

THE LOCALIZATION OF HOMOLOGOUS PLASMA PROTEINS IN THE
TISSUES OF YOUNG HUMAN BEINGS AS DEMONSTRATED
WITH FLUORESCENT ANTIBODIES*

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PLATES 7 TO 10

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It is now generally accepted that tissue proteins and plasma proteins are in a state of dynamic interchange (1-3). Parenterally administered plasma proteins can serve as the sole source of nitrogen for the body (4), while the tissues can mobilize plasma protein in response to acute stress (5). In an effort to learn more about the nature of this interchange both in health and disease and about the relationships between the plasma proteins and the tissues, a series of investigations have been conducted in this laboratory. Using as antigens purified protein fractions of human plasma obtained by low temperature ethanol-water fractionation (6, 7), specific antisera have been prepared in rabbits against human plasma albumin, γ -globulin, β -lipoprotein, β_1 -metal-combining globulin, and fibrinogen. By conjugating these antisera with fluorescein isocyanate by the method of Coons *et al.* (8, 9), specific reagents for the detection of each of these proteins in sections of human tissue by fluorescence microscopy have been obtained. This paper presents the results of such an immunohistochemical study of the distribution of these particular plasma proteins in tissues obtained at biopsy or postmortem examination from a group of infants and children in whom striking changes in the circulating plasma proteins would not be expected.

Materials and Methods

Preparation of Antisera.—All plasma proteins employed as antigens for this study were obtained from pooled plasma of normal adults by the low temperature ethanol-water fractionation methods developed by Cohn and his colleagues (6, 7).

Albumin.—Preparation decanol 10 was crystallized from fraction V and has been characterized physicochemically (10) and immunochemically (11, 12).

γ -Globulin.—Preparation 413-II-1, 2 was found to contain 99 per cent γ -globulin and about 1 per cent albumin both electrophoretically and immunochemically (11).

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β -Lipoprotein.—Preparation BSX was obtained by ultracentrifugation as described earlier (13). By quantitative immunochemical precipitation, this preparation was found to contain about 0.2 per cent albumin.

β_1 -Metal-Combining Globulin.—Preparation 197-4x was obtained from Dr. D. Surgenor and had been crystallized four times (14). It was found by quantitative precipitin tests to contain about 2 per cent γ -globulin and 0.02 per cent albumin.

Fibrin was prepared from fraction I using the following final concentrations: fibrinogen 0.5 per cent, thrombin 0.1 unit/ml. The reaction proceeded at room temperature and the fibrin strands were wound about a glass rod as they formed. After 90 minutes, the fibrin was blotted with filter paper and then stripped off the rod into a Waring blender in which it was washed twice with 0.15 M NaCl, then twice with water, and then twice again with 0.15 M NaCl.

The purified preparations of plasma albumin, γ -globulin, and β_1 -metal-combining globulin were individually dissolved in small volumes of 0.15 M NaCl and the solutions then added to 6 to 9 volumes of a washed suspension of aluminum hydroxide (15); the final concentration of plasma protein was approximately 25 mg. of protein per ml. of suspension. The fibrin was teased into very small particles and suspended in washed aluminum hydroxide, 25 mg. fibrin per ml. of final suspension; this preparation could easily be passed through a No. 19 needle. The β -lipoprotein was employed as a saline solution and its use as an antigen begun within a week of its preparation in the ultracentrifuge.

Groups of 3 or 4 rabbits were given a subcutaneous injection of 25 mg. of the given antigen per kilo of body weight every 10 to 12 days for 4 injections. The rabbits given β -lipoprotein were bled after this course, but the remainder of the animals after a rest period of 3 weeks were given 2 more injections. 10 days after the last injection, the animals were bled by cardiac puncture and the sera from each group of animals were pooled.

Characterization of the Antisera.—Despite the relatively high degree of purity of the antigens employed, it was necessary to study the antisera to determine their specificity. With the exception of the rabbit antisera vs. human plasma albumin, the antisera were found to contain antibodies against more than one species of protein by means of quantitative precipitin curves (16, 17), use of the Oudin method (18), and by adsorption studies. Antisera vs. γ -globulin contained precipitins for albumin, as did antisera vs. β -lipoprotein and antisera vs. β_1 -metal-combining globulin. Antisera vs. β_1 -metal-combining globulin also contained precipitins for γ -globulin and β -lipoprotein. The antisera studied were, therefore, adsorbed with small increments of the appropriate proteins to render the resulting antiserum specific for the major protein species. Complete adsorption of the antibodies against trace components with purified homologous proteins did not affect the quantitative precipitation curve for the principal antigen, indicating that the latter did not significantly cross-react with the antibodies removed. The antisera vs. fibrin precipitated fibrinogen quantitatively, giving a typical precipitation curve for rabbit antibody with the latter; these antisera did not react with any of the components of normal human serum.

Preparation of Labelled Antisera.—Fluorescein amine was prepared and purified according to the method of Coons and Kaplan (9) with minor modifications. The antisera were fractionated with an equal volume of saturated ammonium sulfate and the precipitate dialyzed vs. 0.15 M NaCl until completely free of NH_4^+ . The resulting antibody solution was used for conjugation, using 0.05 mg. fluorescein amine II per mg. of protein (9). The labelled antibody preparations were dialyzed against several changes of 0.15 M NaCl buffered to pH 7.3, 0.01 M phosphate, for 5 days. The antibody preparations were then precipitated 3 times at pH 5.1, $\Gamma/2 = 0.15$, ethanol 30 per cent by volume, -10°C . and then subsequently dialyzed against buffer-saline ($\Gamma/2$ NaCl = 0.1, $\Gamma/2$ phosphate buffer pH 7.3 = 0.05). The antisera were then adsorbed twice with bovine liver powder and twice with rabbit liver powder to remove non-specific staining material (9); although bovine fibrinogen (or fibrin) and bovine

plasma albumin partially cross-react with rabbit antisera against their human analogues, very little antibody was removed by this adsorption and the sera retained most of their potency. Frequently, rat liver powder was substituted for bovine liver powder in adsorbing antisera *vs.* fibrinogen.

Preparation of Tissues for Study.—Both biopsy and autopsy materials were studied. Biopsy specimens of normal tissue included liver, spleen, kidney, thyroid, adrenal, lymph node, aorta, skin, muscle, and fascia. The tissues were frozen immediately after removal by dropping them into small dry bottles immersed in a dry-ice-alcohol mixture at approximately -70°C . Necropsy material included heart, aorta, trachea, lung, liver, kidney, spleen, thymus, adrenal, thyroid, pancreas, lymph node, tongue, esophagus, stomach, duodenum, colon, appendix, diaphragm, skeletal muscle, cartilage, bone marrow, brain, and spinal cord. Necropsy tissues were obtained from 1 to 5 hours after the death of the patient and placed at -20°C . in dry bottles; this study group included four patients with erythroblastosis fetalis, three patients with congenital heart disease, one patient with bronchiolitis, and two patients with brain tumors. The three cardiac and two neurological patients succumbed suddenly during the course of a surgical procedure. The tissues studied were taken from patients whose ages ranged from 2 days to 8 years and 3 months with the single exception of one sample of brain tissue taken from an adult.

All tissues were kept at -20°C . until sectioned at -30°C . at 4 to 8μ . Adhesive to keep the sections on the slides was found to be unnecessary. As a routine all sections were placed in 95 per cent ethanol at 37°C . for 30 minutes and then placed in acetone at room temperature for 20 minutes. Methanol or ethanol at 5°C . was occasionally used for primary fixation of the sections, but in our hands, this tended to increase the degree of non-specific staining. To remove some of the plasma protein in some instances and thus avoid excessive staining, sections were treated with 75 per cent or 50 per cent ethanol at 37°C . in place of 95 per cent ethanol. This worked particularly well for albumin.

Identification of Plasma Proteins in Tissues.—The dry treated sections were covered *completely and rapidly* with a few drops of the given labelled antiserum and then placed in a moist chamber at 25°C . for 30 minutes. The slides were then washed 4 times with cold saline-buffer (NaCl $\Gamma/2 = 0.14$, phosphate buffer pH 7.3 $\Gamma/2 = 0.01$) over a period of 20 minutes. A drop of reagent glycerol, buffered with pH 7.3 phosphate buffer, was then placed on the sections. As controls for the specificity of *each* of the labelled antisera in *each* tissue studied, sections were treated with labelled antiserum that had been adsorbed with small increments of homologous antigen; additional sections were treated with labelled rabbit antisera after pretreatment with unlabelled normal or immune rabbit sera.

A standard microscope equipped with non-fluorescing glass lenses was used; a quartz Abbe condenser (9) was employed interchangeably with a bispheric darkfield condenser (19). The light source was a Zeiss carbon arc of 20 ampere capacity. The filter system described by Coons *et al.* (9) was first employed, but due to discontinuance of manufacture of the Wratten 2A filter, a double thickness of Wratten 2B gelatin was employed in the microscope barrel above the objectives. With the latter filter, a stock thickness of Corning No. 5840 was used in conjunction with the quartz Abbé condenser and a $\frac{1}{2}$ stock thickness of Corning No. 5840 was used with the bispheric condenser. Selected fields were photographed and located on the slide by coordinates established with a graduated mechanical stage. Sections were then placed in 10 per cent formalin and stained with hematoxylin and eosin; the fields photographed were relocated by use of the coordinates, so as to permit careful histologic study of the tissues.

RESULTS

While the proteins studied demonstrated many obvious similarities in distribution, a number of differences were observed. For the most part, antigen

was detected with anti- β -lipoprotein (anti-B) in the nuclei of all cell types examined, while with antifibrinogen (anti-F), antigen was restricted largely to the lymphatic and vascular channels, the connective tissues, and the interstitial spaces. Antigen revealed with anti- γ -globulin (anti-G), anti-albumin (anti-A), and anti- β_1 -metal-combining globulin (anti-FE) was localized in lymphatics and blood vessels, connective tissues and interstitial spaces, and in the nuclei of many cells. Antigen in connective tissue appeared to occur between collagen fibers, as if in the matrix or fine spaces rather than within the fibers themselves.

The results with the various antisera will be reported for each organ examined. Results were consistent in tissues from different patients with a few specific exceptions which will be mentioned.

Liver:

Anti-G.—For the most part, specific fluorescence was limited to sinusoids (Figs. 1 and 2) and portal areas. Occasional endothelial and Kupffer cells displayed small amounts of antigen in the cytoplasm, and occasionally in nuclei (Fig. 2). Occasional hepatic cell nuclei revealed a trace of antigen.

Anti-B.—The sinusoids fluoresced faintly, the antigen apparently being concentrated in hepatic and sinusoid cell nuclei (Fig. 3); the nuclear fluorescence was diffuse and outlined nucleoli in many instances.

Anti-A.—Large amounts of antigen were found in sinusoids, and in connective tissue and blood vessels of portal areas. The hepatic cell nuclei fluoresced intensely (Figs. 4 to 6), the nucleoli frequently being outlined. The cytoplasm of the hepatic cells in some instances exhibited small amounts of antigen. Nuclei of sinusoidal cells in many instances did not reveal specific fluorescence under these conditions (Fig. 6).

Anti-FE.—Sinusoidal and portal area fluorescence was quite strong. Approximately half the hepatic cell nuclei exhibited central fluorescent spots (Fig. 7).

Anti-F.—Although intense fluorescence was demonstrated in sinusoids and portal areas, only traces of antigen could be found elsewhere. Occasionally hepatic nuclei displayed fluorescence (Figs. 8 and 9). Under these conditions, little if any antigen was detected in the cytoplasm of hepatic cells.

Kidney:

Anti-G.—Some glomerular nuclei, both endothelial and epithelial, fluoresced faintly. Traces of antigen were also present in some tubular nuclei, particularly those of convoluted tubules. The greatest concentrations of antigen were demonstrated in blood vessels, especially along medullary rays, and in the interstitial tissue, particularly in the cortex. A section through the medulla is shown in Fig. 16.

Anti-B.—Many glomerular nuclei were quite prominent (Figs. 12 and 13), but not so intense as those of tubular epithelium. Nuclei of all levels of tubules showed intense diffuse fluorescence (Figs. 10, 11, and 14). No definite specific cytoplasmic fluorescence was observed. Medullary rays and interstitial tissue displayed faint to moderate fluorescence.

Anti-A.—The qualitative distribution was similar to that seen with anti-B. The fluorescence of tubular and glomerular nuclei was weaker than for anti-B, while that of interstitial connective tissue and blood vessel contents was much more intense. While almost all the tubular nuclei fluoresced, an occasional nucleus did not. In some instances the presence of traces of

antigen in the cytoplasm of some of the proximal convoluted tubular cells was suspected. A section through the renal medulla is shown in Fig. 15.

Anti-FE.—The over-all picture was much like that seen with anti-A, most tubular nuclei and some glomerular nuclei exhibiting small amounts of antigen with larger amounts in blood vessels and interstitial tissue.

Anti-F.—Antigen was limited to blood vessel contents and connective tissue. On occasion, a rare glomerular nucleus fluoresced faintly, but otherwise the tubules and glomeruli were negative (Figs. 17 and 18).

Spleen:

Anti-G.—Nuclei and cytoplasm of cells in both red and white pulps contained considerable amounts of antigen. In the white pulp, the cytoplasm and nuclei of lymphocytes and lymphoblasts, particularly those in "germinal centers" (Fig. 19), revealed specific fluorescence. In the red pulp some macrophages and many reticular cells contained antigen in both cytoplasm and nucleus. Trabeculae and adventitia of small arteries fluoresced strongly.

Anti-B.—Although the cytoplasm of the various cells and the intercellular material showed little fluorescence, the nuclei of almost all cells in both white and red pulps contained antigen (Figs. 22 and 23). Nuclei of reticular cells and lymphoblasts appeared to fluoresce more strongly and diffusely than those of small lymphocytes.

Anti-A and Anti-FE.—The general distribution was much like that found with anti-G.

Anti-F.—Fluorescence was limited almost completely to intercellular spaces in both red and white pulps, to sinusoids, and to connective tissue. There was little nuclear fluorescence in either red or white pulps.

Lymph Nodes:

In the case of anti-G, anti-B, anti-A, and to a lesser extent, anti-FE, the distribution of antigen in the lymphatic nodules was like that observed in the lymphatic nodules of the spleen (Figs. 20 and 21). In the medulla, reticular cells were particularly prominent, with diffuse strong nuclear fluorescence like that seen in lymphoblasts.

With anti-F, intercellular material and the contents of sinusoids fluoresced strongly, but little nuclear fluorescence was noted.

Thymus:

Anti-G.—Antigen was found in many lymphocytes and reticular cells, and Hassall's bodies contained considerable amounts (Fig. 24). Although most Hassall bodies fluoresced intensely, some did not fluoresce at all.

Anti-B.—Nuclei of the lymphocytes and reticular cells displayed impressive fluorescence, the lymphocytic nuclei revealing a centrally placed area of fluorescence and the reticular cell nuclei exhibiting more intense diffuse fluorescence (Fig. 25).

Anti-FE and Anti-A.—Distribution of antigen was much like that obtained with anti-G, but Hassall's bodies, in general, displayed less prominent fluorescence.

Anti-F.—There was intense fluorescence of intercellular material, but little nuclear fluorescence. In most instances, little antigen could be demonstrated in Hassall's bodies.

Lung:

Anti-G.—Some endothelial nuclei contained antigen (Fig. 26). The pulmonary capillaries also contained antigen, as did edema fluid when present.

Anti-B.—Almost all cell nuclei seen fluoresced moderately to strongly (Fig. 27).

Anti-A.—The distribution was much like that observed with anti-G, but fluorescence of capillary contents was more intense and that of nuclei relatively less intense.

Anti-FE.—Antigen was most abundant in capillaries. Nuclei of many endothelial cells were outlined by fluorescent cytoplasm and occasional endothelial nuclei revealed the presence of antigen.

Anti-F.—Antigen was present in capillaries and many endothelial cells as with anti-FE.

Trachea:

Anti-G.—Antigen was present in nuclei of pseudostratified epithelium, in tracheal glands, and in lacunar cells of tracheal cartilages. The lamina propria and perichondrium were rich in antigen, but no antigen could be found in the hyaline matrix of the cartilages.

Anti-B.—The lacunar cell nuclei fluoresced brightly (Fig. 28). The nuclei of all glands and the pseudostratified epithelium were very prominent (Figs. 29 and 30).

Anti-A.—The distribution of antigen was similar to that found with anti-G: nuclear fluorescence was more striking here (Fig. 31) although not so strong as that with anti-B. Both cytoplasm and nuclei of lacunar cells contained antigen.

Anti-FE.—Small amounts of antigen were found in nuclei of tracheal glands and epithelium. The lacunar cells were variable, but in most instances little specific fluorescence was noted. The lamina propria contained large amounts of antigen.

Anti-F.—Aside from intense fluorescence in the lamina propria, the only specific fluorescence was found in an occasional nucleus of the tracheal glands or epithelium.

Thyroid:

Anti-G.—The stroma was rich in antigen, and small amounts were found in nuclei of the acinar epithelial cells. The amount of antigen in colloid varied from none to slight.

Anti-B.—The stroma contained some antigen, but fluorescence of epithelial cell nuclei and of colloid was most striking.

Anti-A.—Both epithelial nuclei and cytoplasm, the colloid, and the stroma contained antigen.

Anti-FE and Anti-F.—Large amounts of antigen were present in the stroma, but the colloid resembled that observed with anti-G, and only occasional epithelial nuclei displayed fluorescence.

Adrenal:

Anti-G.—Nuclei of cells in both cortex and medulla revealed specific fluorescence as did the sinuses. Nuclei of adult cortex were more prominent than those of fetal cortex.

Anti-B.—All nuclei fluoresced (Fig. 39).

Anti-A.—The appearance was similar to that seen with anti-G. The cytoplasm of some cortical cells contained antigen.

Anti-FE.—Many nuclei of both fetal and adult cortices contained antigen, but nuclear fluorescence tended to be less diffuse than with the other antisera (Fig. 38). There appeared to be tendency for antigen to be distributed more toward the central part of the cortex than toward the peripheral portions.

Anti-F.—The blood vessels were intensely fluorescent. Occasional nuclei in the medulla and about one-tenth of the nuclei of the cortex contained antigen.

Heart:

The cardiac muscle did not reveal intracellular antigen with any of the antisera studied, but the endothelium, and connective tissue of the myocardium, endocardium, and pericardium contained considerable amounts of all antigens.

Skeletal Muscle:

Antigen could be detected in large amounts in the perimysium with all antisera (Fig. 32). In a patient whose circulating γ -globulin was almost completely absent, fluorescence in the interstitial connective tissue was absent after testing with anti-G, although the other proteins studied were present (Figs. 33 and 34). Antigen could not be detected within the muscle cells with any of the antisera studied.

Tongue:

The lamina propria and the perimysium contained antigen for all antisera. Epithelial nuclei were very prominent after treatment with anti-B, were less prominent after anti-A, were negative after anti-F, and variable after anti-FE and anti-G.

Gastrointestinal Tract:

Antigen could be found in the lamina propria, submucosa, and serosa with all the antisera employed. While the smooth muscle cells were apparently devoid of antigen, fluorescence scattered between the cells was quite apparent. The distribution of antigen in the cells of the stratified epithelium of the esophagus was like that observed for the epithelium of the tongue. The nuclei of glands of the colon and duodenum were noted to contain antigen with all antisera except anti-F, and in some cases, antigen in the cytoplasm of the colic glands was noted with anti-G, anti-FE, and anti-A. A complete survey of the cytologic distribution of antigen in the gastrointestinal tract awaits further study.

Pancreas:

The acinar and centro-acinar cells were fluorescent after treatment with all antisera except anti-F, the nuclei being most striking.

Bone Marrow:¹

On smear, many cells contained antigen with the antisera studied, but certain identification of the cell types could not be made.

Brain:

Sections of cerebral cortex revealed antigen in ganglion cell and glial cell nuclei (Figs. 35 to 37), with all the antisera studied except anti-F. Large amounts of antigen were detected with all antisera in the meninges and capillaries.

Skin:

The stratum corneum always exhibited strong yellow non-specific fluorescence. The distribution of antigen in the cells of the epithelium was much like that seen in the tongue. The connective tissue of the papillary, reticular, and subcutaneous layers contained large amounts of all antigens.

DISCUSSION

The use of labelled antisera as specific tracers for unlabelled homologous antigens in tissue sections involves a number of serious difficulties in quantita-

¹ Since smears of intact cells were used, the method was considered unsatisfactory because of the question of penetration of labelled antibody. Consequently, studies on bone marrow sections prepared by special methods will be reported at a future date.

tion and interpretation, the difficulties being related to protein-protein interaction (20), the precipitation characteristics and reversibility of the antigen-antibody reaction (21, 22), the solubility of such antigens even after alcohol and acetone fixation, and the alterations in the immunochemical properties of antigens after such fixation. As has been noted, careful consideration had to be given to the presence or suppression of fluorescence in control sections treated in a variety of ways to determine the specificity of the reaction with labelled antisera. This has been pointed out by Coons *et al.* on a number of occasions (8, 9). With respect to quantitation, there are a number of serious technical difficulties.

1. Since some of the plasma proteins studied remained soluble in 0.15 M NaCl after treatment with alcohol and acetone, relative antigen excess has, on occasion, led to local absence or loss of fluorescence due to solubility of the antigen-antibody complex thus formed.

2. For the same reason, since the rapidity of precipitation of an antigen by homologous antiserum varies with the antibody concentration, antisera relatively low in antibody content could permit solution of soluble portions of antigen from sections before fixation by the antiserum occurred.

3. Inhibition of fluorescence at the site of antigen-antibody combination may occur under certain circumstances; *e.g.*, there is some evidence that high concentrations of hemoglobin tend to quench specific fluorescence.

4. Since the antibody-antigen ratio, and hence the degree of fluorescence per unit of antigen, increases as the amount of antibody relative to antigen in the system increases, comparison of results obtained with two different antisera becomes a complex problem. Variation in the amount of fluorescein bound per molecule of antibody in the different antisera during conjugation is an additional factor. It is entirely possible and probable, however, that these methods can be made more quantitative through the application of the principles described by Heidelberger and Kendall (16).

5. The optical system employed for the visualization of fluorescence and the ultraviolet output of the arc will have profound effects on the intensity of the fluorescence observed.

It must be emphasized, therefore, that the results described are those obtained with the maximum sensitivity that our apparatus and technique provided. At higher levels of sensitivity, it is not inconceivable that in tissues or cells thought not to contain antigen small amounts may be demonstrated. It has been our experience that slight increases in optical efficiency have resulted in the observance of fluorescence in cells previously thought to be devoid of antigen.

There is little question but that the specific fluorescence found in the interstitial connective tissue was due to unaltered homologous plasma protein. In a patient with almost complete absence of circulating γ -globulin (23), little fluorescence in the connective tissue of muscle or skin biopsies was observed

after testing with anti-G, although fluorescence with the other antisera tested was normal. The intravenous administration of γ -globulin in the form of human plasma to this patient resulted in the prompt appearance of specific fluorescence in the connective tissue upon testing with anti-G.

The nature of the intracellular antigens observed cannot be answered so clearly. In an extensive immunochemical study of the plasma proteins, part of which is reported here, it has been found that individual plasma proteins are apparently immunochemically specific and that cross-reactions with antisera of the type studied here occurred only between related subspecies of proteins (24, 25) or their catabolic or artificial derivatives. Great care was taken to render the antisera studied in this report protein-specific. The fact that heterologous plasma proteins can penetrate cell nuclei of many organs in mice (26) and rabbits (27, 28) supports the concept that the intracellular antigens observed were plasma proteins.

As has been noted, nuclear concentrations of antigen in many cells were more impressive than cytoplasmic concentrations. The reasons for such differential localization are unknown. If the protein was catabolized by such cells, this may be a reflection of a faster cytoplasmic rate of plasma protein turnover. If the proteins were synthesized in some of these cells, it would appear that the passage of plasma protein from cytoplasm to plasma involves travel against a concentration gradient. There is little reason to presuppose that intracellular plasma proteins are so firmly bound to other proteins in the cytoplasm as to be completely masked immunochemically, since the number of haptenic groups which would have to be bound is large. It has been demonstrated that heterologous plasma proteins localized intracellularly in animals retain their reactivity with labelled antisera (26). Inhibition of specific fluorescence by cytoplasmic components, however, does remain a possibility.

As this paper was being prepared for publication, Wang *et al.* (29) reported the isolation of lipoproteins from nuclei of animal tissues. Our results appear to be in accord with their findings.

The intranuclear presence of γ -globulin is of interest for another reason. The question of "tissue" immunity, as opposed to "humoral" immunity may find a partial answer in such intracellular antibody. Perhaps only a few molecules of specific antibody per cell nucleus can provide immunity against those infectious agents which require the intact cell, and particularly cell nuclei, for survival and reproduction. Under these circumstances, it would seem possible for manifestations of tissue immunity to appear when circulating antibody levels are extremely low or below detectable ranges.

The general absence of intracellular fibrinogen suggests either that cells specifically reject fibrinogen or that the rate of intracellular degradation of fibrinogen is quite rapid. The half-life of administered fibrinogen in 2 patients with afibrinogenemia was about 3.5 days (30); this evidence suggests a more rapid turnover of fibrinogen than of γ -globulin (31, 32), or albumin (33).

Cohn and his colleagues (34) could not demonstrate bovine plasma albumin in electrophoretic studies of extracts of perfused bovine liver and concluded that the albumin in liver differs from plasma albumin, probably being combined with other proteins. Similar electrophoretic findings were obtained by Sarof and Cohen (35) during studies of rabbit liver proteins. It had been our privilege to test some of the liver fractions used by Cohn's group; on immunochemical analysis, small amounts of material reacting with rabbit antiserum *vs.* bovine plasma albumin were found in both the soluble and residue fractions (36), the bulk being in the residue fraction. The bovine plasma albumin in the soluble fraction comprised 0.5 per cent or less of the proteins in this fraction, an amount too small to be readily recognized on electrophoresis of a complex mixture. In the same study, traces of bovine γ -globulin were also found in the residue fraction. These immunochemical findings are in accord with the small amounts of intracellular albumin and γ -globulin found in sections of human liver in this study. The presence of much of the bovine albumin in the residue fraction is in accord with our finding of antigen in liver cell nuclei.

It must be recognized that the data reported here do not allow differentiation between sites of degradation and synthesis of these proteins or among sites where these proteins are found as structural components, where they are selectively concentrated, or where they are passive transients. The widespread occurrence of intracellular plasma proteins, however, suggests certain possibilities. Synthesis of certain plasma proteins by more than one cell type may explain the immunochemical heterogeneity of certain protein species, such as β -lipoproteins (24) and γ -globulins (25). Such heterogeneity may, however, simply reflect the complexity of the protein molecule, alternate pathways of synthesis being used by the same cell or by different individuals of the same cell type.

The data reported here, showing relatively large amounts of plasma proteins in connective tissue, and to a lesser extent in cells, indicate that these structures may provide a reservoir for the maintenance of relatively constant levels of circulating plasma proteins. The magnitude of the extravascular plasma protein, and consequently the extent of such buffer capacity were indicated by Sterling (33) who found that the total exchangeable plasma albumin pool was approximately twice the mass of the circulating plasma albumin. Similar findings have been obtained for fibrinogen in 2 patients with afibrinogenemia (30) and for γ -globulin in a patient with relative absence of circulating γ -globulin (23). With such relatively large, rapidly equilibrating pools of plasma protein, the effects of temporary disequilibrium between anabolism and catabolism would be minimized. Conversely, the existence of such a pool must be considered during attempts at rapid parenteral replacement of plasma proteins, since a considerable portion of the plasma protein infused may disappear into this extravascular pool.

SUMMARY

Employing fluorescent antibodies for the detection of homologous plasma proteins in tissue sections, the distribution of plasma albumin, γ -globulin, β -lipoprotein, β_1 -metal-combining globulin, and fibrinogen has been studied in the tissues of infants and children. Plasma albumin, γ -globulin, and β_1 -metal-combining globulin were found in many cells and particularly cell nuclei, connective tissues and interstitial spaces, lymphatics, and blood vessels. β -Lipoprotein was found mostly in the nuclei of all cell types while fibrinogen was restricted largely to the lymphatic and vascular channels, connective tissues and the interstitial spaces.

The widespread distribution of these plasma proteins in cells and connective tissues indicates the magnitude of the extravascular plasma protein pool which is in equilibrium with circulating plasma. Unfortunately, these results do not permit accurate localization of the sites of production of these plasma proteins, but do give some idea of their intimate relationship to the tissues.

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PLATES

EXPLANATION OF PLATES

All sections were cut at 4μ . White areas in the photographs represent the areas of fluorescence.

PLATE 7

Sections from biopsy of normal liver of a child, age 2 years and 8 months, with signs of extrapyramidal disease.

FIG. 1. Treated with labelled anti-human γ -globulin (anti-G) showing presence of antigen in sinusoids and general absence from hepatic cells. $\times 210$.

FIG. 2. Central area of Fig. 1 showing fluorescent spots in occasional hepatic nuclei (H) and rare Kupffer cell (upper K) and fluorescent cytoplasm of occasional Kupffer cells (K). $\times 430$.

FIG. 3. Treated with labelled anti-human β -lipoprotein (anti-B) showing antigen in numbers of liver cell nuclei. $\times 210$.

FIG. 4. Treated with anti-human plasma albumin (anti-A) showing general pattern of antigen in the sinusoids and hepatic cell nuclei. Most of the antigen has been washed out of the sinusoids. $\times 210$.

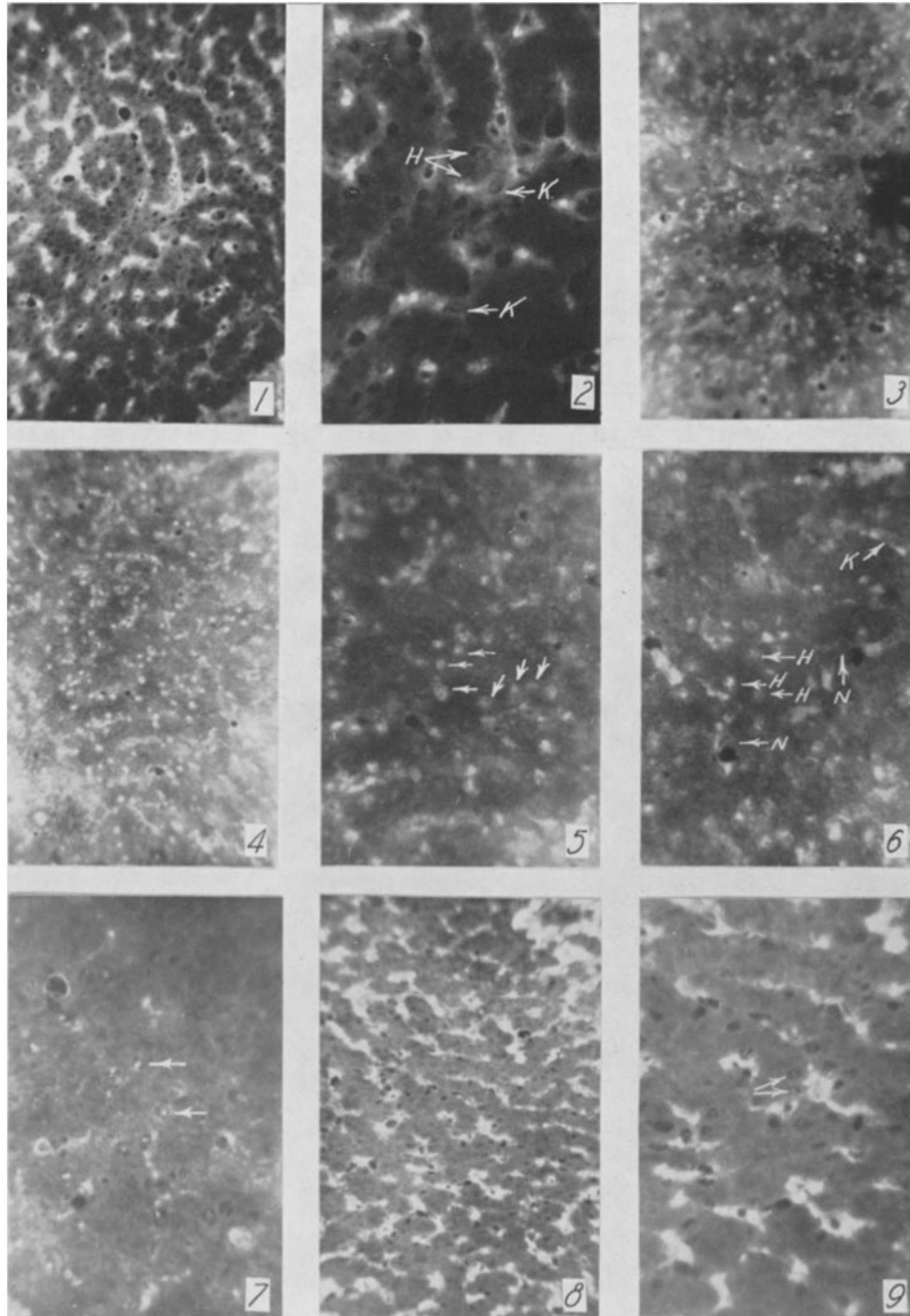
FIG. 5. Central area of Fig. 4 showing antigen in hepatic cell nuclei (arrows). $\times 430$.

FIG. 6. Another area of same section showing antigen in hepatic cell nuclei (H) and in the cytoplasm of a Kupffer cell (K). Antigen was absent in two endothelial nuclei indicated by N. $\times 430$.

FIG. 7. Treated with anti-human β_1 -metal-combining globulin (anti-FE) showing fluorescence in scattered hepatic cell nuclei (arrows). $\times 430$.

FIG. 8. Treated with anti-human fibrinogen (anti-F) showing antigen in sinusoids; antigen was not demonstrable in hepatic cell cytoplasm. $\times 210$.

FIG. 9. Central area of Fig. 8 showing antigen in several hepatic cell nuclei (two are indicated by arrows). $\times 430$.



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PLATE 8

Sections from biopsy of normal kidney of a 9 month old child with hydrocephalus.

FIG. 10. Treated with anti-B showing antigen in nuclei of distal (D) and proximal convoluted tubules and loops of Henle; antigen is also demonstrated in glomerular nuclei. $\times 210$.

FIG. 11. Central area of Fig. 10 showing nuclei of a proximal convoluted tubule. $\times 430$.

FIG. 12. Treated with anti-B showing antigen in some glomerular nuclei (plain arrows) and in nuclei of a distal tubule (D). A glomerular arteriole is also shown (A). $\times 430$.

FIG. 13. Treated with anti-B showing antigen in nuclei of a glomerulus (outlined by arrows). $\times 210$.

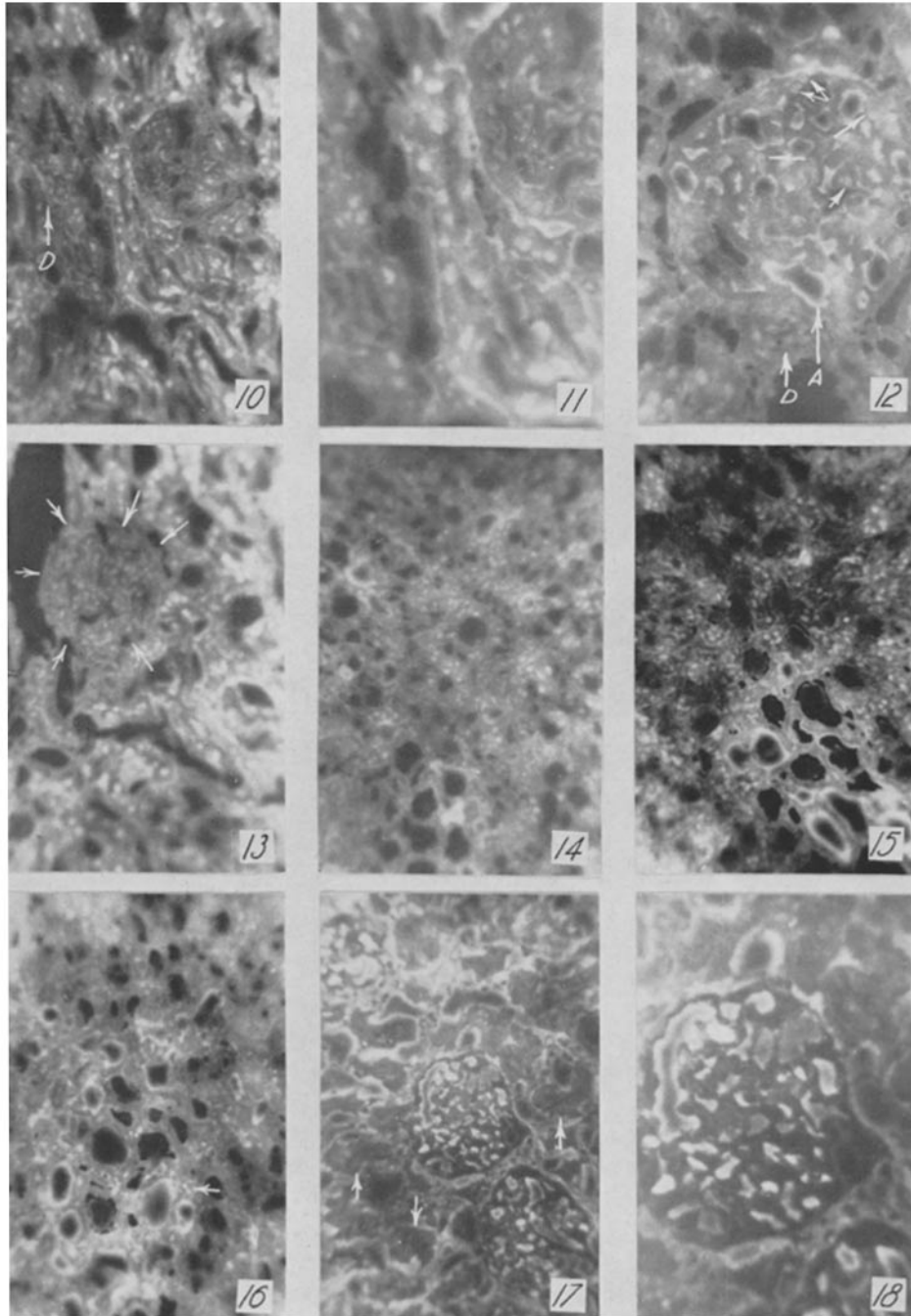
FIG. 14. Treated with anti-B. Antigen is localized in nuclei of loops of Henle and collecting tubules. $\times 210$.

FIG. 15. Treated with anti-A. Section through renal medulla showing antigen in nuclei of loops of Henle and collecting tubules, and in lumens of blood vessels. $\times 210$.

FIG. 16. Treated with anti-G. Section through renal medulla; occasional nuclei of loops of Henle containing antigen (arrow). $\times 210$.

FIG. 17. Treated with anti-F showing antigen principally in interstitial tissue and blood vessels. Arrows point to convoluted tubules in which antigen could not be detected. $\times 210$.

FIG. 18. Glomerulus of Fig. 17 showing antigen in capillary tuft, but no nuclei discernible. $\times 430$.



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PLATE 9

FIG. 19. Section from spleen obtained at splenectomy performed on a 4 year old child with acquired hemolytic anemia. Treated with anti-G; this area of white pulp shows abundant antigen in cytoplasm and nuclei of lymphocytes and lymphoblasts. $\times 430$.

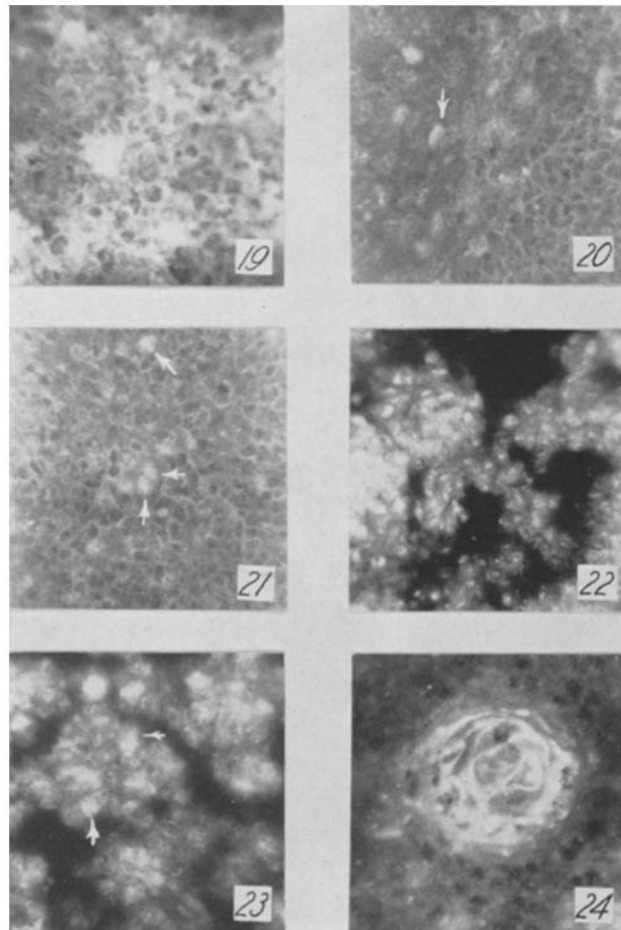
FIG. 20. Section from biopsy of inguinal lymph node of a normal 9 month old child. Treated with anti-G; area shown is the edge of germinal center, with antigen in cytoplasm and nuclei. Particularly prominent are nuclei of lymphoblasts (arrows). $\times 430$.

FIG. 21. Section from same lymph node as Fig. 20. Area shown is on periphery of a lymphatic nodule, again demonstrating antigen in nuclei of lymphoblasts (arrows). $\times 430$.

FIG. 22. Section from spleen of an infant dying of erythroblastosis fetalis on the 2nd day of life (patient 52-20). Treated with anti-B and showing antigen in nuclei of cells of white pulp. Nuclei of lymphoblasts are most prominent. $\times 430$.

FIG. 23. Section of a spleen obtained at splenectomy from a 4 year old patient with acquired hemolytic anemia. Treated with anti-B. Area of white pulp is shown, again with antigen in nuclei of lymphocytes and lymphoblasts; antigen in the latter is very apparent (arrows). $\times 430$.

FIG. 24. Section of thymus obtained from patient 52-20. Treated with anti-G, showing antigen in a Hassall body and nuclei of several lymphocytes.



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PLATE 10

FIG. 25. Same thymus as Fig. 24, section treated with anti-B; antigen is in nuclei of lymphocytes (fine spots) and in nuclei of reticular cells (larger white spots). $\times 210$.

FIG. 26. Section from lung of patient 52-20 treated with anti-G; antigen shown in nuclei of endothelial cells, two of which are indicated by arrows. $\times 430$.

FIG. 27. Same lung as Fig. 26, section treated with anti-B. Arrows indicate several endothelial nuclei containing antigen. $\times 430$.

FIG. 28. Section of tracheal cartilage obtained from patient 52-20 treated with anti-B showing antigen in lacunar cells. $\times 430$.

FIGS. 29 and 30. Section of tracheal glands (patient 52-20) treated with anti-B, showing antigen in nuclei. $\times 210$ and 430 respectively.

FIG. 31. Section of pseudostratified tracheal epithelium of patient 52-20 treated with anti-A, showing antigen in nuclei, several of which are indicated by arrows.

FIG. 32. Biopsy of pectoral muscle of a 4 year old child with patent ductus arteriosus. Section treated with anti-G, showing abundant antigen in connective tissue. $\times 210$.

FIG. 33. Biopsy of gastrocnemius muscle of a 9 year old patient with relative agammaglobulinemia. Section treated with anti-G showing relative absence of antigen in connective tissue (outlined by arrows). $\times 210$.

FIG. 34. Section from same biopsy as Fig. 33, treated with anti-F showing antigen in connective tissue. $\times 210$.

FIGS. 35 to 37. Section from biopsy of cerebral cortex of adult taken at time of removal of cortical tumor. Treated with anti-A; all $\times 430$.

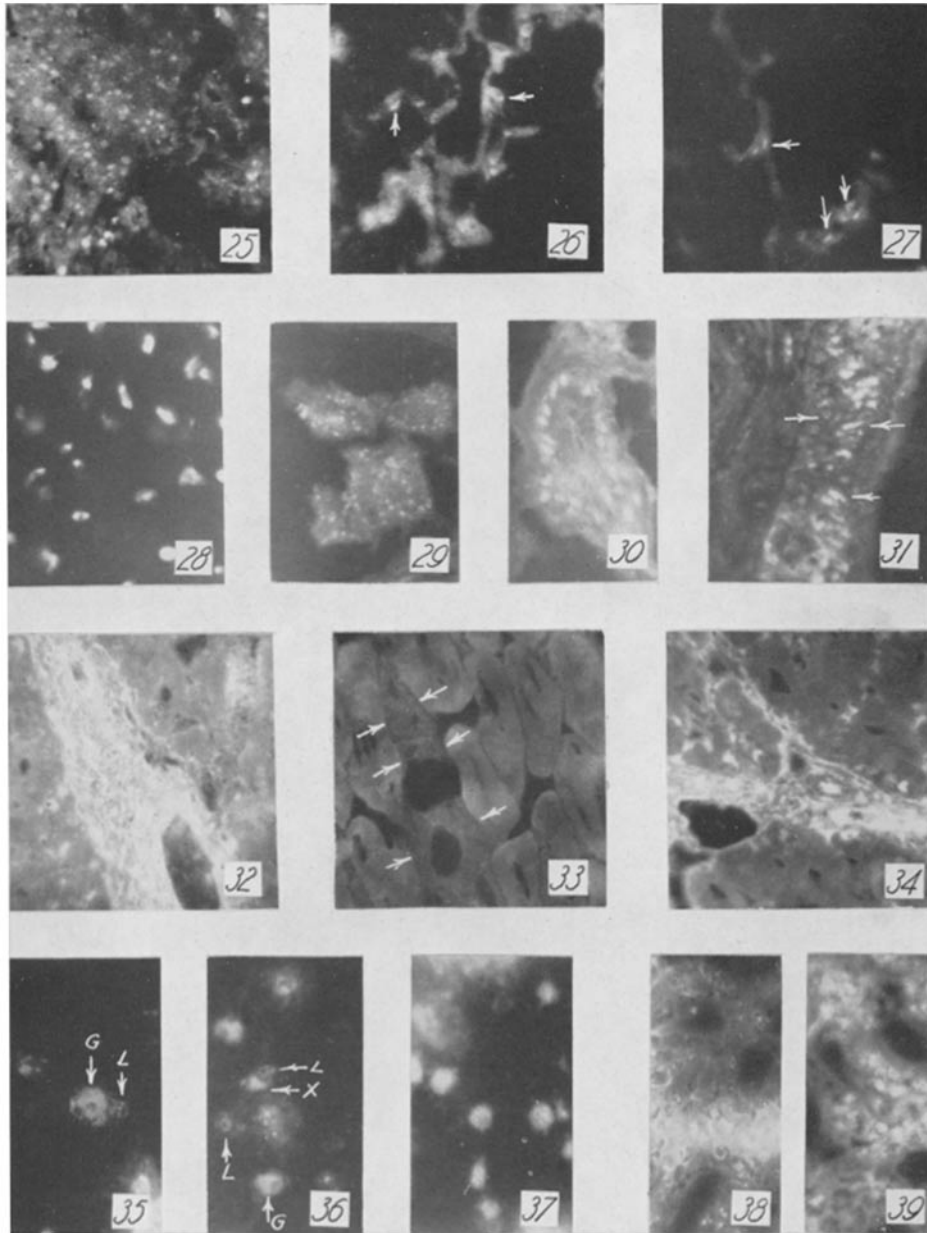
FIG. 35. A ganglion cell nucleus (G) containing abundant antigen, the fluorescence outlining the nucleolus. Also shown is antigen in a satellite cell nucleus (L). Minute spots of fluorescence about the ganglion cell nucleus are orange fluorescence due to unidentified substances.

FIG. 36. Antigen in ganglion cell nucleus (G) again outlining a nucleolus and in nucleus of satellite cell (L). Structure indicated by x appeared to be portion of ganglion cell nucleus.

FIG. 37. Glial cells showing antigen in their nuclei.

FIG. 38. Fetal adrenal cortex of patient 52-20, section treated with anti-FE. Antigen is present as spots in many nuclei. $\times 430$.

FIG. 39. Adult adrenal cortex of patient 52-20. Section treated with anti-B showing antigen in nuclei. $\times 430$.



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