THE HISTOGENESIS OF BASEMENT MEMBRANES*, ‡

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Plates 9 to 16

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There is no doubt that basement membranes of blood vessels are of mesenchymal origin, but the widely held concept that basement membranes surrounding epithelial structures are formed from condensations of connective tissue ground substance has been questioned recently (1–4). We have shown that parietal yolk sac cells of the mouse, which are an embryonic type of epithelium, secrete the thick basement membrane upon which they rest (4). This information was obtained in parallel experiments employing electron and fluorescence microscopy of normal parietal yolk sac cells and a tumor ultimately proven to be a parietal yolk sac carcinoma. These tumors secreted large quantities of hyaline material which was shown to be similar histochemically and electron microscopically to that of the basement membrane (Reichert's membrane) upon which normal parietal yolk sac cells are found.

Antibodies formed in rabbits against this neoplastic hyalin (NH) were found after conjugation with fluorescein to stain reticulin and basement membranes of vessels and glandular structures. In the present study, evidence will be presented indicating that basement membranes of some epithelia of the mouse originate in the endoplasmic reticulum of the epithelial cells, and that this material differs antigenically from collagen, reticulin, and basement membranes of blood vessels.

Materials and Methods

The source of the NH was a parietal yolk sac carcinoma developed by either ascitic conversion (5) or prolonged tissue culture (6) of a murine testicular teratocarcinoma which in turn had been originally acquired from L. C. Stevens (7). The methods employed in developing and handling these tumors have been described (4–6).

The NH was prepared from either the ascites or from subcutaneous transplants according

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to the method for glomerular basement membranes described by Goodman *et al.* (8). A 10 per cent suspension of tumor tissue in 8 per cent saline was made in a Waring blendor; the cells of the suspension were disrupted by sonication for 15 to 30 minutes in a Raytheon sonicator, and the insoluble fraction collected by centrifuging in the cold at 1400 *G* for 30 minutes. The precipitate containing the NH was washed 5 times in 8 per cent NaCl at which time it was free from cytoplasmic or nuclear material when examined light microscopically. The NH was washed in distilled water and lyophilized.

Antisera to the NH were prepared in rabbits employing incomplete Freund's adjuvants (25 mg lyophilized NH in 4 cc of oil in water emulsion per rabbit) injected into the foot-pads and subcutaneously in multiple sites. The animals were test bled and reinjected at monthly intervals until antisera of good quality were obtained.

The rabbit anti-neoplastic hyalin (anti-NH) was characterized by gel diffusion technique after solubilizing the antigen in 1 N NaOH. Since it precipitated from the alkaline solution if the pH was brought below 7.5, the pH was adjusted to 8 with 0.1 N HCl. After absorption of the anti-NH with small amounts of mouse sera to remove antibodies against serum proteins that had contaminated the antigen, a single precipitin band appeared when anti-NH and alkaline-soluble NH were reacted in Ouchterlony plates.

The anti-NH sera were fractionated with half-saturated ammonium sulfate, and the globulins conjugated with fluorescein isothiocyanate (9, 10), and stored frozen. They were absorbed once with activated charcoal and twice with human liver powder.

Some of the conjugated anti-NH sera was absorbed with splenic residue to determine whether the staining of basement membranes of mesenchymal origin observed previously could be abolished. Spleens were dissected fresh from mice and prepared in a manner identical with the preparation of NH. The insoluble splenic residue contained splenic reticulin and basement membranes of vessels. The antiserum was absorbed by shaking a suspension of splenic residue in the conjugate in the cold for 24 hours, centrifuging, and repeating the absorption process twice; whereupon the absorbed conjugate failed to stain blood vessels or reticulin of frozen sections of spleen or myometrium.

The frozen tissues to be examined were cut at 6 microns; fixed in 1:1 ethanol-ether at room temperature for 15 minutes followed by 95 per cent ethanol at 37°C for 15 minutes; washed in 0.02 M phosphate-buffered (pH 7.0) saline; stained with the specific conjugate for 30 minutes at room temperature; washed again in buffered saline, and mounted with 9:1 glycerol-buffered saline. Adjacent control sections were stained either with fluorescein-conjugated normal rabbit globulin or with the specific conjugate following absorption with NH in a manner similar to the absorption with splenic residue.

For immunoelectron microscopic studies the crude globulins obtained from anti-NH serum by ammonium sulfate fractionation were conjugated to ferritin employing the bifunctional reagent *p*,*p*'difluoro- *m*,*m*'-dinitrodiphenylsulfone (FNPS)[1] (11). A reaction mixture containing 200 mg of globulins in 2 ml of normal saline, 4 ml of "Pentex" ferritin (350 mg), 2 ml of 4 per cent sodium carbonate, and 5 mg of FNPS in 1 ml of acetone was stirred for 24 hours at 4°C. The product containing globulin-ferritin conjugate with unreacted ferritin and globulins was dialyzed against normal saline and subjected to electrophoresis in agar medium (12), under conditions described by Das and Giri (13). After a 24 hour electrophoretic run, the agar containing the globulin-ferritin conjugate was frozen below −20°C and allowed to thaw at room temperature. The solution separating from the agar granules contained pure conjugate. It was used in further studies after dialysis against normal saline.

Advantage was taken of the insoluble nature of the antigen in tracing its intracellular site of synthesis with ferritin-labeled antibody. The tumor cells were homogenized as a 10 per

[1] FNPS can now be purchased from General Biochemicals, Laboratory Park, Chagrin Falls, Ohio.

cent suspension in 0.88 M sucrose solution by three 10-second bursts at top speed in a Vertis 45 homogenizer. The homogenate was centrifuged in the cold at 1400 *G* for 30 minutes, the precipitate which contained extracellular NH among other things was saved, and the supernatant was centrifuged in a Spinco model L at 100,000 *G* for 2 hours to secure the microsomal-mitochondrial fraction.

Some of the extracellular NH and the microsomal-mitochondrial fraction were dispersed respectively in ferritin-conjugated antibody. The control fractions were suspended in either ferritin-conjugated antibody that had been absorbed with NH or in non-conjugated ferritin and antibody. After reacting in the cold for 2 hours, these were washed 3 times in saline and fixed for electron microscopy.

Tissues and precipitates for electron microscopy were cut in small fragments in chilled isotonic-buffered OsO_4 and embedded in vestopal W (Martin-Jaeger, Geneva, Switzerland). Sections were cut on a Porter-Blum microtome and, after mounting on collodion-covered, carbon-coated grids, were stained in 2 per cent aqueous uranyl acetate for 24 hours. An RCA EMU-3F electron microscope was employed in the ultrastructural examinations.

RESULTS

With fluorescein-conjugated anti-NH, specific staining was observed in the following: NH; the basement membranes of renal glomeruli and tubules, prostate, testis, and blood vessels; and the reticulin of spleen, lymph nodes, and muscle (Figs. 1 to 5). Collagen from the subcutaneous space and tendon did not stain. A complete study of the antigenicity of basement membranes of the mouse with species cross-reactions will be published separately.

There was marked variation in the degree of staining observed; NH (Fig. 1), Reichert's membrane, the renal glomeruli (Fig. 2), and the basement membranes of vessels (Fig. 5) fluoresced brilliantly while the basement membranes surrounding glandular structures were less densely stained. A fine basket-like web of fluorescence was observed in the fibromuscular stroma of the prostate (Fig. 4) and around myometrial cells, perineural connective tissue cells, and reticular cells of the spleen (Fig. 5) and lymph nodes.

In order to determine whether the fluorescence of reticulin and the basement membranes of blood vessels on the one hand and of NH on the other, were cross-reactions of a single antigen-antibody system or whether the anti-NH contained separate antibodies directed against each of these antigens, the labeled anti-NH was absorbed with splenic residue.

Absorption was continued until fluorescein-labeled anti-NH stained neither reticulin nor the basement membranes of the vessels of the spleen (Fig. 5 *b*). This absorbed antibody failed to stain reticulin and vascular basement membranes of parietal yolk sac carcinoma (Fig. 1 *b*), kidney (Figs. 2 *b* and 3 *b*), prostate (Fig. 4 *b*), testis, and placenta. The NH of the parietal yolk sac carcinoma, its normal embryonic counterpart Reichert's membrane, and the focal intracytoplasmic fluorescence of either benign or malignant parietal yolk sac cells (believed to be the intracytoplasmic sites of secretion of NH) fluoresced with undiminished brilliance.

The fluorescence of basement membranes of the renal glomeruli was somewhat reduced in intensity (Fig. 2 *b*). But this reduction never equalled that observed around renal tubules (Figs. 2 *b* and 3 *b*), prostatic acini (Fig. 4 *b*), or testicular tubules. Instead of the bright band of fluorescence noted previously in these situations, only a thin layer closely applied to the bases of the epithelial cells could be found. It was concluded that a thin lamella composed of an antigen similar to or identical with NH but which was related to neither basement membranes of vessels nor to reticulin of the same organ, lay in juxtaposition to the bases of epithelial cells.

One would expect a reduction in fluorescence of the basement membranes of the renal glomeruli and tubules, prostate, and testis, after staining with fluorescein-labeled antibody that had been absorbed with splenic residue if their basement membranes were composed of a reticular or vascular component in addition to the NH-like component. This thesis was examined by an electron microscopic study of the structures involved.

Basement membranes from an ultrastructural standpoint proved to be composed of a thin lamella of homogeneous, moderately electron-opaqe material, intimately contacting or separated from the bases of the epithelial cells by an electron-lucent space (Fig. 6 *a* and 6 *b*). It should be made clear that the epithelial lamella at the bases of epithelial cells was a remarkably constant finding. It closely resembled NH and Reichert's membrane ultrastructurally, differing from the latter only in its lack of fasciculation. It should be recalled, however, that NH and Reichert's membrane when in small amount also lacked the fascicular appearance.

The relationships external to this epithelial lamella varied with the organ studied. In some situations, squamous epithelium of the skin or visceral yolk sac, for instance, (Fig. 6 *c*), reticulin fibers (or collagen fibers?) identifiable by their characteristic periodicity were observed blending with the epithelial lamella. When capillaries were present in juxtaposition to the epithelium, an endothelial lamella morphologically identical with its epithelial counterpart was observed intimately contacting the endothelium (Fig. 6 *b*). The endothelial lamella either blended with the epithelial cells resulting in a relatively thick homogeneous band or was separated from it by an electron-lucent space. In the glomerulus, we have observed fibrils in the endothelial component of the glomerular basement membranes, confirming the observations of Farquhar *et al.* (3).

The lamellae of epithelial structures have also been observed contacting similar lamellae of smooth muscle cells (renal tubules to arterioles) (Fig. 6 *a*) or they have contacted the lamellae of other epithelia (lamellae of renal tubules to those of Bowman's capsular epithelium) (Fig. 6 *a*).

In view of the ultrastructural relationships observed, it seems reasonable to conclude that after abolishing the fluorescent staining of endothelial lamellae and reticulin, the remaining fluorescence observed at the bases of the renal

tubular cells or around prostatic acini must be the result of localization of fluorescein-labeled antibody on the lamella immediately adjacent to the bases of the epithelial cells. In further support of the idea that the fluorescein-labeled antibody localizes on the lamella and not on the adjacent electron-lucent zone is the observation, to be described in the next section, that this antibody when ferritin-labeled, localized on extracellular NH.

Intracellular Origin of Reichert's Membrane.—In a previous report a correlation was established between the distribution and amount of the focal fluorescence observed when the tumor cells were stained with fluorescein-labeled anti-NH and the distribution and amount of the endoplasmic reticulum as observed electron microscopically (4). Although this suggested that the NH was produced in the endoplasmic reticulum, we wished more direct evidence, — the localization of ferritin-labeled anti-NH in the endoplasmic reticulum for instance.

In performing this experiment advantage was taken of the relatively insoluble nature of the antigen. The tumor cells were homogenized and extracellular-nuclear fractions as well as microsomal-mitochondrial fractions were obtained. Ferritin-labeled anti-NH was applied to these fractions, thereby eliminating the problem of penetration of large molecules through cellular membranes.

Ferritin-labeled anti-NH localized exclusively in the extracellular NH and did not react with nuclei of the extracellular-nuclear fraction. Deep penetration of ferritin-labeled anti-NH into the NH was not observed and probably reflects the binding of the tagged antibody by antigen before deep penetration could occur. (Fig. 7 *a*).

The immunologic specificity of this observation was confirmed because localization of ferritin-labeled anti-NH was almost completely abolished by prior absorption of the labeled antibody with NH (Fig. 7 *b*). The few ferritin molecules observed in the NH after staining with absorbed antibody reflects the insignificant degree of non-specific binding of ferritin-labeled rabbit gamma globulin to NH.

The application of ferritin-labeled anti-NH to the subcellular components of the cytoplasm of the tumor cells resulted in localization of ferritin molecules on the endoplasmic reticulum (Fig. 8). The mitochondria were rarely labeled, in fact those that contained any ferritin molecules were usually so badly damaged that elements of the ergastoplasm could have been admitted to them. Ferritin-labeled anti-NH did not localize on cell and nuclear membranes (Fig. 10). The localization of ferritin could be almost completely abolished by prior absorption of the antibody with NH again indicating the specificity of this reaction (Fig. 9).

It was concluded from this experiment that one of the antigens of extracellular NH was localizable in the endoplasmic reticulum of the tumor cells and in accord with current theory was probably synthesized in that organelle.

DISCUSSION

The classic concept of basement membranes, based upon light microscopy (review by Lillie) has been that a basement membrane is a homogeneous hyaline band intervening between epithelium and connective tissue or between vessels and connective tissue that takes silver stains for reticulum and is PAS-positive (14). It has been postulated that they are formed by polymerization of glycoproteins of ground substance and thus are of connective tissue origin (15).

From an electron microscopic standpoint, basement membranes from various parts of the body, kidney (2, 3, 16, 17), thyroid (18), lung (19), skin (20), and endocrine glands (21), have as a common component a moderately electron-opaque band of amorphous material of variable width, immediately adjacent to or separated from the base of the epithelial or endothelial cell by a narrow electron-lucent zone. On the opposite surface and often immediately adjacent or blending with it are "reticulin" (collagen) fibers recognized by their periodicity. In highly vascularized epithelial tissues such as the lung or renal tubules, one finds either twin or single hyaline bands, the latter formed by fusion of adjacent epithelial and endothelial lamellae. Reticulin fibers are occasionally found between these bands.

Basement membranes have been extensively studied from an immunopathologic standpoint. A common cross-reacting antigen, for instance, has been observed in studies of tissue-specific antigens and has been attributed to a common vascular antigen, presumably originating in the basement membranes (22). It has been demonstrated that renal glomerular basement membranes are antigenic when injected in heterologous species (8, 23); the antibody elicited is capable of causing severe anatomical and functional derangement of the renal glomeruli when injected into the original species (1, 8, 23–25). When the mechanism of this experimental glomerulonephritis was investigated using fluorescent antibody techniques, basement membranes around epithelia and within vessels fluoresced specifically as did reticulin (25). This common antigenicity was held in accordance with the theory that these structures originated as condensations of ground substance (25). The critical experiment to test the theory was not performed, however.

When these data from widely divergent areas of technology are considered together, many questions remain unanswered. Are the basement membranes surrounding epithelial structures the same as their endothelial counterparts and are they formed by the condensation of ground substance? Does the epithelial cell play a part in the production of its basement membrane? If so, is this a product formed within the cell or is it a cell membrane or extracellular phenomenon as is suggested by the studies upon reticulin formation? These are the questions asked of the model employed in the present series of experiments.

In establishing the parietal yolk sac carcinoma as the model for these studies, using tissue culture and electron and fluorescence microscopy, we proved that

parietal yolk sac cells of the mouse embryo secrete in the total absence of connective tissue or its products the thick basement membrane upon which they rest (4). Evidence was obtained strongly suggesting the endoplasmic reticulum as the organelle of formation of this membrane. This contention was substantiated in this presentation by the localization of ferritin-labeled anti-NH to the endoplasmic reticulum and extracellular hyalin of parietal yolk sac carcinoma.

Since one basement membrane was proven to be an epithelial secretion, it seemed reasonable to expect that those of the other epithelia may also be a secretion of the epithelial cells in question and that mesenchymal basement membranes might prove to be antigenically different from them.

When fluorescein-labeled anti-NH was absorbed with splenic pulp (until the staining of reticulin and basement membranes of blood vessels was abolished), the antibody stained Reichert's membrane with undiminished brilliance. This provided a tool that could distinguish with the precision of immunochemical specificity, a basement membrane of proven epithelial origin from basement membranes of vessels and antigens of connective tissue.

When fluorescein-labeled epithelial-specific anti-NH was applied to other basement membranes including those of the renal glomeruli, renal tubules, and prostatic acini, positive but reduced fluorescence was observed when compared to staining obtained with non-epithelial-specific anti-NH. When these results were interpreted in light of the ultrastructural evidence, the only visible antigen comparable to Reichert's membrane available for staining would be the moderately electron-opague lamella adjacent to the bases of the epithelial cells. This idea was in turn strongly supported by the observations that ferritin-labeled anti-NH localized in NH and Reichert's membrane, and not over electron-lucent zones corresponding to those often observed between cellular membranes and their basement membranes.

Since it has been proven that the basement membrane of parietal yolk sac originates in the endoplasmic reticulum of that cell type, and that fluorescein-labeled antibodies specific for this material stain the basement membranes of epithelial structures, it is probable that all epithelial cells secrete their own basement membranes. We have not demonstrated an endoplasmic reticulum in renal tubular cells capable of producing basement membrane material but believe the explanation is one of turnover. Parietal yolk sac cells secrete the hyalin of Reichert's membrane during a 10 day interval; the intracellular synthesis during this period is proceeding rapidly. Since the catabolism of basement membrane material in renal tubules is extremely slow (unpublished data), renal tubular cells, after first forming the membrane, may make only infinitesimal amounts for maintenance. In fact the cells may lose the ability to synthesize, which may account for the lack of renal tubular regeneration when there is extensive damage to both the epithelium and its basement membrane. Other observations (1, 3) have shown that the endoplasmic reticulum of the

normal glomerular epithelium may occasionally contain basement membrane material.

SUMMARY

A parietal yolk sac carcinoma of the mouse that secretes large quantities of basement membrane–like material has been used to study the formation of basement membranes. Suitably characterized fluorescein-labeled antibodies against this material stained basement membranes of epithelial structures and vessels, as well as reticulin. When absorbed with reticulin and vascular basement membranes of the spleen until these structures no longer fluoresced, the antibody still stained the basement membrane–like material of the tumor, its normal embryonic counterpart (Reichert's membrane), and the basement membranes at the bases of epithelial cells.

The observation made previously that parietal yolk sac cells secreted, in the absence of connective tissue and reticulin, the basement membrane (Reichert's membrane) upon which they rested has been confirmed through the localization of ferritin-labeled antibody to the endoplasmic reticulin of the secreting cells.

Since a basement membrane proven to be an epithelial secretion is antigenically similar to basement membranes at the bases of all epithelial cells studied but antigenically different from connective tissue elements, it is postulated that the basement membranes at the bases of epithelial cells in general are an epithelial secretion, and are not a condensation of ground substance as is commonly believed.

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EXPLANATION OF PLATES

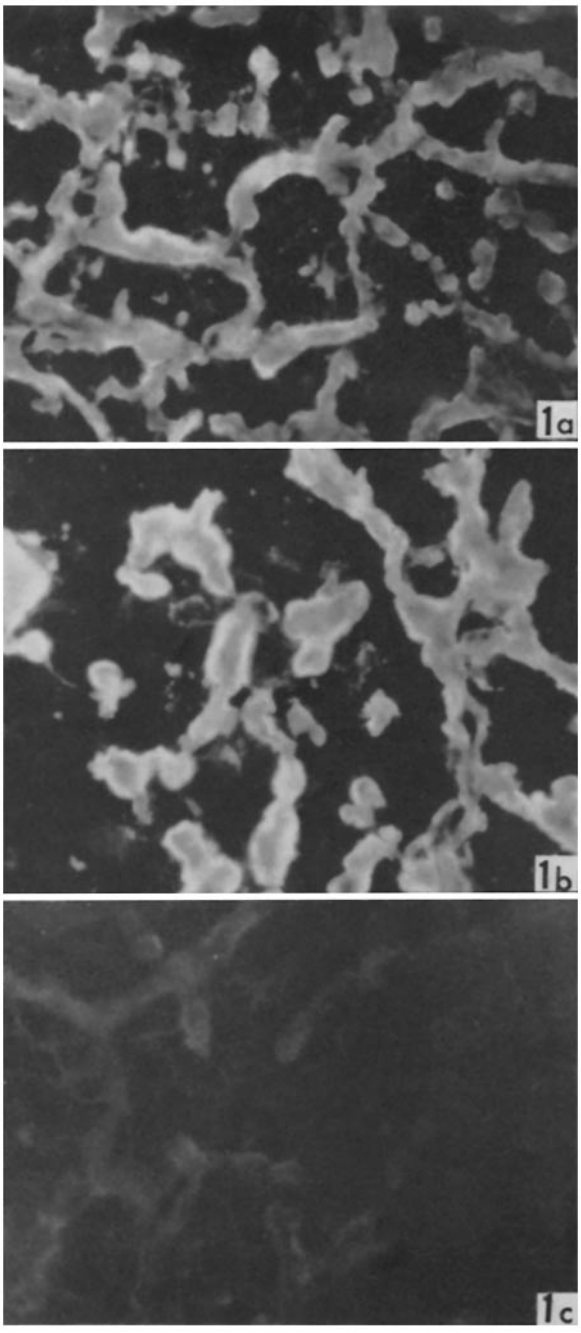
PLATE 9

This is a series of frozen sections of the parietal yolk sac carcinoma of the mouse stained with (*a*) fluorescein-labeled antibody, (*b*) fluorescein-labeled antibody absorbed with splenic residue prior to staining, and (*c*) fluorescein-labeled anti-NH that had been absorbed with NH prior to staining. × 494.

FIG. 1 *a*. The extracellular hyalin stains brilliantly and specifically.

FIG. 1 *b*. There is little or no reduction in the amount of staining of extracellular hyalin when fluorescein-labeled anti-NH absorbed with reticulin is used.

FIG. 1 *c*. The specificity of the reactions is indicated by abolition of staining by absorbing the fluorescein-labeled anti-NH with NH.



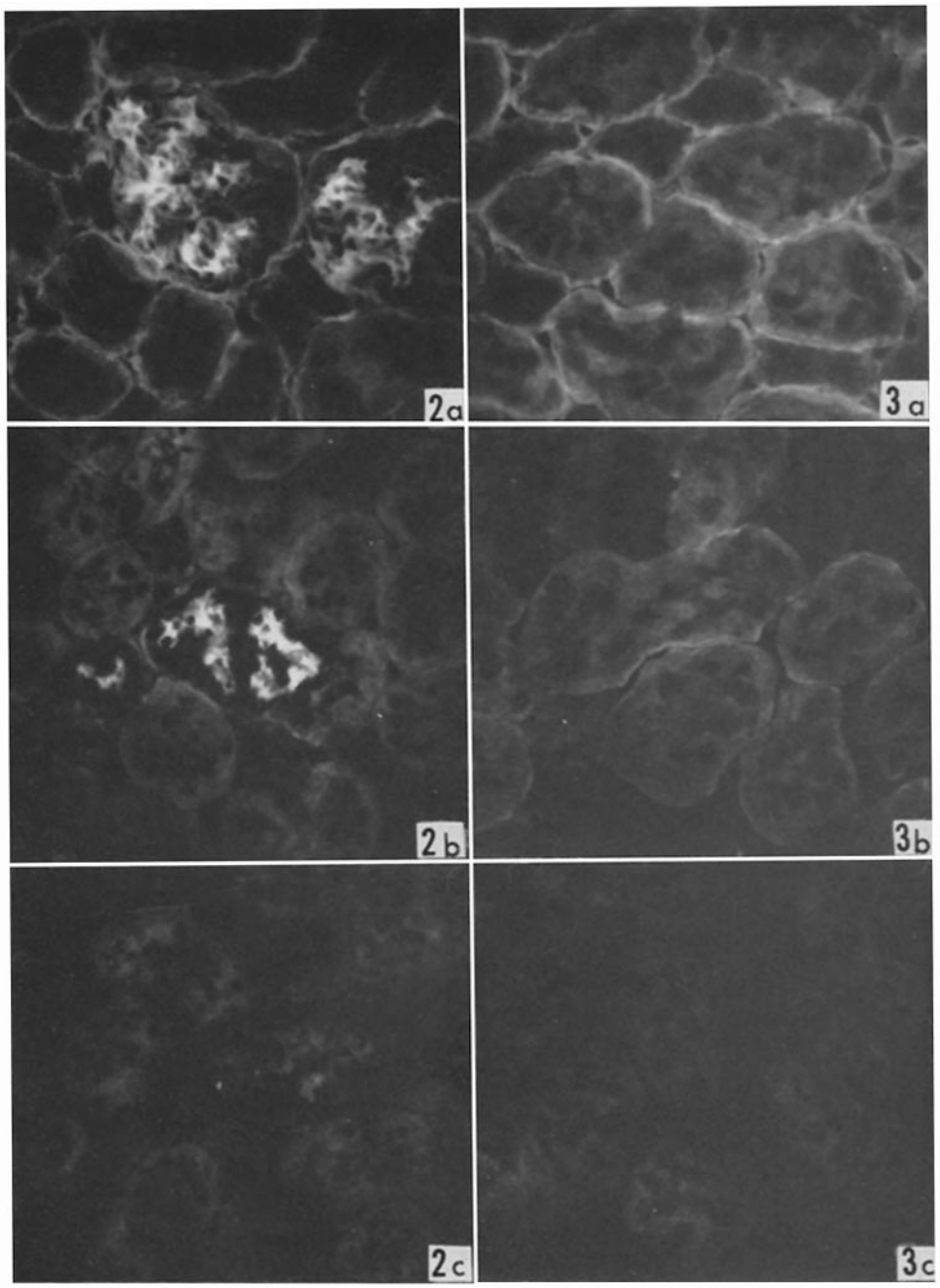
(Pierce *et al.*: Basement membranes)

PLATE 10

This is a series of serial frozen sections of kidney from the mouse, stained with (*a*) fluorescein-labeled anti-NH, (*b*) fluorescein-labeled anti-NH that had been absorbed with splenic residue prior to staining, and (*c*) fluorescein-labeled anti-NH that had been absorbed with NH prior to staining.

FIGS. 2 *a* to 2 *c*. Glomerular, capsular, and tubular basement membranes stain brilliantly in Fig. 2 *a* but in Fig. 2 *b* staining after prior absorption of the antibody with splenic residue resulted in diminished fluorescence. This is most apparent in the basement membranes of Bowman's capsule and of renal tubules. Absorption of the fluorescein-labeled anti-NH with NH prior to staining abolishes all fluorescence, indicating thereby the specificity of the reaction. ×312.

FIGS. 3 *a* to 3 *c*. These are renal tubules from the same series as Figs. 2 *a* to 2 *c*, but photographed at greater magnification. It will be noted that after absorption of fluorescein-labeled anti-NH with splenic residue a faint band of fluorescence still remains at the bases of the renal tubular cells (*b*). It is greatly reduced in comparison to that observed when non-absorbed antibody is employed (*a*). Non-absorbed antibody stains reticulin and basement membranes of vessels in addition to the epithelial component. After prior absorption of the antibody with NH all fluorescence is abolished (*c*). × 494.



(Pierce *et al.*: Basement membranes)

PLATE 11

FIGS. 4 *a* to 4 *c*. These are serial frozen sections of prostate from the mouse, stained with (*a*) fluorescein-labeled anti-NH, (*b*) fluorescein-labeled anti-NH that had been absorbed with splenic residue prior to staining, and (*c*) fluorescein-labeled anti-NH that had been absorbed with NH prior to staining.

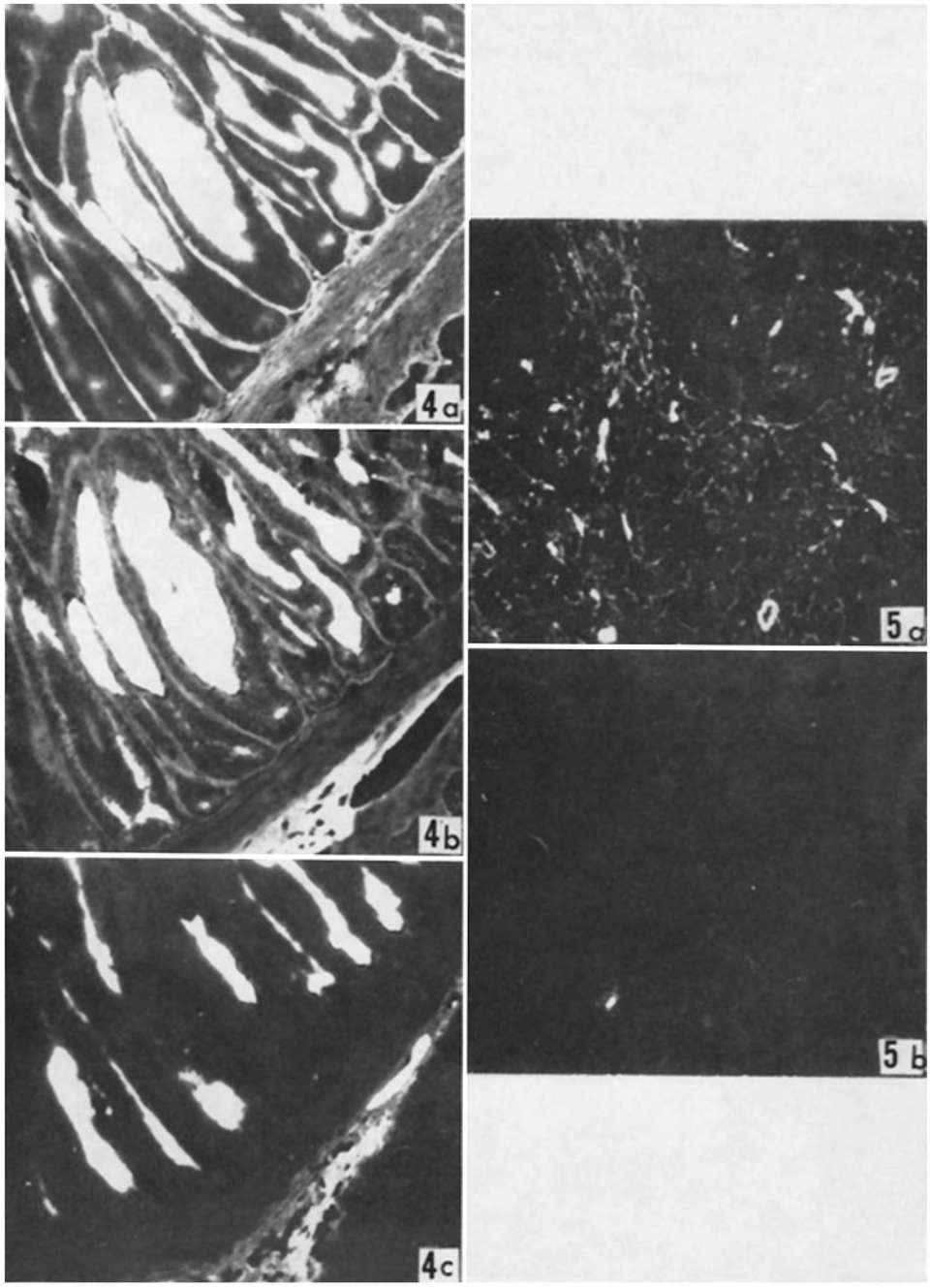
FIG. 4 *a*. Note the intense autofluorescence of the secretions occupying the lumens of prostatic acini. The reticulin of the fibromuscular stroma of (*a*) stains brilliantly as do vessels lying between the acini. The basement membranes fluoresce radiantly. × 125.

FIG. 4 *b*. After absorption of the antibody with splenic residue prior to staining, vascular and reticular staining is abolished while that of the basement membranes of the acini, although reduced in amount, is still brilliant. × 125.

FIG. 4 *c*. Absorption of the antibody with NH prior to staining abolishes all specific fluorescence. Only autofluorescent secretions are seen in this photograph. × 125.

FIG. 5 *a*. This is a frozen section of spleen stained with fluorescein-labeled anti-NH illustrating the degree of reticular staining present. Notice, too, the intense staining of basement membranes of the blood vessels. × 125.

FIG. 5 *b*. This is an adjacent section to that of 5 *a*, stained with fluorescein-labeled antibody that had been absorbed with splenic residue. Only background staining remains. × 125.



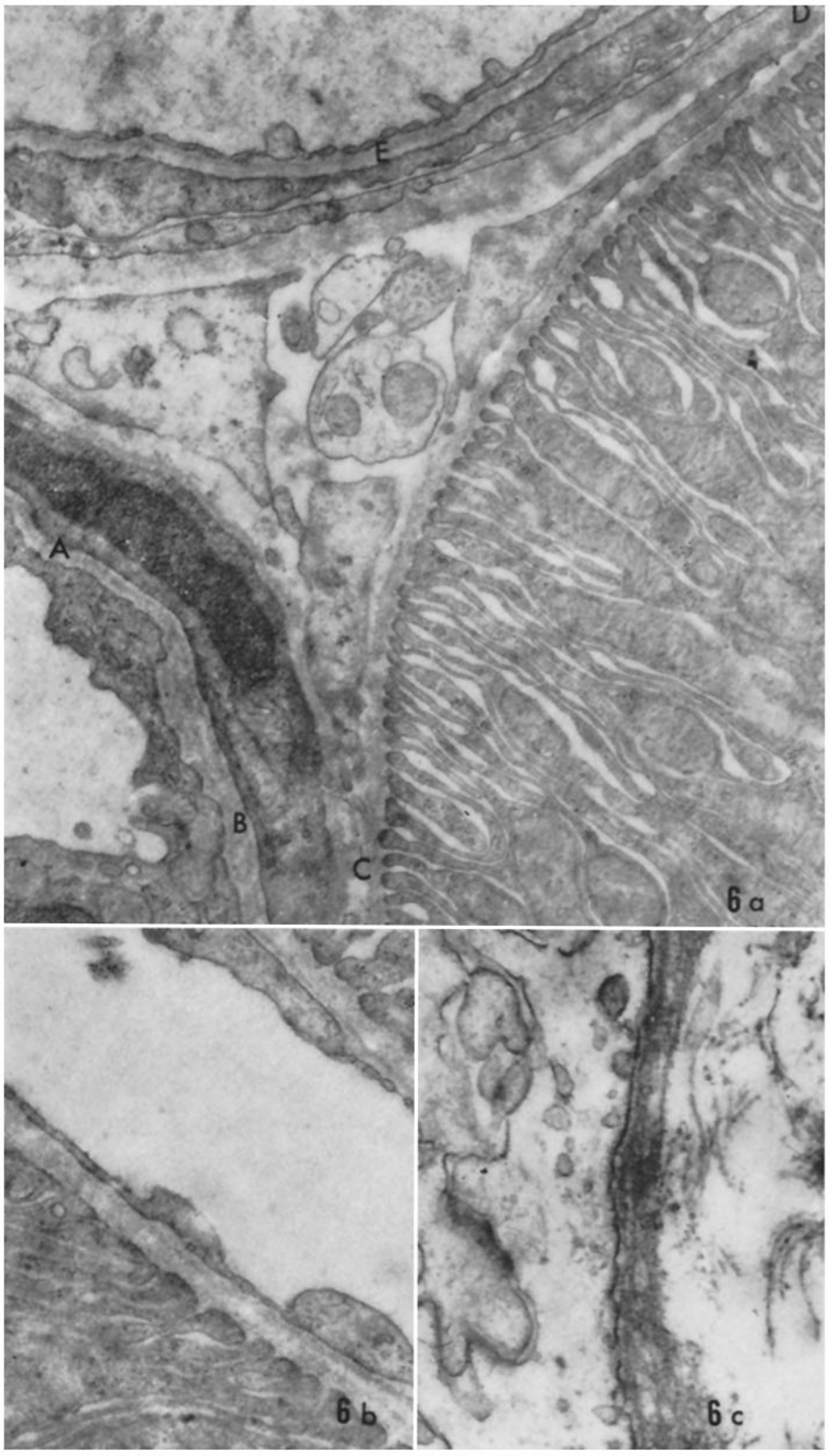
(Pierce *et al.*: Basement membranes)

PLATE 12

FIG. 6 *a*. This electron micrograph illustrates portions of a glomerulus, tubule, and arteriole of the murine kidney. The endothelialas bement membrane of the arteriole may be seen at *A* where it is composed of an electron-opaque lamella bordered by electron-lucent zones. At *B* double lamellae, one adjacent to endothelium, the other to muscle, may be seen. These fuse at *A*. At *C* the basement membrane of smooth muscle of the arteriole blends with that of the renal tubular epithelium and is indistinguishable from it. At *D* the basement membrane blends with that of Bowman's capsule. At *E* the basement membrane lying between the glomerular epithelium and endothelium has the appearance of a dense central band with electron-lucent borders. × 14,000.

FIG. 6 *b*. This is a section through the base of a renal tubular cell illustrating the relationships of the epithelial lamella to that of endothelium. × 18,000.

FIG. 6 *c*. This is a section through the base of a visceral yolk sac cell of the embryo illustrating the basilar lamella with reticulin (collagen) fibrils external to and blending with it. × 18,000.

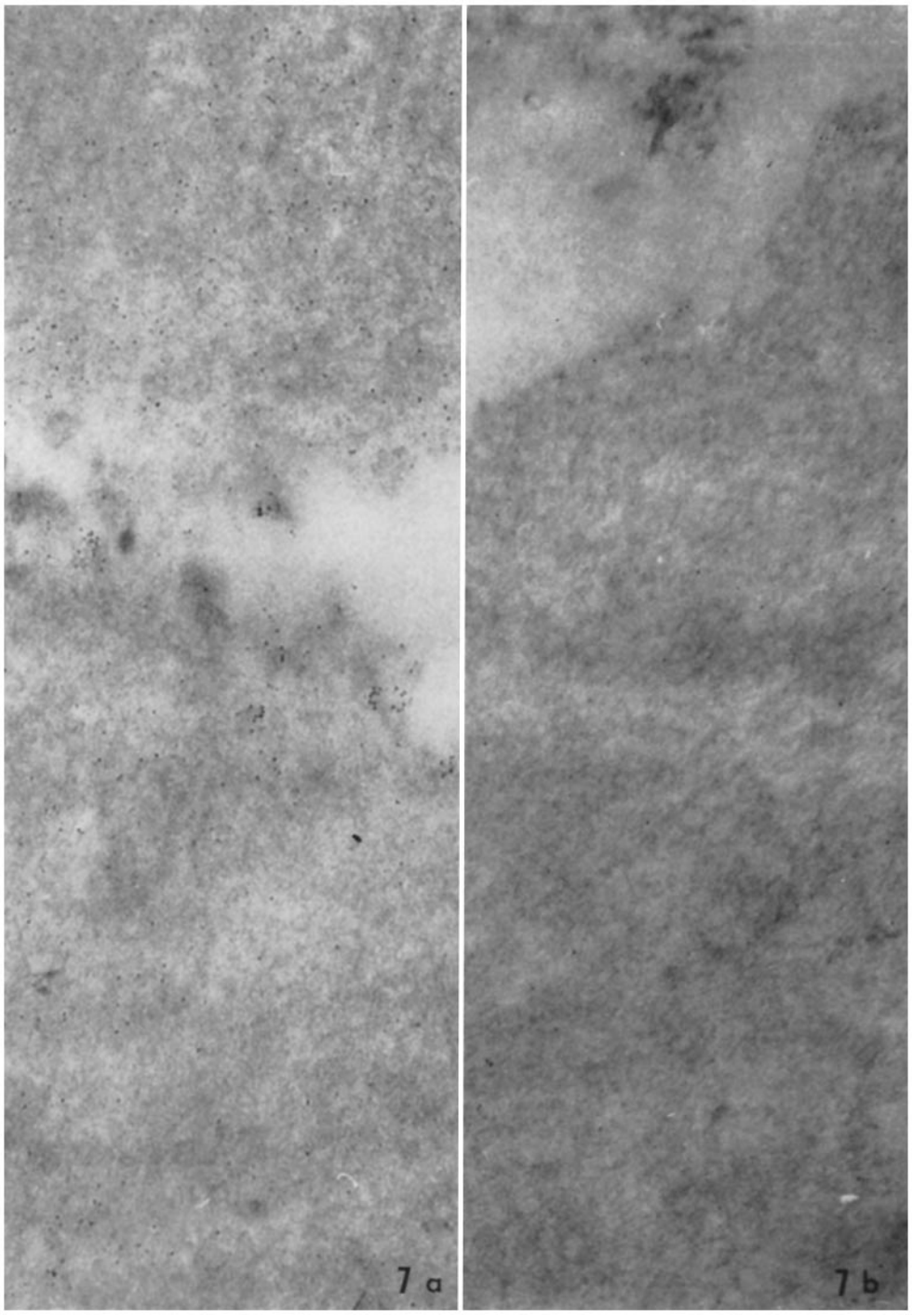


(Pierce *et al.*: Basement membranes)

PLATE 13

FIG. 7 *a*. This is a section of extracellular NH that was treated with ferritin-labeled anti-NH. The labeled antibody has localized on NH, not on the electron-lucent zones. × 44,000.

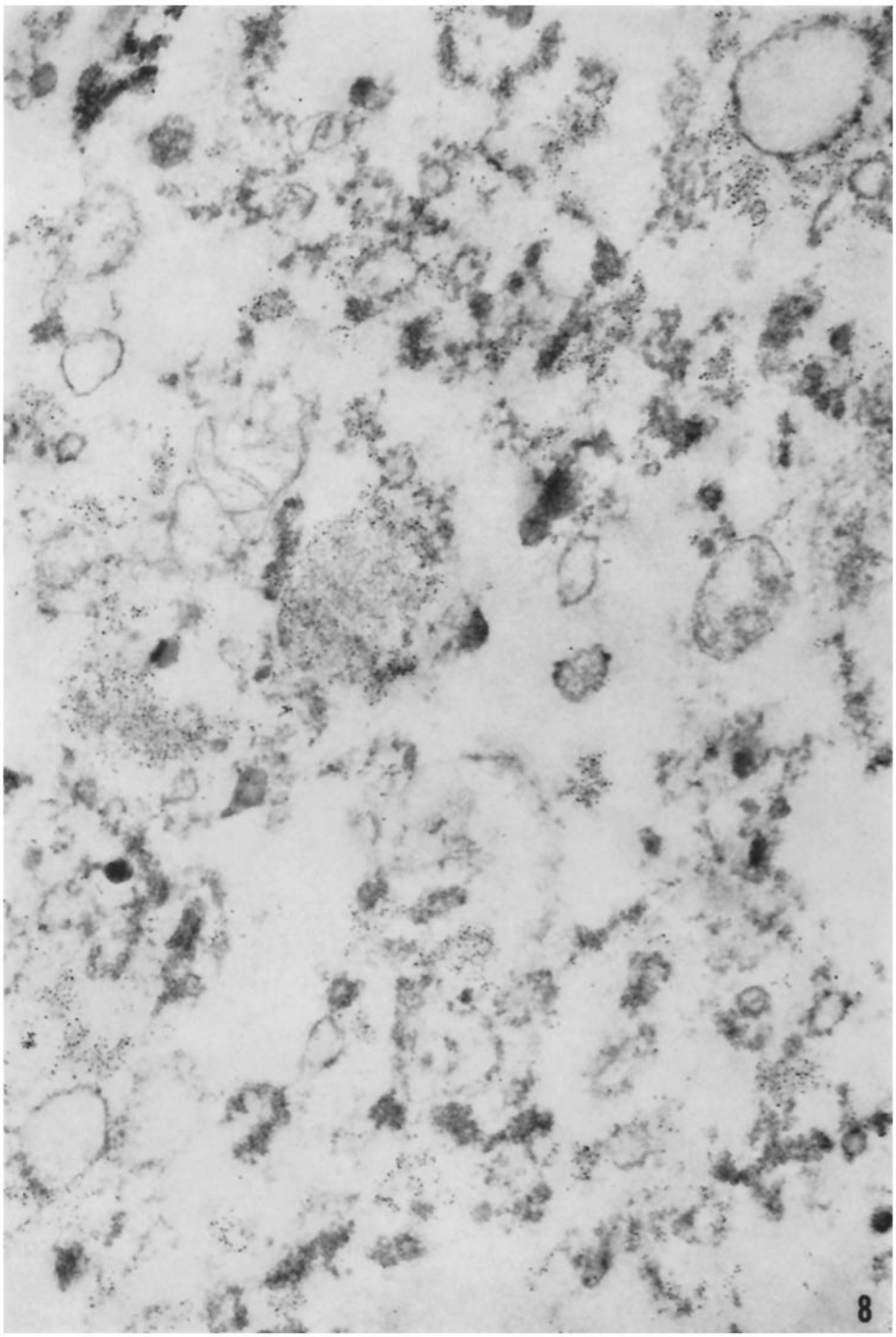
FIG. 7 *b*. This is the control section for 7 *a*. In this illustration the ferritin-labeled antibody was absorbed with NH prior to staining. The degree of ferritin localization observed reflects the degree of binding of ferritin-labeled non-specific rabbit gamma globulin. × 44,000.



(Pierce *et al.*: Basement membranes)

PLATE 14

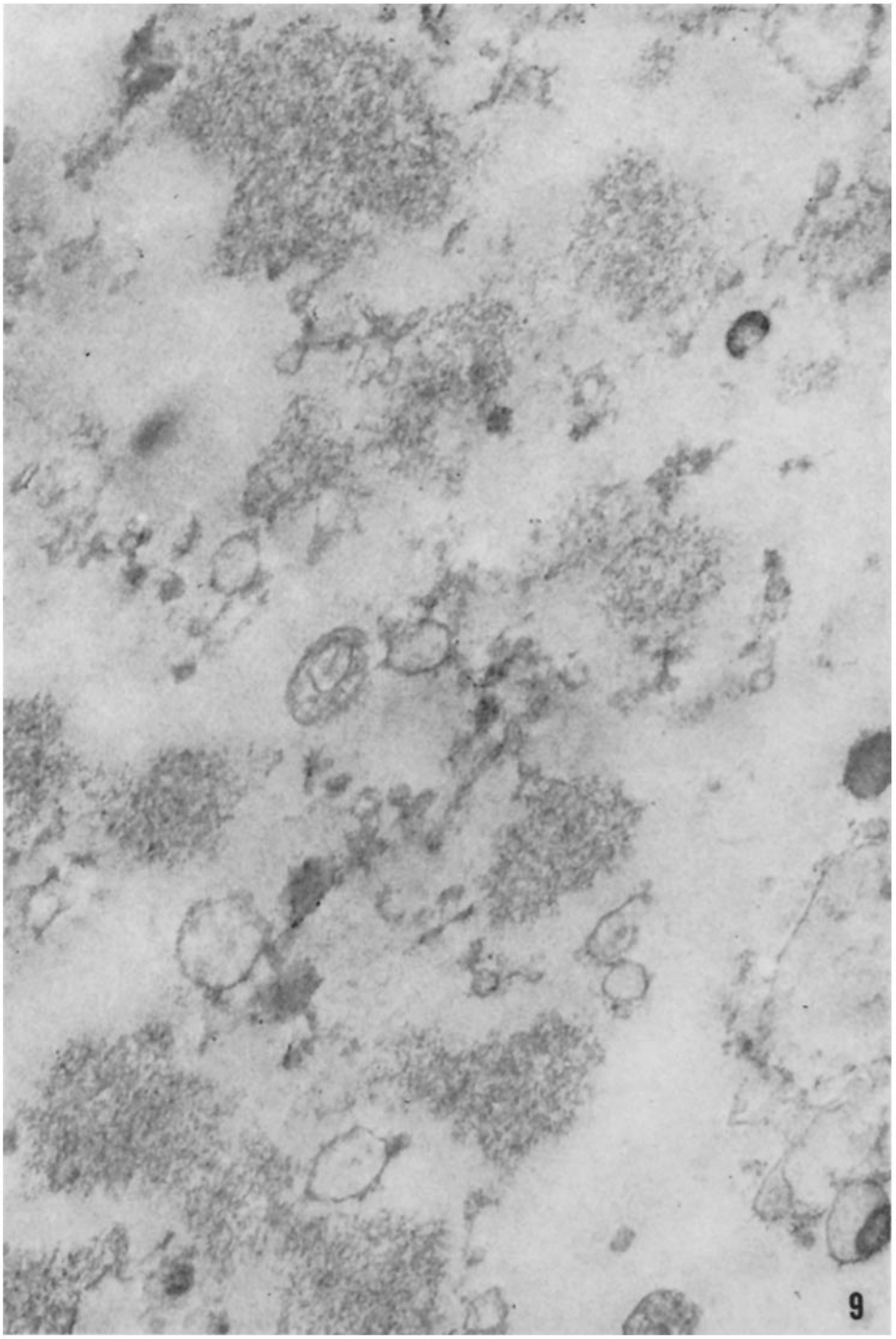
FIG. 8. This is a section from the microsomal-mitochondrial fraction stained with ferritin-labeled antibody. Mitochondria are not labeled. The label is over the endoplasmic reticulum. × 36,000.



(Pierce *et al.*: Basement membranes)

PLATE 15

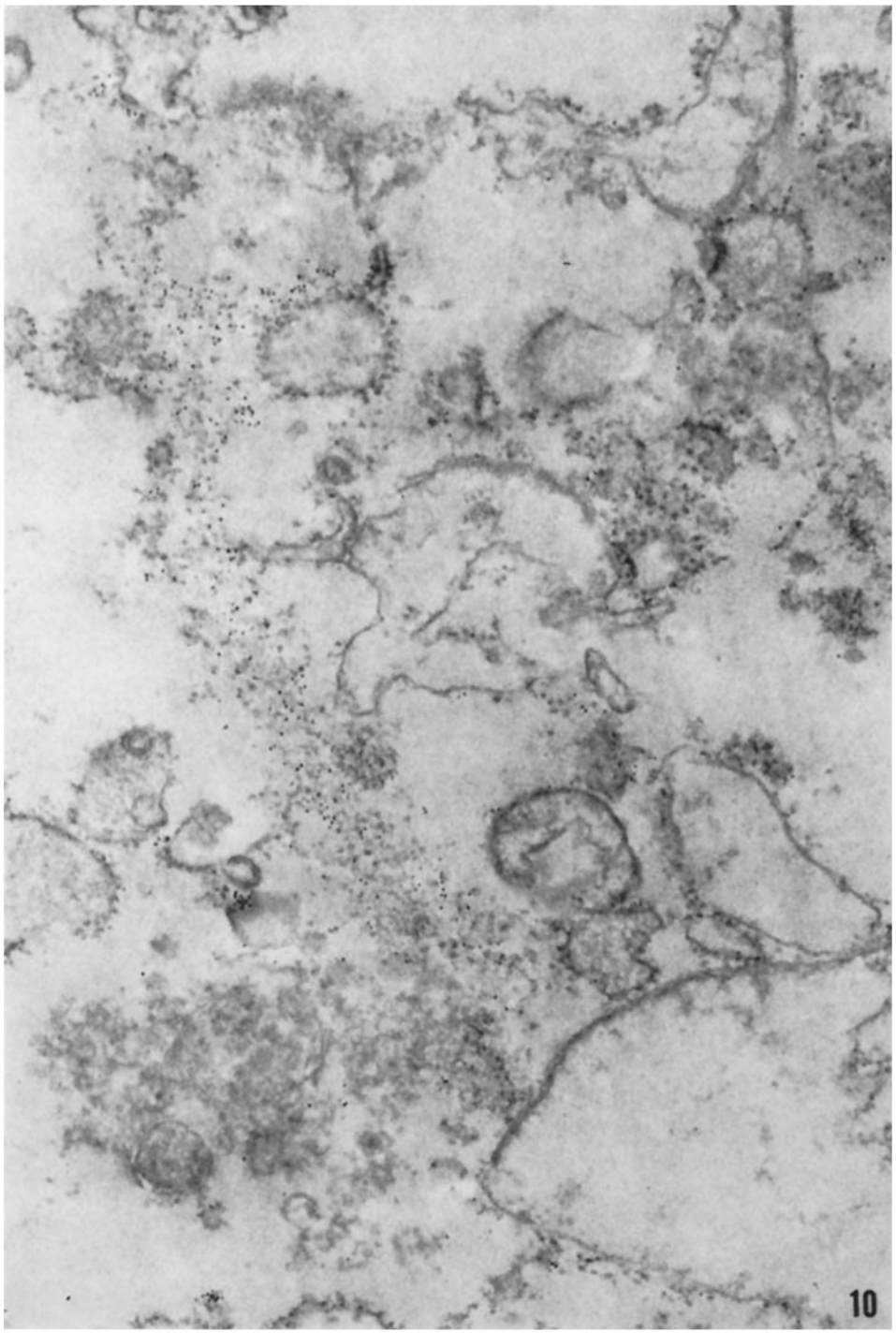
FIG. 9. This is the control for Fig. 8, a section of microsomal-mitochondrial preparation stained with ferritin-labeled antibody that had been previously absorbed with NH. Only occasional molecules of ferritin are visible. × 36,000.



(Pierce *et al.*: Basement membranes)

PLATE 16

FIG. 10. This is a section of cellular homogenate containing many membranous structures, presumably of cellular and nuclear origin stained with ferritin-labeled antibody. There is no localization upon the membranes, only on the granular form of the endoplasmic reticulum. × 44,000.



(Pierce *et al.*: Basement membranes)