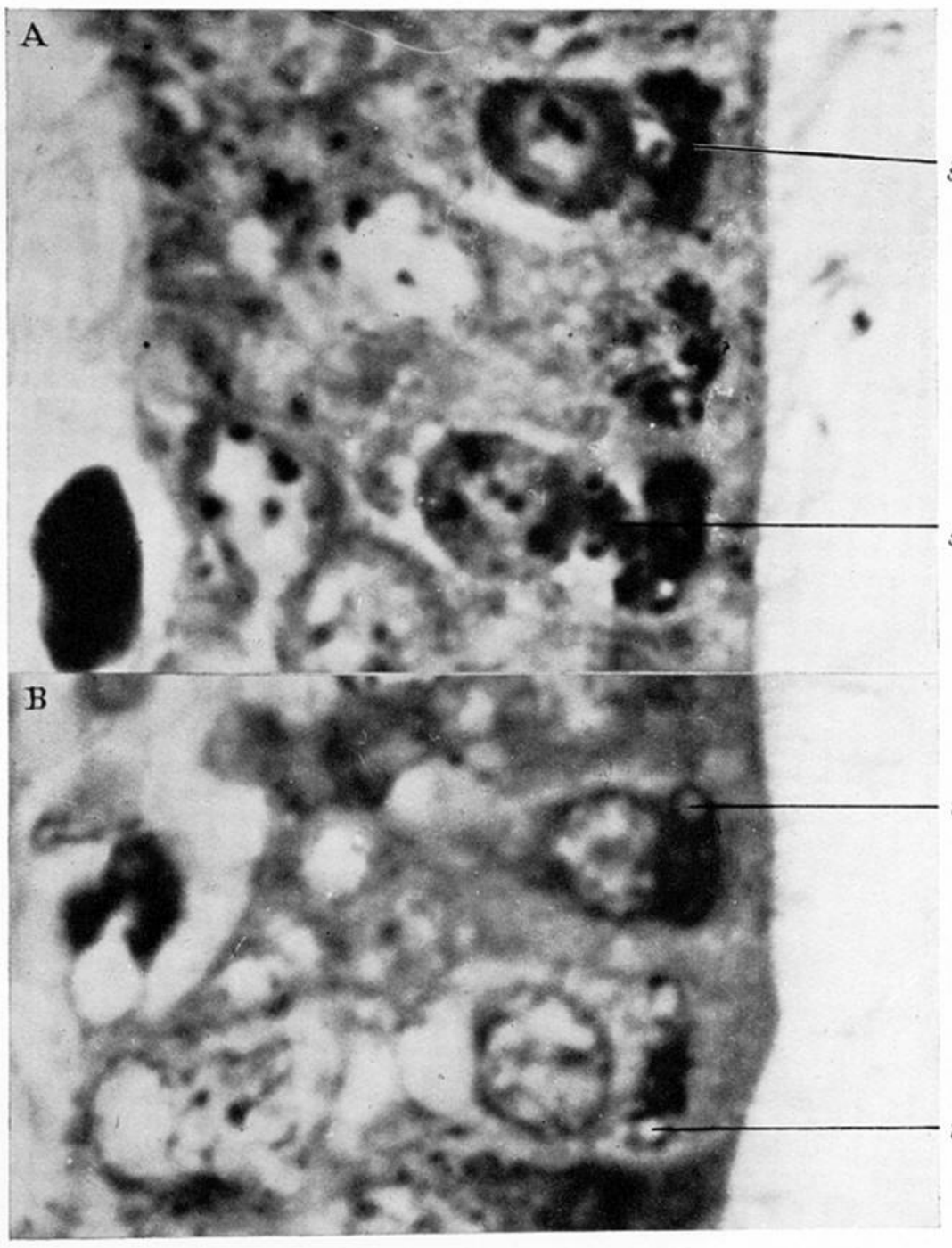

FIG. 31

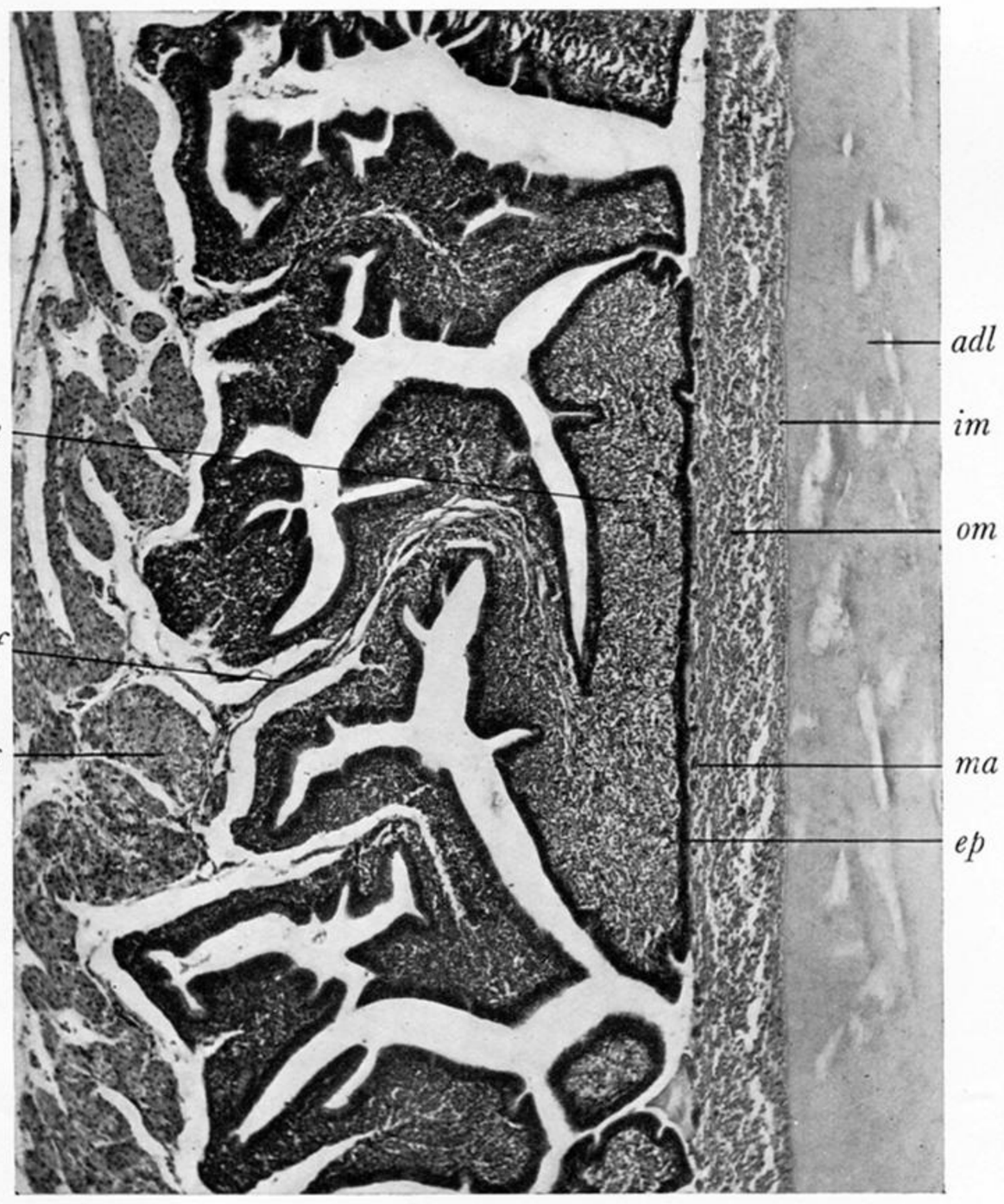

FIG. 32

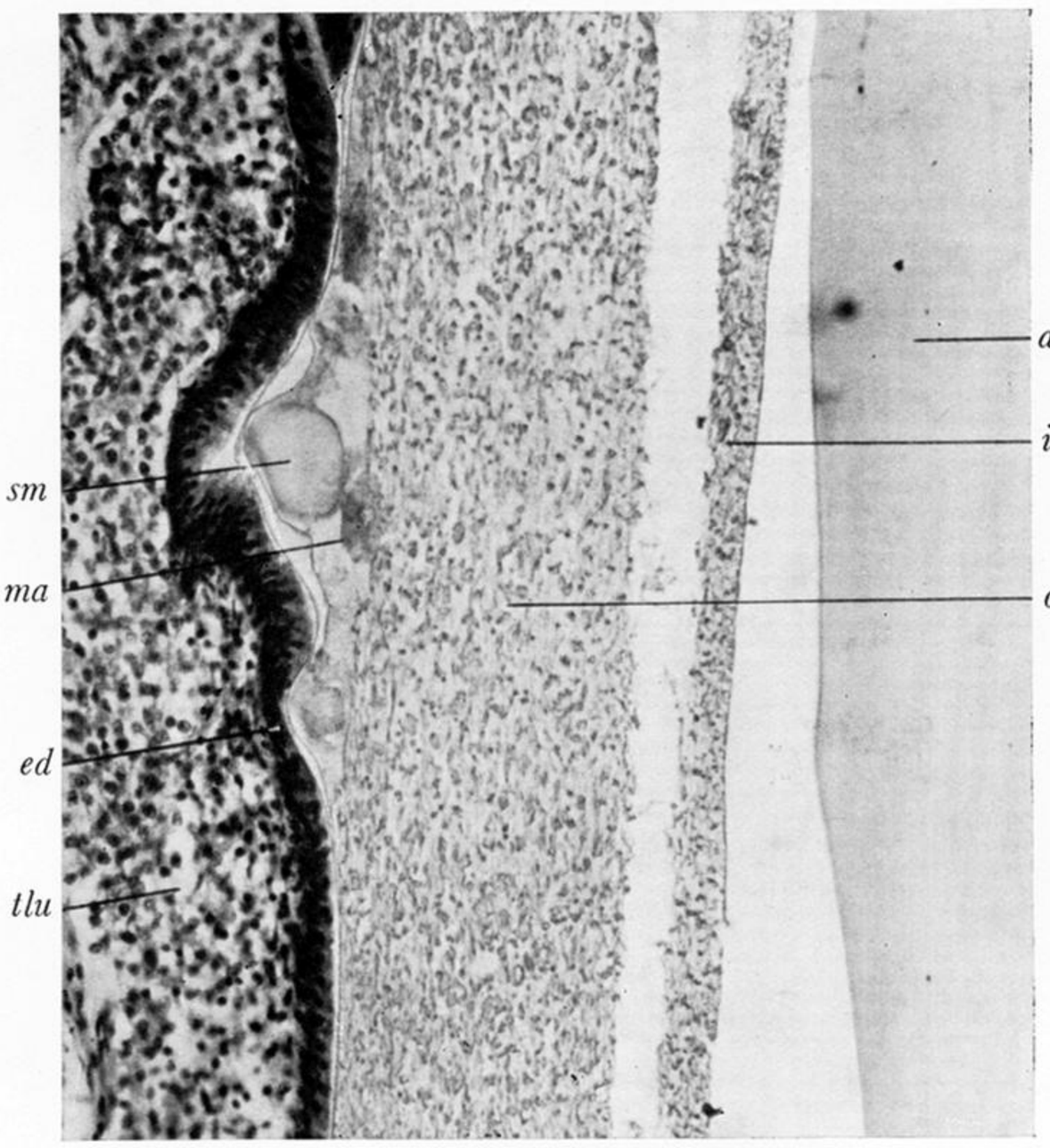

FIG. 33

PLATE 20

FIG. 30—Fowl 10. Portion of a silver impregnation of the uterine epithelium. Note the Golgi reticulum does not extend towards the apical cell margins and that it is reduced in size in the basal cells. × 3000.

FIG. 31—Fowl 2, egg laid several hours previously. Showing the Golgi apparatus in the uterine epithelium. In " A " the aggregation of granules, stained with iron hæmatoxylin, is shown in a vacuolated area between the Golgi reticulum and the nucleus. In " B " clear spaces in the Golgi reticulum, which possibly represent secretory material formed within its meshes, are illustrated. × 3000.

FIG. 32—Fowl 33, egg with completed shell membranes just migrated into the uterus. Showing a section through portion of the uterine muscle coat, the folds of the mucosa lying in contact with the shell membranes. Note the intimate contact between the epithelium covering the flattened surface of the fold at *x*, and between it and the shell membrane the mammillæ of the shell matrix are clearly shown. × 55.

FIG. 33—Fowl 33.—An adjacent area to that illustrated in fig. 32. The layers of the shell have become separated in sectioning and the uterine epithelium has been brought into contact with its surface in the final print. A mass of special secretion, *sm*, staining faintly blue with Delafield's hæmatoxylin lies between the epithelium and the mammillæ. × 265.

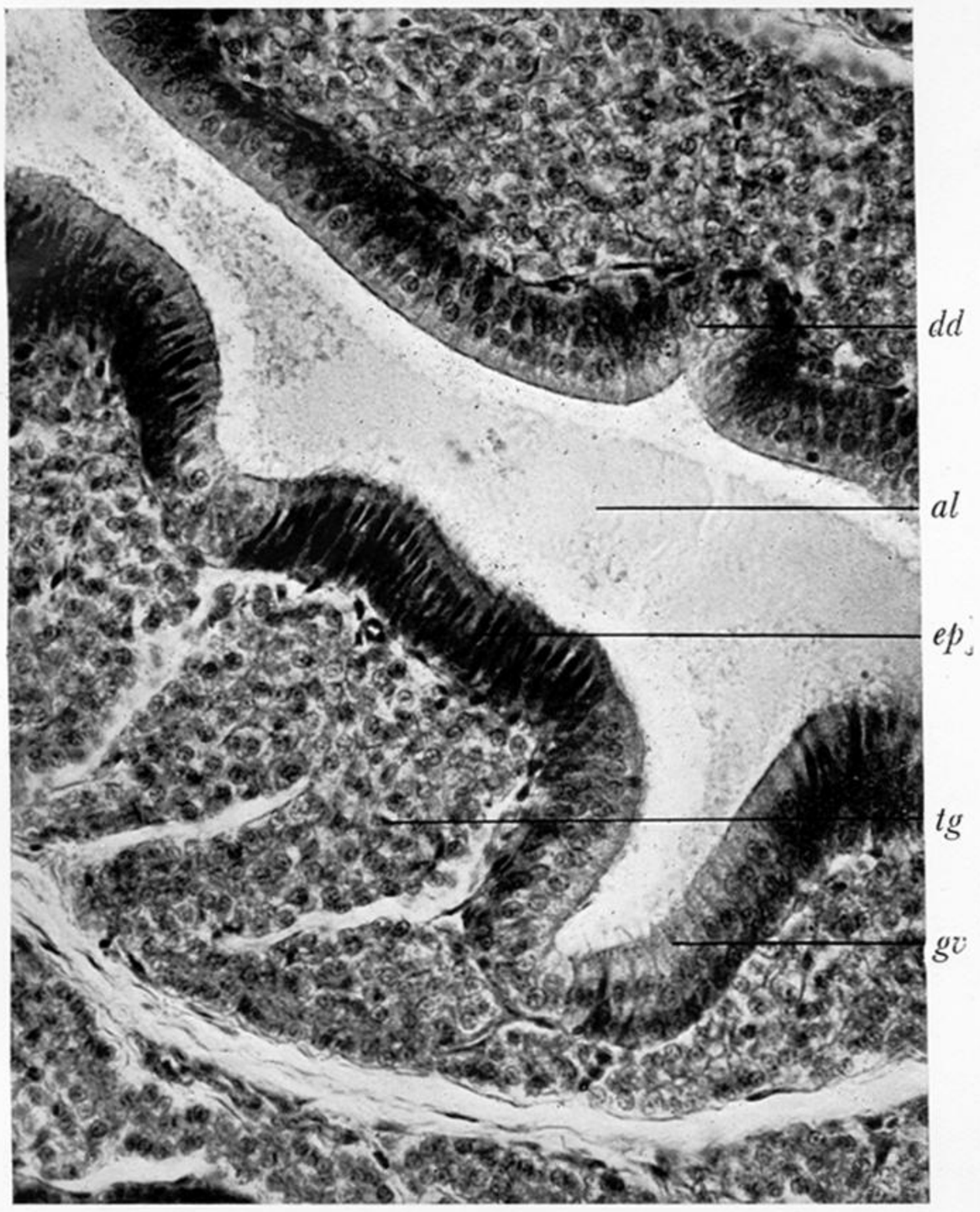

FIG. 34

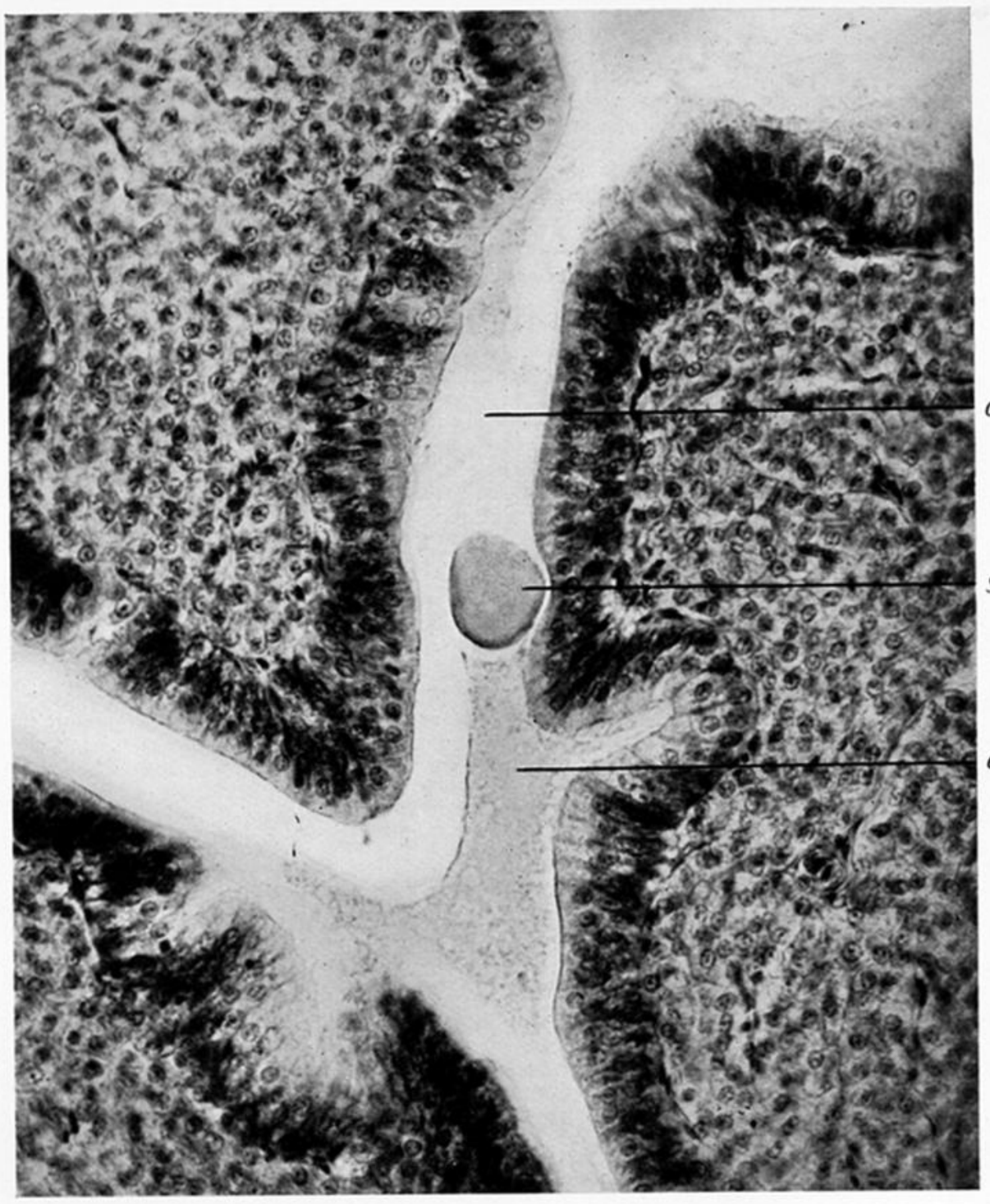

FIG. 35

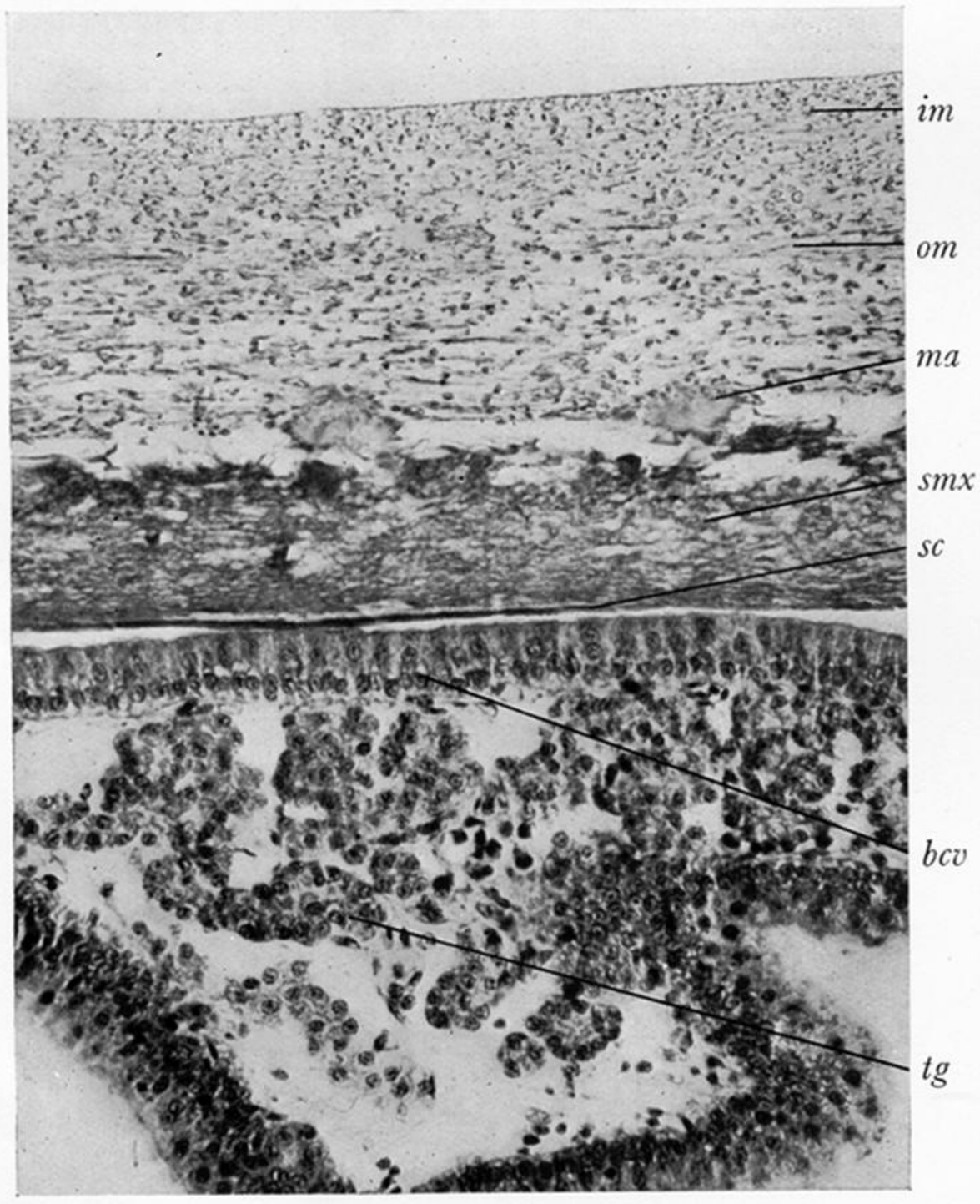

FIG. 36

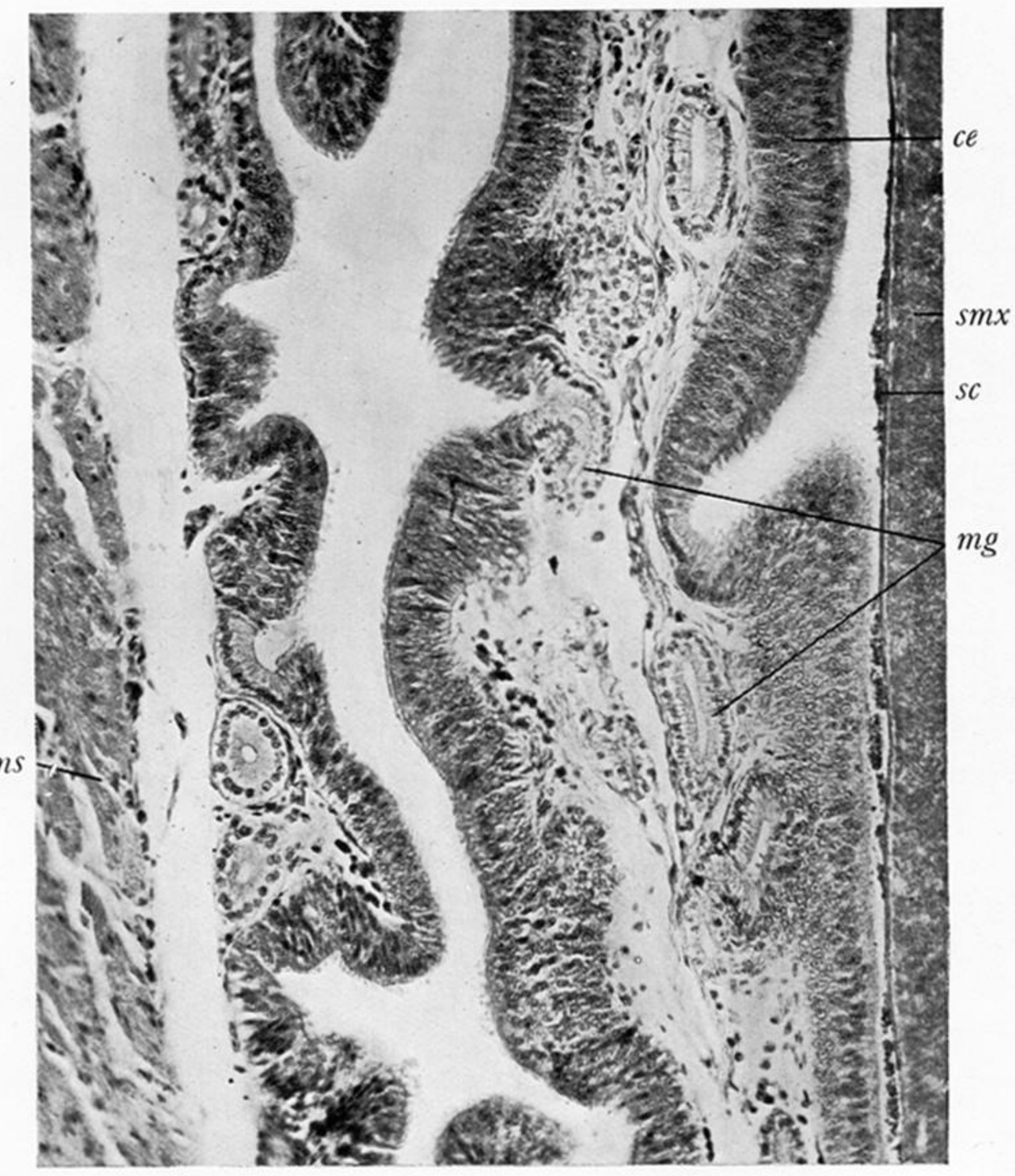

FIG. 37

PLATE 21

FIG. 34—Fowl 33. Showing portions of two adjacent uterine folds some distance from the egg region. A patch of modified epithelium, *ep*, is shown in a phase of excessive secretion. × 400.

FIG. 35—Fowl 33. A mass of shell matrix secretion lying between uterine folds, which in fig. 33 is demonstrated as lying in contact with the developing shell, is contrasted in texture and staining reactions with albumen issuing from a tubular gland. × 400.

FIG. 36—Fowl 34. Portion of a section taken from a uterus containing a completely calcified shell fixed *in situ* and subsequently decalcified. The thickness of the shell matrix and the sparseness of the mammillæ are well illustrated and the condition of the uterine epithelium should be contrasted with that in fig. 32. Note the well-defined cuticular layer on the shell surface which is granular in composition in fig. 37, from the same specimen. The basal cell vacuoles in the epithelium are characteristic of this final secretion stage. × 375.

FIG. 37—Fowl 34. Portion of the uterine wall in contact with the shell surface, of which only portions of the matrix and cuticle are illustrated, where folds characteristic of the vagina occur. The scattered tubular mucous glands are seen in the corium and the thickness of the vaginal epithelium should be noted with its absence of glandular phenomena. × 230.