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Experimental Pulmonary Disease and Autoimmune Nephritis in the Rabbit Produced by Homologous and Heterologous Choroid Plexus (Experimental Goodpasture's Syndrome)[1,2] (38314)

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The syndrome of pulmonary hemorrhage and autoimmune nephritis, "Goodpasture's syndrome" is a well recognized clinicopathological entity (1). The role of antiglomerular basement membrane antibodies in the pathogenesis of this syndrome is well recognized (2). Clinical observations, characterization of antibodies eluted from the diseased glomeruli and a variety of experimental models with homologous and heterologous antigens suggest that in addition to glomerular basement membrane immunologically related structures play a role in this disease (4–10). Despite its immunochemical and morphologic similarity (3) the choroid plexus membranes have not been frequently mentioned in the pathogenesis of this entity. Experimental analogues have not consistently duplicated the combination of pulmonary and renal disease. We have observed autoimmune injury to the choroid plexus in Goodpasture's syndrome (12) and suggested that this anatomic site may be involved in this entity. This study reports investigations of the effects of the morphological and immunopathological changes induced by administration of homologous and heterologous choroid plexus.

Materials and Methods. Preparation of choroid plexus. Sheep and rabbit choroid plexus were obtained from Pel-Freeze Biologicals (Rogers, Arkansas). They were trimmed, homogenized, washed by centrifugation until the concentrated supernatant no longer contained serum proteins, and choroid plexus basement membrane was solubilized as described by Kefalides (3). The soluble choroid plexus was freeze dried and used for immunization.

Five groups of five New Zealand white rabbits, 2.5 kg, and one group of 12 animals were used in the study.

Group I. These animals received 5 mg rabbit choroid plexus in Freund's adjuvant by footpad injection and then 5 mg subcutaneously every 2 weeks. The animals were checked daily for epistaxis and urine was tested qualitatively weekly for blood and protein by Labstix (Ames Labs). Serum was obtained weekly for detection of anti-GBM antibodies by double layer immunofluorescence using normal rabbit kidney and Fluorescein conjugated goat anti-rabbit IgG (FITC-GARIgG). Animals were sacrificed when proteinuria was 30 mg% or greater, hematuria moderate or large, anti-GBM antibodies detected, epistaxis present or any combination of these. Two animals in this group, Nos. 1 and 3 were nephrectomized 24 hr prior to sacrifice. All animals were sacrificed by three months after onset of immunization. Serum was obtained just before sacrifice for transfer experiments. Nephrectomy was performed to obtain a higher titer of antibody.

Group II. These animals were treated similarly to Group I except sheep choroid plexus was

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used rather than rabbit for immunization. Animals Nos. 9 and 10 were nephrectomized prior to sacrifice.

Group III. Each of these animals received 10cc of pooled serum intravenously from animals from Group I. Urine testing was performed as in Groups I and II. The same criteria were used for sacrifice.

Group IV. Each of these animals received 10cc of serum intravenously from animals from Group II. Similar criteria for sacrifice were used.

Group V. A. Four animals received 10cc of normal rabbit serum. B. Four animals received immunizations of lyophylized normal rabbit serum in a dose schedule similar to Group I. C. Four animals received immunization of lyophilized sheep serum in a dose schedule similar to that of Group II. Animals in this group were tested as those in the other groups. All were sacrificed at 3 mo.

Group VI. A. Two normal animals were nephrectomized and serum obtained 24 hr later just prior to sacrifice. Serum was obtained for transfer to other animals. B. Three animals. Each animal received 10cc of pooled serum from rabbits in Group VI A. Animals were tested as other groups and sacrificed at 3 mo.

At nephrectomy and sacrifice renal cortex was snap frozen in liquid nitrogen and stored at −70° for immunohistologic studies. Another portion was fixed in Bouin's solution and 4 micron sections were used for light microscopy and for staining with Hematoxylin and Eosin (H&E) and periodic acid Schiff (P. A. S.). The remainder of the kidneys from each immunofluorescent positive group were pooled and antibody eluted from isolated glomeruli as described previously (11). Immunohistologic studies using Fluorescein conjugated goat anti-rabbit IgG (FITC-GARIgG), Fluorescein conjugated goat anti-rabbit B_1C (FITC-GARB_1C) and Fluorescein conjugated rabbit anti-sheep IgG (FITC-RASIgG) were performed on 4 μm kidney cortical sections. Antibody eluted from glomerulonephritic kidneys was tested for anti-GBM antibodies by double layer immunofluorescence on normal rabbit and sheep kidney. In addition, the eluted antibody was tested for choroid plexus and lung antibody by double layer immunofluorescence on normal sheep and rabbit lung and choroid plexus. These techniques have been described by us in detail (11). Lung was examined grossly and sections fixed in Bouin's solution for light microscopy. Immunohistologic studies were performed on four to six micron sections of frozen lung tissue using FITC-GARIgG and FITC-GARB_1C. A portion of choroid plexus was snap frozen in liquid nitrogen for immunohistologic studies. The remainder was fixed in Bouin's solution for light microscopy. Direct immunofluorescent staining with FITC-GARIgG, FITC-GARB_1C and FITC-RASIgG was performed.

Results. Table I summarizes the clinical findings in all groups prior to sacrifice or nephrectomy. Animals receiving rabbit or sheep choroid plexus, Group I and II developed proteinuria and hematuria, occasionally epistaxis and frequently detectable anti-GBM antibodies between 6 and 12 weeks. Animals in Groups III and IV, receiving post nephrectomy serum from rabbits in Groups I and II respectively were found to have urinary changes within 1–2 weeks, but no epistaxis or anti-GBM antibodies were detected. Control animals in Groups V and VI showed no abnormalities (Table I).

The incidence of morphological and immunohistological changes in the various groups are summarized in Table II.

Only in the groups receiving choroid plexus or serum transferred from choroid plexus treated animals were changes noted (Group I-V). A moderate to severe glomerulonephritis (Fig. 1), pulmonary inflammation and hemorrhage (Fig. 2) were the predominant changes. The glomerular lesions were milder in Groups III and IV as compared to Groups I and II. Linear deposits of host IgG and B_1C but no sheep IgG were noted in the glomeruli in all rabbits in Group I-IV (Fig. 3). Similarly linear choroid plexus deposits of host IgG were noted in rabbits in Groups I and II (Fig. 4). However, no alterations in choroid plexus morphology were detected in any of the groups. Pulmonary alterations and choroid plexus immunofluorescence were not observed in Groups III and IV. No morphologic or immunohistologic alterations were detected in control animals Groups V and VI (Table II). Only three animals, No. 1 (Group I) and Nos. 7 and 8 (Group II) showed definite linear alveolar basement membrane localization of host IgG and B_1C. Staining was not bright. The other animals in Groups I and II showed extremely faint staining of questionable significance. Alveolar basement membrane staining was completely negative in all other groups.

TABLE I. Summary of Urinary, Clinical and Serum Findings in all Animals Prior to Sacrifice or Nephrectomy.

Group	Animal No.	Received	Urine		Epistaxis	Anti-GBM	Time of sacrifice or nephrectomy weeks
			Blood	Protein			
I.	1	Rabbit CP	Medium	30 mg%	Negative	+	8
	2	Rabbit CP	Large	100 mg%	Negative	Negative	12
	3	Rabbit CP	Moderate	30 mg%	+	+	6
	4	Rabbit CP	Moderate	30 mg%	+	+	12
	5	Rabbit CP	Moderate	100 mg%	Negative	Negative	12
II.	6	Sheep CP	Large	30 mg%	Negative	Negative	12
	7	Sheep CP	Large	100 mg%	+	+	8
	8	Sheep CP	Large	300 mg%	+	+	6
	9	Sheep CP	Large	300 mg%	Negative	+	8
	10	Sheep CP	Moderate	100 mg%	+	+	6
III.	11	Group I serum	Moderate	30 mg%	Negative	Negative	1
	12	Group I serum	Moderate	30 mg%	Negative	Negative	1
	13	Group I serum	Moderate	30 mg%	Negative	Negative	1
	14	Group I serum	Moderate	30 mg%	Negative	Negative	1
	15	Group I serum	Moderate	30 mg%	Negative	Negative	2
IV.	16	Group II serum	Light	30 mg%	Negative	Negative	1
	17	Group II serum	Light	30 mg%	Negative	Negative	1
	18	Group II serum	Moderate	100 mg%	Negative	Negative	1
	19	Group II serum	Large	300 mg%	Negative	Negative	1
	20	Group II serum	Moderate	30 mg%	Negative	Negative	1
V. A	21-24	Normal rabbit serum	All negative		All negative	All negative	12
V. B	25-28	Lyophilized rabbit serum	All negative		All negative	All negative	12 12
V. C	29-32	Lyophilized sheep serum	All negative		All negative	All negative	12
VI. A	33-34	Nephrectomized normal animals	All negative		All negative	All negative	0.14
VI. B	35-37	Serum from group VI A	All negative		All negative	All negative	12

TABLE II. Summary of the Incidence of Morphologic and Immunohistologic Alterations in all Groups.

Group	Lung Abnormalities		Choroid Plexus				Kidney			
	Gross	Microscopic	Microscopic changes	Immunohistology*			Glomerulonephritis	Immunohistology*		
				RIgG	RB_1C	SIgG		RIgG	RB_1C	SIgG
I.	3/5	5/5	0/5	5/5	5/5	0/5	5/5	5/5	5/5	0/5
II.	2/5	5/5	0/5	5/5	5/5	0/5	5/5	5/5	5/5	0/5
III.	0/5	0/5	0/5	0/5	0/5	0/5	5/5	5/5	5/5	0/5
IV.	0/5	0/5	0/5	0/5	0/5	0/5	5/5	5/5	5/5	0/5
V. A	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
V. B	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
V. C	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
VI. A	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
VI. B	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

* Immunohistologic localization of rabbit IgG (RIgG), rabbit (RB_1C), and sheep IgG (SIgG).

Antibody was eluted from the kidneys of animals in Groups I–IV. The amount of antibody was appreciably greater in Groups I and II. Eluted antibody fixation to both sheep and rabbit glomerular and to a lesser extent tubular basement membrane as well as choroid plexus and alveolar basement membrane was demonstrated by double layer immunofluorescence.

Discussion. Nephritis due to anti-GBM antibodies may occur in humans either as an isolated kidney disease or in association with pulmonary lesions (Goodpasture's syndrome) (2). Recently we have shown a role for the choroid plexus in Goodpasture's syndrome (12). Experimental animal models using heterologous nephrotoxic serum (13) and using homologous or heterologous lung, kidney, urine or placenta have usually produced anti-basement membrane nephritis (4, 5, 6, 8, 10, 14). However, reproduction of all the clinicopathologic features of Goodpasture's syndrome has been difficult to accomplish.

Although the choroid plexus membranes show chemical, structural, functional and immunological similarities with the renal glomerular basement membrane (3, 15) this anatomic site has not been prominent in studies in either clinical or experimental nephritis. Moreover, this structure has been implicated in immune complex disease (16–18).

In this study nephritis, hemorrhagic pneumonitis and immune deposits on the choroid plexus basement membrane is quite analogous to Goodpasture's syndrome. The transfer of nephritis and demonstration of anti-GBM antibody in the sera of these animals further demonstrate immunological cross reactivity between glomerular and choroid plexus basement membrane. Although host immunoglobulin and B_1C deposition on the alveolar basement membrane was less common and less prominent than that in the kidney, injury to the lung by cross reacting antibody appears to be a feature in this model. This observation of less striking lung involvement may possibly be due to a relatively smaller amount of the antibody being delivered to the lung. In human, anti-GBM nephritis may be associated with minimal, mild or no morphologic or immunohistologic lesions.

These studies suggest the possibility that the choroid plexus may be the site of the antigen in some cases of Goodpasture's syndrome and in-

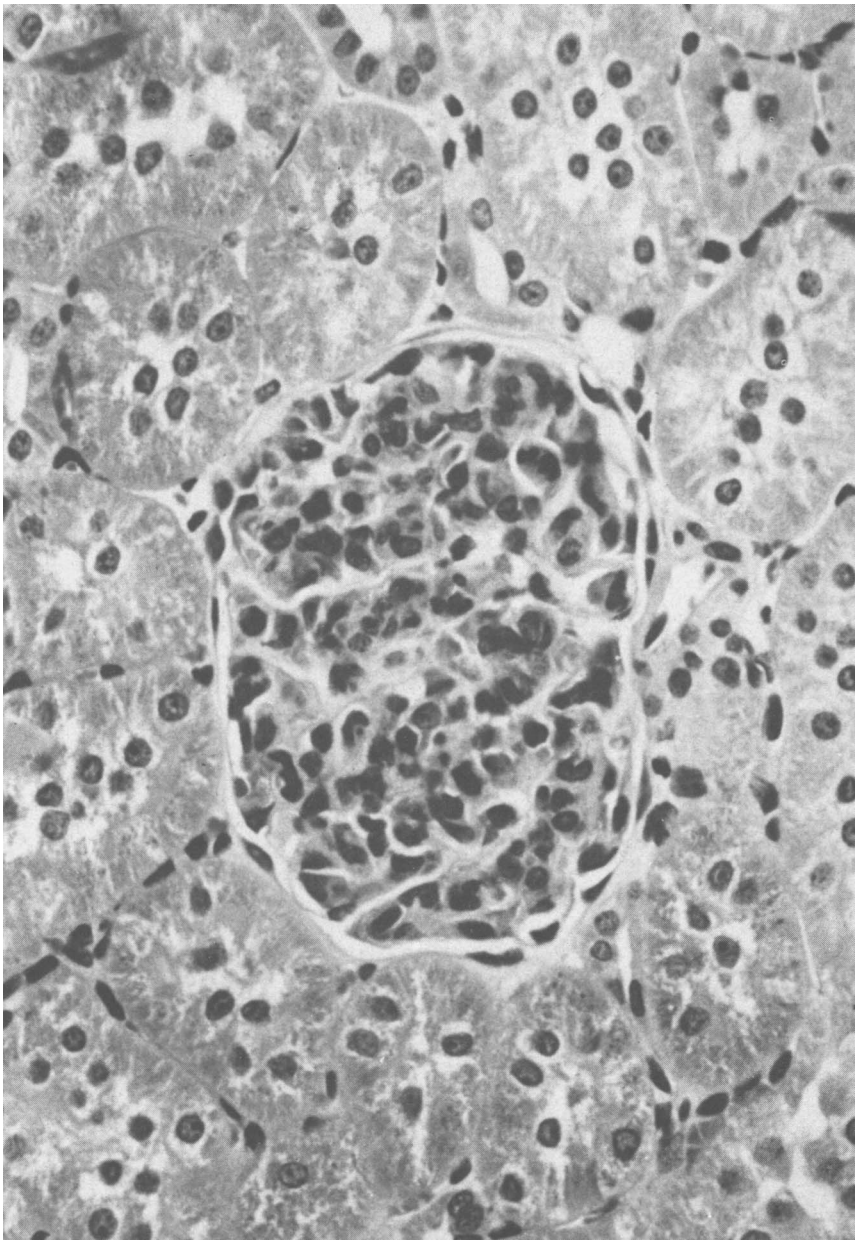

FIG. 1. Shows light microscopy for representative glomerulus from animal from Group II.

jury to this structure may lead to release of normally sequestered antigens, antibodies to which lead to the disease complex. Similar mechanism has been postulated for chemical or infectious injury to the lung.

Summary. The choroid plexus basement membrane bears striking immunologic, structural, chemical and functional similarities to glomerular basement membrane and has been suggested to play a role in anti-glomerular basement membrane nephritis. In these investigations serial injections of homologous and heterologous choroid plexus basement membrane in Freund's adjuvant to rabbits was as-

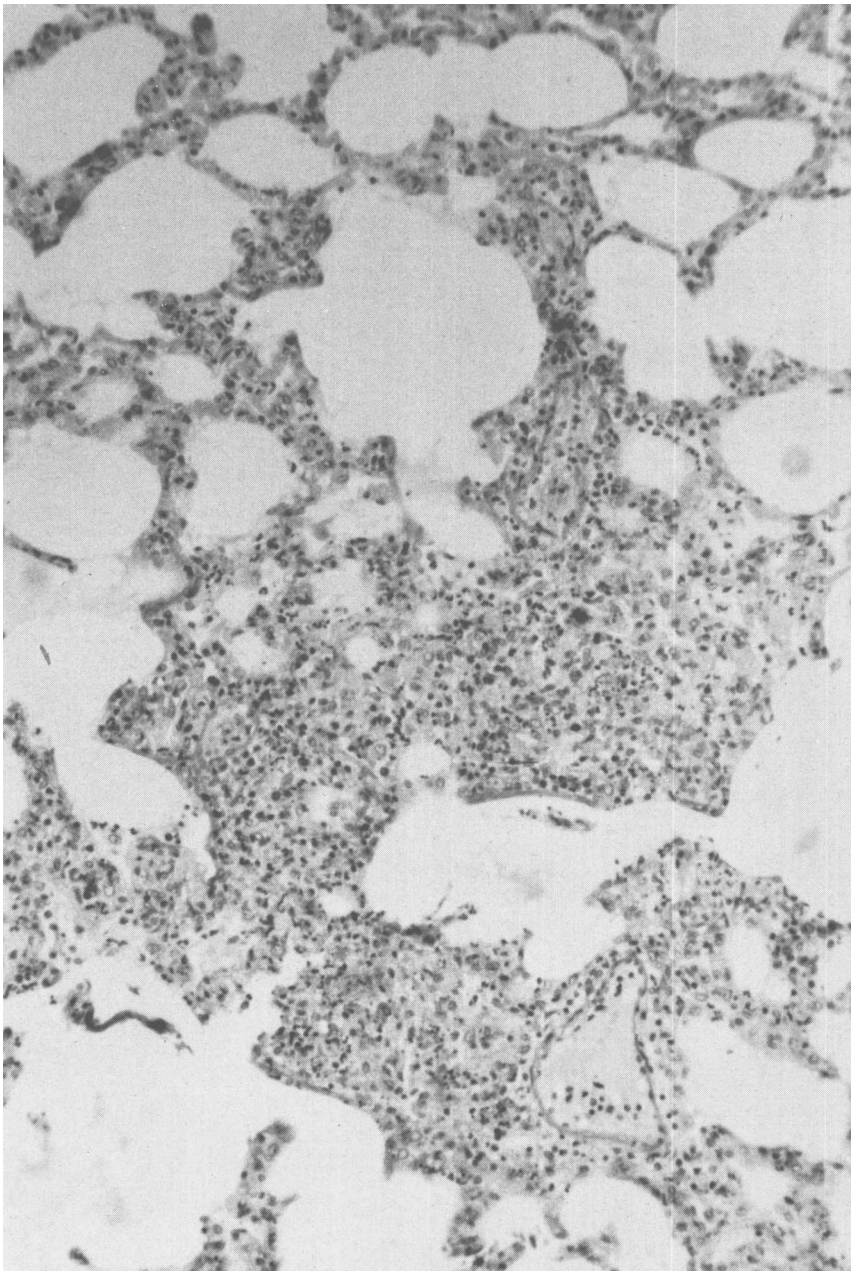

FIG. 2. Shows light microscopy of the lung from animal from Group I.

sociated with nephritis, hemorrhagic pneumonitis and linear deposits on the choroid plexus and to a lesser extent alveolar basement membrane in rabbits. Proteinuria, hematuria and serum anti-GBM antibodies were prominent. Epistaxis was occasionally present. The nephritis was characterized by linear deposition of host IgG and B_1C on the glomerular capillary walls and glomerular eluates contained anti-GBM anti-choroid plexus, alveolar and renal tubular basement membrane antibody. Nephritis was transferred by serum. The studies suggest a role for the choroid plexus in Goodpasture's syndrome.

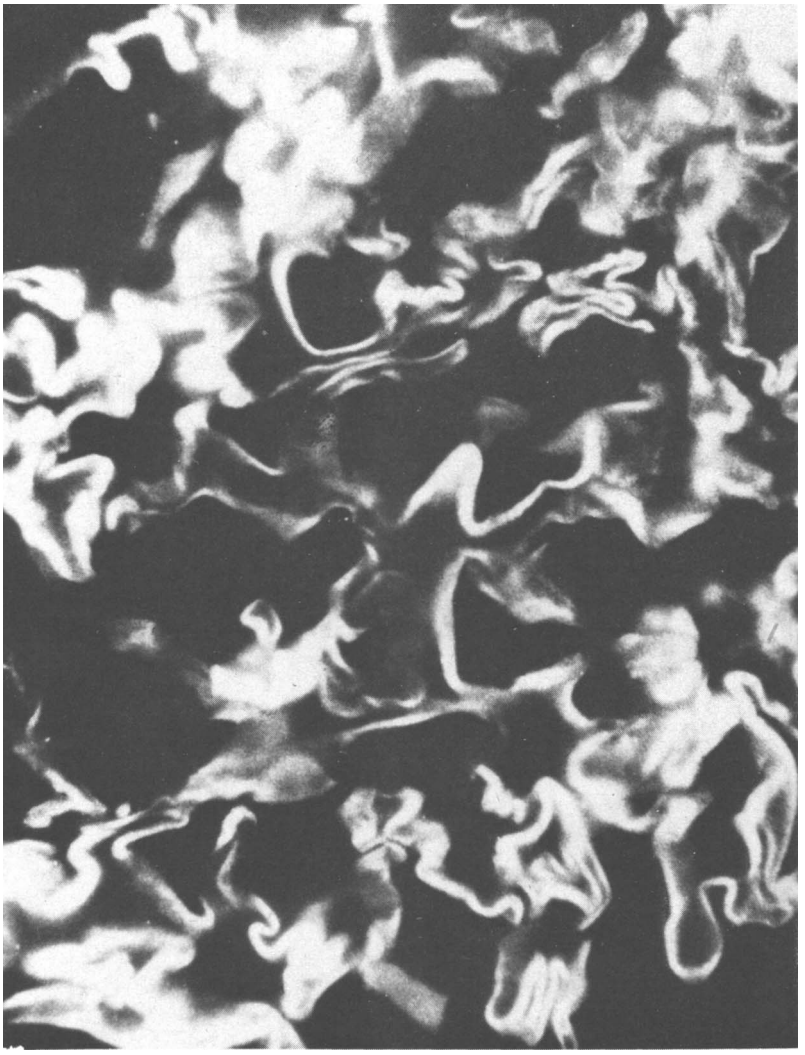

FIG. 3. Shows linear deposition of host IgG along glomerular capillary walls in animal from Group I. Glomerulus was stained with Fluorescein conjugated goat anti-rabbit IgG.

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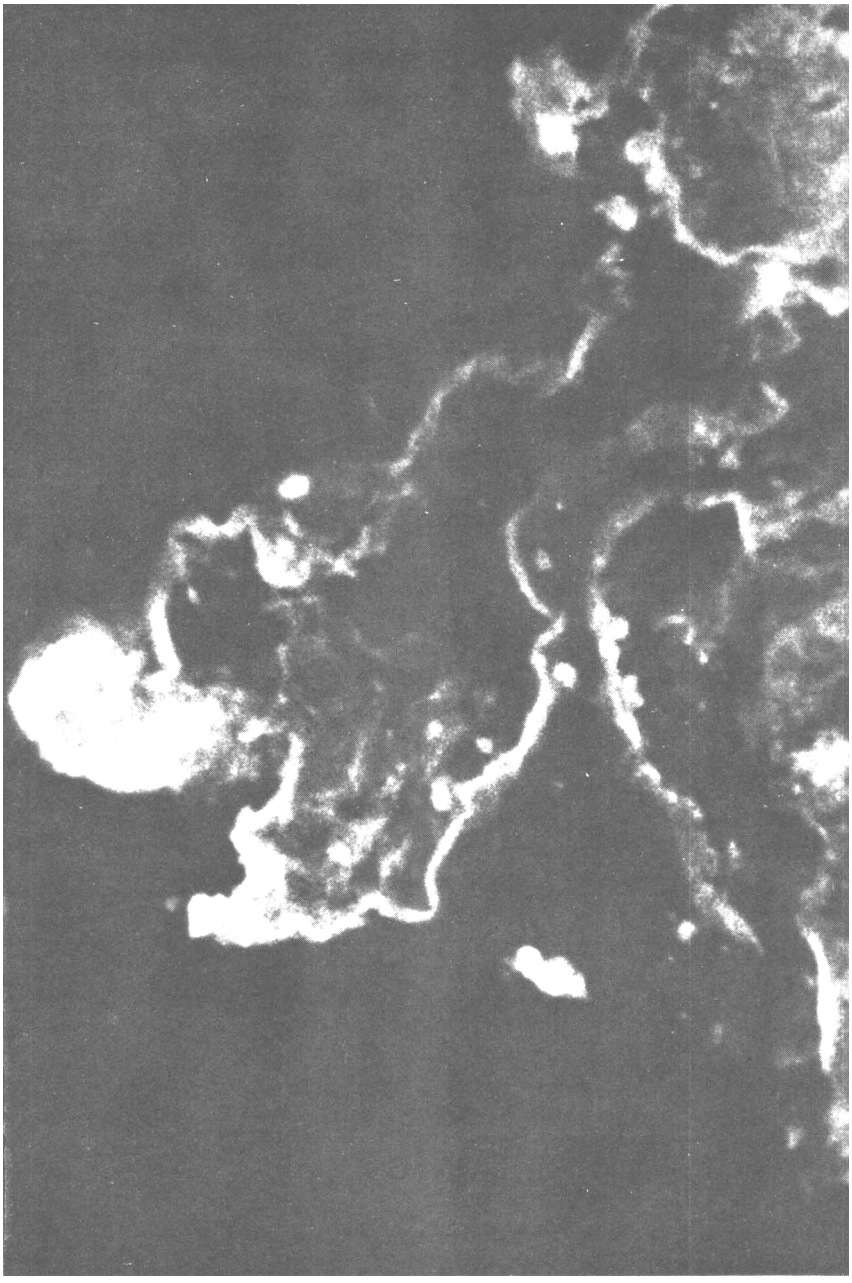

FIG. 4. Shows linear deposition of host IgG on choroid plexus of animal from Group I.

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