IMMUNOHISTOCHEMICAL ANALYSIS OF BASEMENT MEMBRANES OF THE MOUSE

A. R. Midgley, Jr., M.D., and G. B. Pierce, Jr., M.D.
From the Department of Pathology, The University of Michigan Medical Center, Ann Arbor, Mich.

The widely accepted theory that basement membranes are derived from a polymerization of connective tissue ground substance [1] must be modified since a basement membrane of the murine embryo, Reichert's membrane, originates as a secretion of the adjacent epithelial cells.[2] In addition Reichert's membrane was shown to contain an antigen in common with the basement membranes of the epithelial components of prostate and kidney. Since this antigen was unlike the antigens of the connective tissues in these organs, it was postulated that basement membranes bordering epithelia were of epithelial origin.[3]

As a step in determining the validity of this hypothesis, a survey of the antigenicity of basement membranes in the mouse has been made. It has been observed that basement membranes adjacent to most epithelia contain this epithelial antigen which is distinct from those in connective tissue.

Method

Since Reichert's membrane, which is the secretion of parietal yolk sac cells of the embryo, is produced in minute amounts, we employed a parietal yolk sac carcinoma as the source of antigen for these experiments. The methods for preparing this neoplastic hyalin (NH) and fluorescein-conjugated rabbit anti-NH globulins (designated crude fl-anti-NH) have been described.[2]

Crude fl-anti-NH was absorbed with splenic pulp until it failed to stain splenic reticulin and vascular basement membranes.[3] The specificity of the resulting reagent has been tested extensively. It produced brilliant staining of basement membrane material known to originate within epithelial cells [intra- and extra-cellular NH of parietal yolk sac carcinoma (Fig. 1) and Reichert's membrane], but did not stain reticulin or vascular basement membranes in any organ and did not stain anything in non-epithelial tissues including: splenic pulp; peripheral nerve; smooth, cardiac and skeletal muscle (Fig. 2); adipose tissue; cartilage; fibroelastic connective tissue (sclera, dermis, submucosa, etc.); posterior pituitary; adrenal medulla; thymic pulp and Descemet's membrane of the cornea. On the basis of this survey it was concluded that fl-anti-NH absorbed with splenic pulp did not react with reticulin or non-epithelial basement membranes. This reagent was therefore designated "epithelial-specific fl-anti-NH."

Control reagents included fluorescein-labeled normal rabbit globulins and fl-anti-NH which had been absorbed with NH until no staining could be detected in sections of parietal yolk sac carcinoma.

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From each tissue studied, serial frozen sections were cut, each at a thickness of 6 μ, air-dried, fixed for 10 minutes in acetone at room temperature, and washed in 0.02 M phosphate buffered saline (pH 7.0). Adjacent serial sections were stained respectively with: crude fl-anti-NH; epithelial-specific fl-anti-NH; fl-anti-NH absorbed with NH; and fluorescein-labeled normal rabbit globulins. The sections were then washed in phosphate-buffered saline, and mounted in Elvanol.[4] To allow for precise histologic identification, a serial section was fixed in 10 percent formalin with 1 per cent acetic acid and stained with hematoxylin and eosin.

To provide information on the relative amount of antigen in each basement membrane, a subjective appraisal of the intensity of fluorescence was made for the basement membranes in each tissue and recorded on a scale ranging from 1+ (specific fluorescence present) to 4+ (maximal fluorescence). Fluorescence no greater than background was recorded as 0.

Results

Crude fl-anti-NH stained reticulin and all basement membranes of the mouse that were examined. This staining was specific since no reaction was observed in sections treated with either fluorescein-labeled normal rabbit globulins or fl-anti-NH absorbed with NH.

Immunofluorescent staining of basement membranes bordering epithelium, with crude and epithelial specific fl-anti-NH, is listed in Table I. In general, basement membranes reacting with epithelial-specific fl-anti-NH appeared narrower and sharper than the same basement membranes stained with crude fl-anti-NH. This suggested that the reaction of the epithelial-specific antibody was confined to an anatomically narrow, well-delineated band at the base of the epithelial cells while the reaction of the crude antiserum involved this narrow epithelial-specific antigen as well as adjacent reticulin and connective tissue antigens (Figs. 3 to 10).

The fluorescence of epithelial basement membranes stained with epithelial-specific fl-anti-NH varied greatly. For example the basement membrane of proven epithelial origin (Reichert's membrane), stained brilliantly while some epithelial basement membranes in apposition with connective tissue stained well with crude fl-anti-NH but minimally (Fig. 8) or not at all with epithelial-specific fl-anti-NH. Since, by electron microscopy, similar basement membranes have been demonstrated to border nearly all epithelia, it would seem that some of the epithelial basement membranes which failed to stain with epithelial-specific fl-anti-NH (lung for example) might contain epithelial-specific material but in a band too narrow to be detected by present immunofluorescent techniques.

As expected, vascular basement membranes within the subarachnoid space failed to stain with the epithelial-specific fl-anti-NH. However, epithelial-specific basement membrane material was localized to the pia-glial membrane on the surface of the cerebral and cerebellar cortex,

TABLE I

IMMUNOFLUORESCENT STAINING OF BASEMENT MEMBRANES BORDERING EPITHELIA WITH CRUDE AND EPITHELIAL SPECIFIC FL-ANTI-NH

	Crude fl-anti-NH	Epithelial specific fl-anti-NH
Gastrointestinal system		
Salivary glands	4+	1+
Esophagus	3+	1+
Stomach	3+	1+
Duodenum	3+	1+
Jejunum	3+	1+
Ileum	3+	1+
Colon	4+	1–2+
Pancreas	3+	1+
Liver (portal areas and central veins)	2–3+	0
(sinusoids)	1+	0
Genitourinary system		
Kidney (glomeruli)	3–4+	2–4+
(tubules)	1–3+	0–2+
Urinary bladder	4+	1+
Testes	4+	3+
Prostate	4+	2–3+
Ovary (Graafian follicles)	4+	2+
Uterus	3+	1+
Respiratory system		
Lung	3+	0
Trachea	3+	0
Extraembryonic structures		
Placenta (spongy and labyrinthine layers)	3+	1+
(Reichert's membrane)	4+	4+
(Parietal yolk sac carcinoma (NH)	4+	4+
Visceral yolk sac	3–4+	1+
Eye		
Lacrimal glands	4+	2+
Capsule of the lens *	4+	4+
Ciliary limiting membrane	4+	3+
Bowman's membrane	2–3+	0
Bruch's membrane	3+	0
Brain (pia-glial membrane, see text)	3–4+	3+
Endocrine		
Thyroid	4+	0
Anterior pituitary (pars distalis)	4+	3+
(pars intermedia)	4+	2+
Adrenal cortex (glomerulosa)	4+	3+
(fasciculata and reticularis)	2–3+	0

* This membrane, which stains in a manner similar to NH and Reichert's membrane, is bordered solely by epithelium.

and to basement membranes surrounding venules and arterioles within the spinal cord, cerebrum, and cerebellum (Fig. 9). By careful study of serial sections, it was shown that the latter basement membranes represented extensions of the pia-glial membrane (Fig. 10) which is known to invest most arterioles and venules of the central nervous system.[5] A few small intracerebral annular structures also stained with the epithelial-specific reagent. The low resolution of the immunofluorescent technique made it impossible to determine whether these areas represented capillaries invested by an epithelial-specific basement membrane, or portions of the perivascular pia-glial membrane surrounding larger vessels.

Discussion

In a survey of murine basement membranes, fl-anti-NH absorbed with splenic pulp reacted with most of the basement membranes bordering epithelia but not with connective tissue antigens, including vascular basement membranes and reticulin. These observations appear incompatible with the widely held belief that basement membranes of epithelia originate as condensations of connective tissue ground substance.[1] Since the antigen of epithelial basement membranes has been proven in one case to be a secretion originating in the endoplasmic reticulum of the adjacent epithelial cells, it appears that epithelial cells in general secrete their basement membranes.

Basement membranes have been considered by light microscopists to be narrow, hyaline, argyrophilic, periodic acid-Schiff-positive bands interposed between epithelia and connective tissue. Since argyrophilia is a property of reticulin, it has been assumed that all basement membranes contain reticulin and, therefore, probably arise from a condensation of connective tissue ground substance. This concept seems unlikely since Reichert's membrane, which is of proven epithelial origin, contains no reticulin: it is not bordered by cells capable of producing reticulin, it contains no fibrils with periodicity, and it is not argyrophilic.[2,6,7] Therefore, the apparent argyrophilia of most basement membranes is probably due to reticulin (collagen) fibrils which have been compressed against basement membranes during development. By light microscopy these fibrils would appear as part of the basement membrane. In support of this contention electron microscopic studies of basement membranes bordering epithelium have revealed reticulin (collagen) fibrils lying in juxtaposition to, and occasionally appearing to blend with, an electron-dense band of approximately 300 Å thickness which is separated from the epithelial cells by an electron-lucent space of about the same width.[8]

Thus the narrow electron-dense band alone would appear to be the epithelial basement membrane. This contention has been supported by the demonstration of ferritin-labeled epithelial-specific antibody localized to NH in parietal yolk sac carcinoma; [3] and, in addition, by preliminary experiments in which the same ferritin-labeled epithelial-specific anti-NH was localized to the narrow electron dense band adjacent to epithelial cells and not to the adjacent fibrils (unpublished data). Therefore, the lamina densa adjacent to epithelia, which the electron microscopist calls a basement membrane, is probably an epithelial secretion, and the use of the term "epithelial basement membrane" should be restricted to this narrow band.

The absence of epithelial basement membrane antigens in the liver, an organ in which one might expect rapid exchange of macromolecules between parenchymal cells and serum, and the presence of thick epithelial basement membranes in organs in which one would not expect extensive macromolecular diffusion, such as the testis, suggests that epithelial basement membranes might serve as selective filters. In this light the presence of the epithelial pia-glial membrane (which probably originates within the expanded foot processes of adjacent astrocytic cells) between vessels and parenchyma of the central nervous system might suggest that this basement membrane contributes to the "blood-brain barrier." Tschirgi has provided physiologic support for this contention by demonstrating that topical and intravenously administered trypan blue-protein complexes penetrated to but stopped sharply at the pia-glial membrane.[9] More definitive answers will require combined physiologic and morphologic approaches.

Summary

In an extension of earlier work, suitably characterized fluorescein-labeled antiserum against a murine neoplastic hyalin has been shown to stain reticulin and all basement membranes of the mouse. Exhaustive absorption of this conjugated antiserum with splenic pulp resulted in a reagent which stained nearly all epithelial basement membranes of the mouse (including the pia-glial membrane on the surface of the brain and the extension of this membrane around vessels within the central nervous system), but did not stain reticulin or vascular basement membranes. Because antigens of epithelial basement membranes have been found in the cytoplasm of epithelial cells and not in connective tissue, it has been postulated that epithelial basement membranes in general are produced by the bordering epithelial cells.

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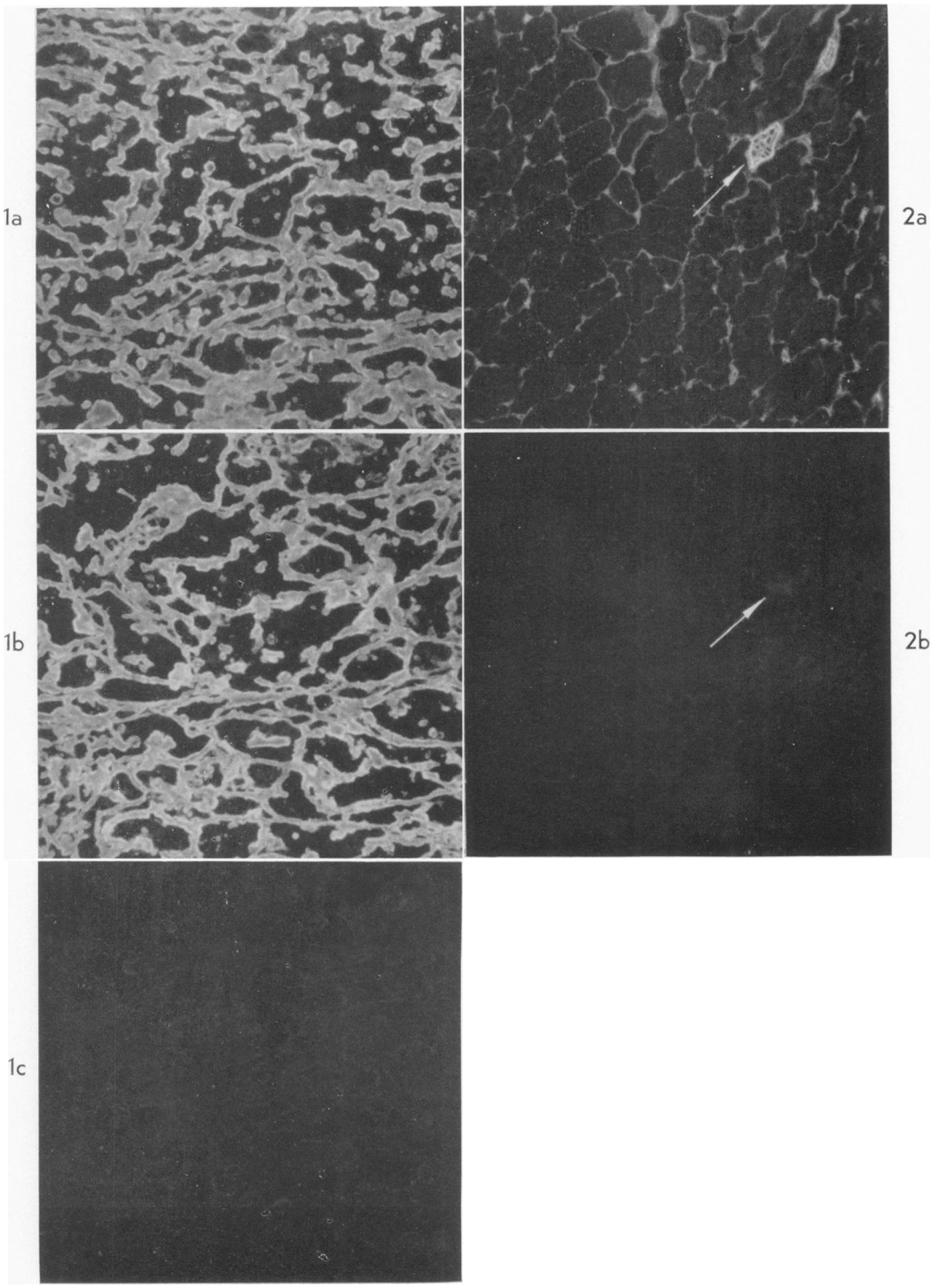
LEGENDS FOR FIGURES

This plate contains a series of photomicrographs from adjacent frozen sections of two tissues stained with (a) crude fl-anti-NH, (b) epithelial-specific fl-anti-NH, and (c) fl-anti-NH that had been absorbed with NH prior to staining.

FIGS. 1a and 1b. Parietal yolk sac carcinoma. No difference can be appreciated between the reaction of extracellular hyalin with epithelial-specific fl-anti-NH (b), and the reaction of the same hyalin with crude fl-anti-NH (a). In each case this hyalin of known epithelial origin stains brilliantly.

FIG. 1c. The lack of staining by fl-anti-NH previously absorbed with NH indicates the specificity of the reactions. × 154.

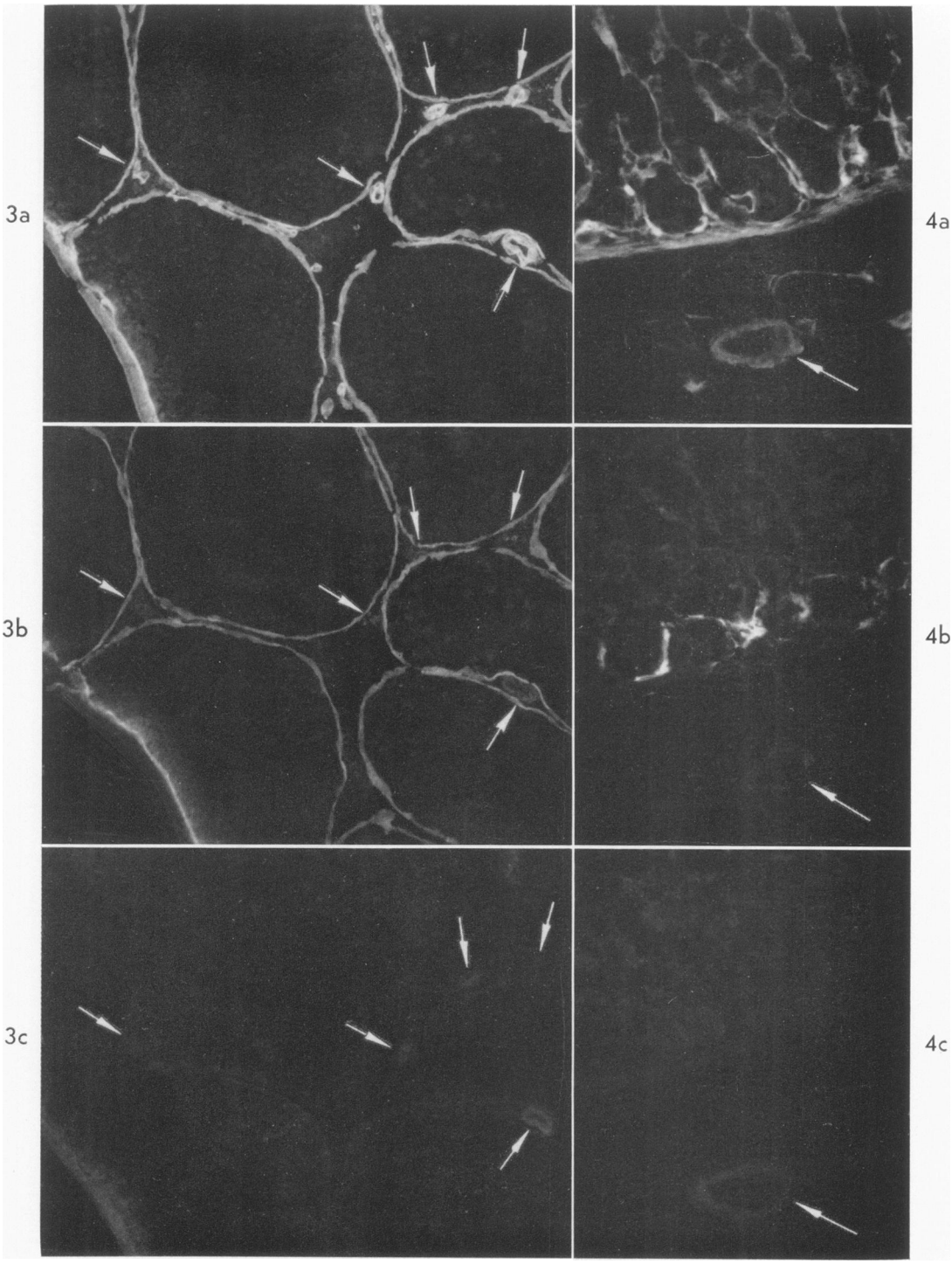
FIGS. 2a and 2b. The bright fluorescence about individual fibers of skeletal muscle and peripheral nerve (arrow) stained with crude fl-anti-NH (a) is absent in the adjacent section stained with epithelial-specific fl-anti-NH (b). This lack of reaction with connective tissue components (Fig. 2b) contrasts with the staining of epithelial-derived extracellular hyalin (see Fig. 1b, stained with the same reagent) and demonstrates the specificity of this reagent for epithelial basement membrane material. × 154.


1a
2a
1b
2b
1c

This is a series of adjacent frozen sections of testis (Fig. 3) and adrenal (Fig. 4), stained with (a) crude fl-anti-NH, (b) epithelial-specific fl-anti-NH, and (c) fl-anti-NH that had been absorbed with NH prior to staining.

FIGS. 3a, 3b and 3c. Reticulin in the fibrous tunica albuginea (lower left), vascular basement membranes (arrows), and tubular basement membranes all stain with crude fl-anti-NH (a). Only the tubular basement membranes stain with epithelial-specific fl-anti-NH (b). × 154.

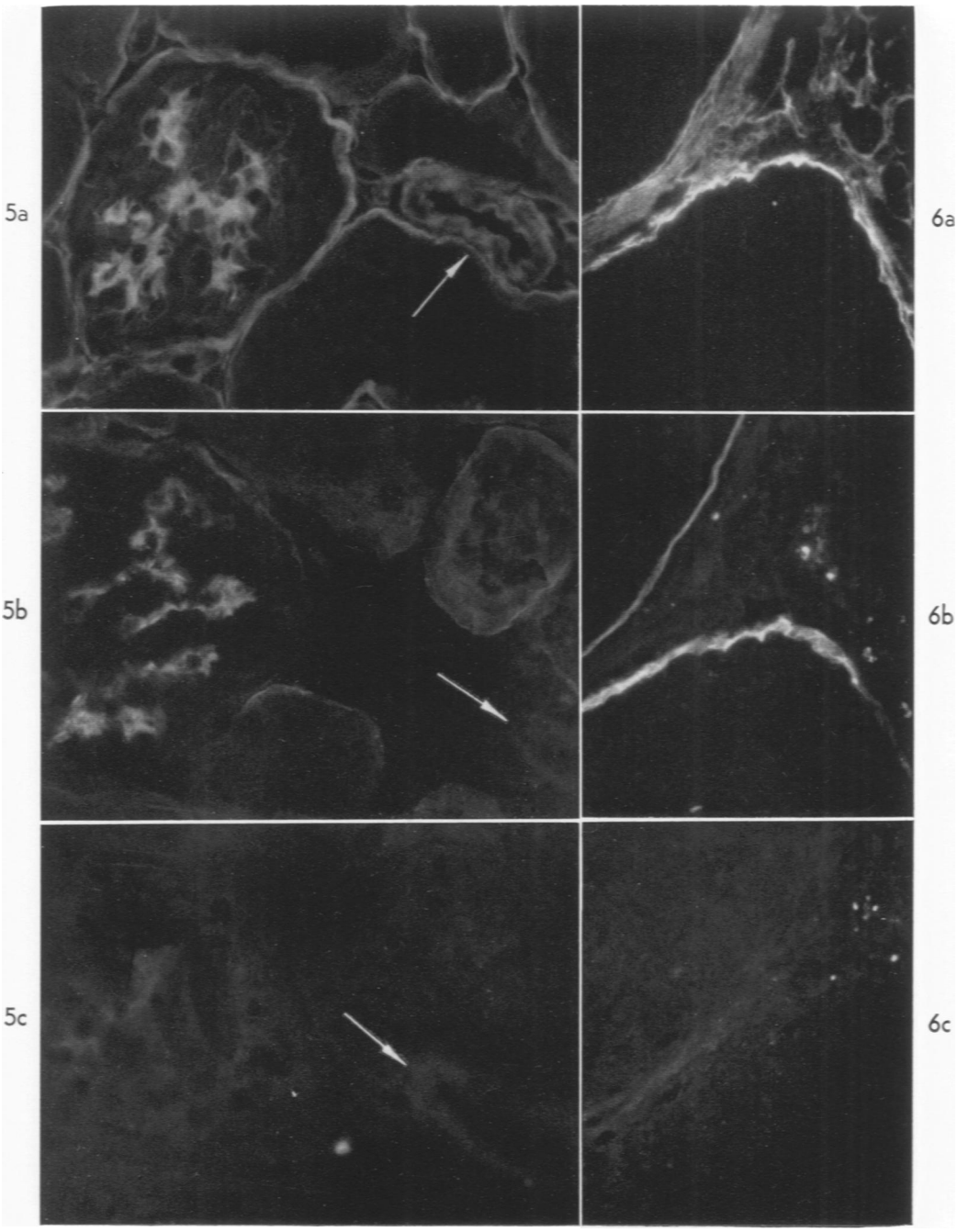
FIGS. 4a, 4b and 4c. Only basement membranes surrounding clusters of cells in the glomerulosa of the adrenal cortex stain with the epithelial-specific fl-anti-NH (b). The reticulin and endothelial basement membranes of the sinusoids in the fasciculata, of peri-adrenal fat, and of the peri-adrenal vessels (arrow) stain brilliantly with crude fl-anti-NH (a). × 385.


3a
4a
3b
4b
3c
4c

These are adjacent frozen sections of kidney (Fig. 5) and ovary (Fig. 6), stained with (a) crude fl-anti-NH, (b) epithelial-specific fl-anti-NH, and (c) fl-anti-NH that had been absorbed with NH prior to staining.

FIGS. 5a, 5b and 5c. There is bright specific staining of the epithelial portion of the glomerular basement membrane; faint narrow staining of portions of the tubular basement membranes; and no staining of vascular basement membranes in an arteriole (arrow) with epithelial-specific fl-anti-NH (b). This contrasts with the bright staining of the arteriole (arrow) and tubular basement membranes with crude fl-anti-NH (a). × 610.

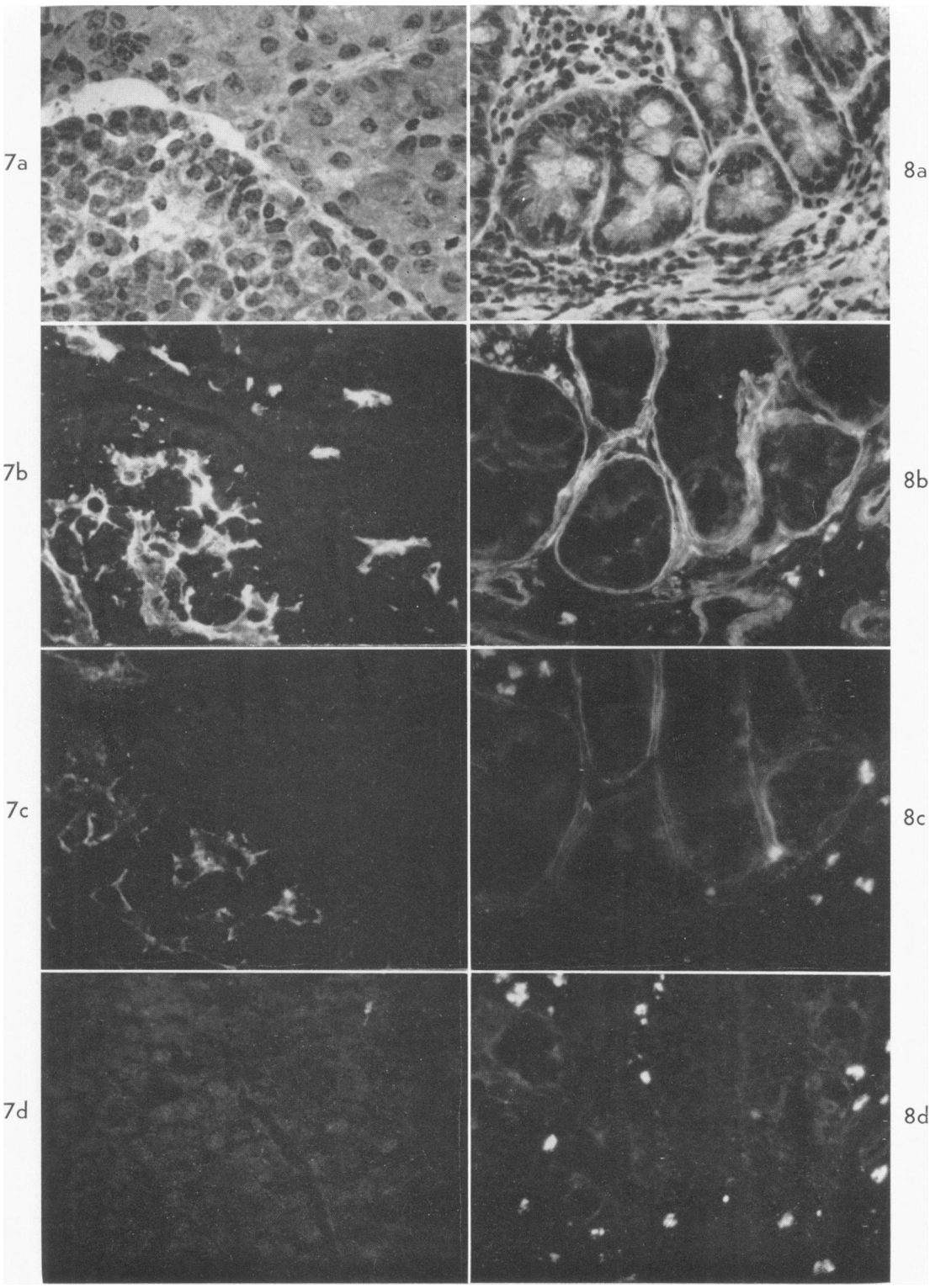
FIGS. 6a, 6b and 6c. Autofluorescent material (steroids?) is present in the stroma in all sections. Epithelial-specific fl-anti-NH has only reacted with the basement membrane at the periphery of germinal follicles (b) and not, as in (a), with reticulin or vascular basement membranes of the ovarian stroma. × 385.


5a
6a
5b
6b
5c
6c

This is a series of frozen sections of anterior pituitary (Fig. 7) and colon (Fig. 8), stained with (a) hematoxylin and eosin stain, (b) crude fl-anti-NH, (c) epithelial-specific fl-anti-NH, and (d) fl-anti-NH that had been absorbed with NH prior to staining.

FIGS. 7a, 7b, 7c and 7d. Epithelial-specific fl-anti-NH has reacted with many basement membranes bordering sinusoids in the pars distalis (lower left), and with a few basement membranes bordering sinusoids in the pars intermedia (upper right). Many basement membranes in the pars intermedia which stain with crude fl-anti-NH (b) apparently are not surrounded by sufficient basement membrane material to be stained by the epithelial-specific fl-anti-NH (c). × 385.

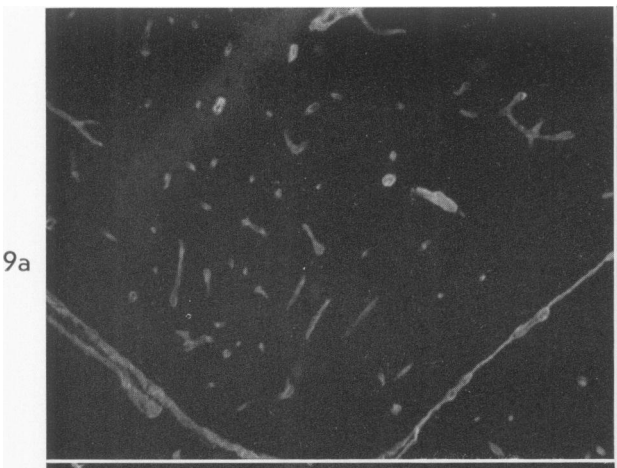
FIGS. 8a, 8b and 8c. Autofluorescent material, best seen in the control section (d), is present in all sections. Staining of reticulin and vascular basement membranes in the submucosa and lamina propria (b) is not seen in the section stained with epithelial-specific fl-anti-NH (c). The mucosal basement membrane, although faintly stained, is readily apparent in (c). × 385.


7a
8a
7b
8b
7c
8c
7d
8d

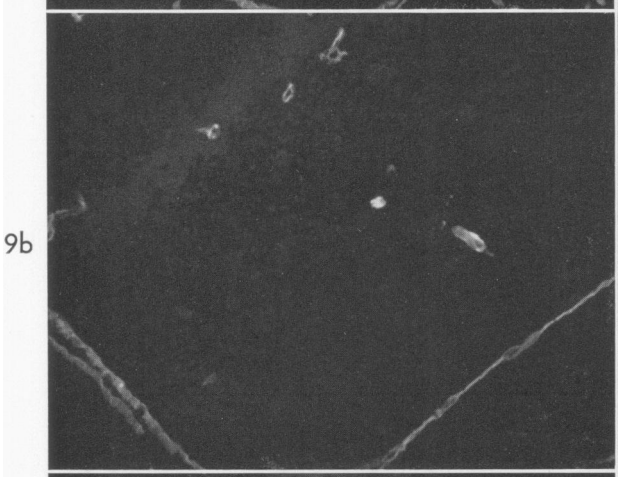
These are adjacent frozen sections of cerebellum (C) and subarachnoid space (SA) stained with (a) crude fl-anti-NH, (b) epithelial-specific fl-anti-NH, and (c) fl-anti-NH which had been absorbed with NH prior to staining.

FIGS. 9a, 9b and 9c. The pia-glial membrane lining the sulci and surrounding several of the vessels stains brilliantly with epithelial-specific fl-anti-NH (b). Staining of reticulin and vascular basement membranes, especially of capillaries, with crude fl-anti-NH (a) is absent in (b). × 154.

FIGS. 10a and 10b. The reaction of vascular basement membranes and reticulin in subarachnoid vessels (arrows) with crude fl-anti-NH (a) is absent in (b). Epithelial-specific fl-anti-NH has reacted only with the pia-glial membrane on the surface of the cerebellar cortex and its extensions about intracerebellar vessels (b). × 385.

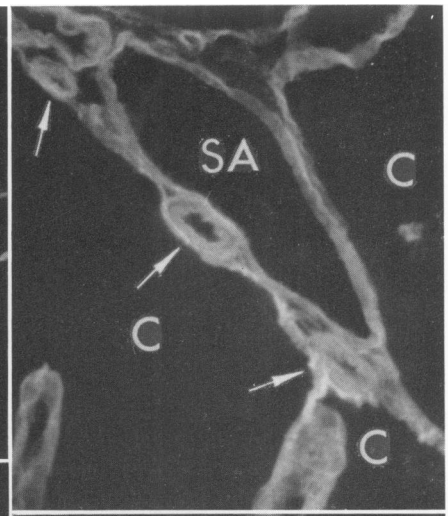


9a

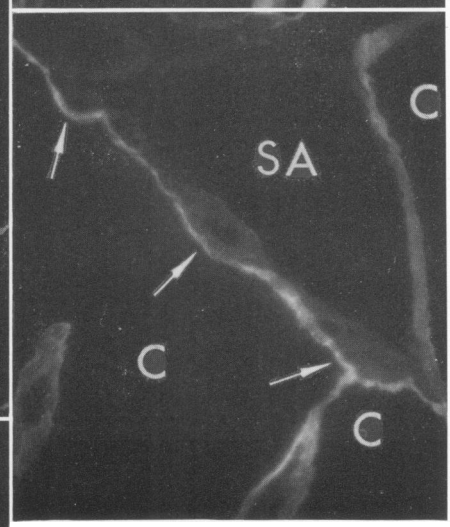


9b

9c



10a



10b