change in regulation by a neural factor, an interpretation which is suggested by the association of this decrease in AChE at the endplate with the large increase in the amount of certain AChE isoenzymes in the soluble fraction of these muscles, and the evidence that there is a parallel change of the latter type in denervated normal muscle (*2*). From this, combined with the foregoing evidence, it appears that those AChE isoenzymes which are increased in dystrophy must be sarcoplasmic. Whether these new soluble AChE types in the dystrophic muscles are the same molecular species as are localized at the endplates in the normal muscle is not yet known.

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Host Immunoglobulin G and Complement Deposits in the Choroid Plexus during Spontaneous Immune Complex Disease

Abstract. *Hybrid (NZB × W)F_1 mice spontaneously develop antibodies to nuclear antigens (ANA) and DNA (ADNA) and are an animal model of human systemic lupus erythematosus. Immunofluorescent and electron microscopic observations of the choroid plexus and renal glomeruli of (NZB × W)F_1 mice reveal deposits of host immunoglobulin G (IgG) and the third complement component which appear shortly after the development of ANA and ADNA in the circulation. Additionally, enhancement of ADNA responses accelerates the appearance and severity of IgG deposits in the choroid plexus. The choroid plexus may be a favored site for the deposition of immune complexes and the neuropsychiatric findings in patients with systemic lupus erythematosus and some patients with acute or chronic infections may be related in part to immune complex disease of the choroid plexus.*

Hybrid (NZB × W)F_1 mice consistently develop a spontaneous disease that closely resembles human systemic lupus erythematosus (SLE) (*1*). Within 1 year, most of the females die of a glomerulonephritis that is characterized mainly by deposits of DNA-antibody to DNA (ADNA) and nuclear antigen–antibody to nuclear antigens (ANA), and to a lesser extent by C type virus associated antigens–antibody to C type virus and red blood cell antigens–antibody to red blood cells in glomerular basement membranes and mesangia (*2*). Immunofluorescence studies reveal that host immunoglobulin G (IgG) and the third component of complement (C3) have begun to accumulate in the glomeruli of female mice at 3 to 5 months of age subsequent to the appearance of ADNA and ANA in their circulations (*2*).

In human SLE other organs besides the kidneys are involved producing a multitude of clinical manifestations. Nervous and mental disorders have been recorded in 75 percent of patients with SLE, but neuropathologic correlations are frequently difficult to establish (*3*). Since the vascular endothelium of the choroid plexus is fenestrated and thus morphologically similar to the endothelium in renal glomeruli, the choroid plexus may be another favored site for the deposition of immune complexes. Deposits of such complexes might play a role in the pathogenesis of nervous and mental disturbances.

We now describe the spontaneous accumulation of IgG and C3 in the choroid plexus of (NZB × W)F_1 mice and its relation to the formation of ADNA and ANA. Additional experiments which enhanced ADNA or ANA responses in the circulation also accelerated the development and increased the severity of choroid plexus deposition.

Female (NZB × W)F_1 mice in groups of ten mice at 1, 2, 3, 4, 5, 7, 8, and 9 months of age were killed by exsanguination. The choroid plexus from one-half of the brain and a small slice from the kidney were fixed in 5 percent phosphate-buffered glutaraldehyde for electron microscopy. The remaining brains and kidneys were frozen in liquid nitrogen for immunofluorescence studies. For electron microscopy, the tissue was again fixed in 1 percent phosphate-buffered osmium tetroxide, dehydrated, and embedded in Araldite. Sections were stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop 101 electron microscope. For direct immunofluorescence, frozen sections were stained with fluorescent labeled antibodies against mouse IgG, C3, fibrinogen, and albumin (*2*). Plasma was collected from all animals for the determination of ADNA and ANA. Antibodies to DNA were quantitated by means of [^{3}H]thymidine labeled DNA in a modified Farr antigen binding assay as reported (*4*), and ANA determinations were done on normal mouse kidney sections by the indirect immunofluorescent technique (*2*).

The results showed no IgG deposits in the choroid plexus until the animals were 4 months old. When the animals were 5 and 9 months of age, IgG was

present in the choroid plexus in 50 percent and 80 percent of the mice, respectively; it occurred as irregular scattered granular deposits. The C3 was less consistently detected in the choroid plexus compared to both IgG deposition in the choroid and C3 deposits in affected renal glomeruli. In the glomeruli, the granular IgG and C3 deposits were observed throughout the mesangium and the glomerular loops, whereas the choroid plexus deposits were patchy and often large portions of the plexus showed no deposits (Fig. 1b). Fluoresceinated antibodies to albumin and to fibrinogen did not stain the choroid plexus deposits. Owing to the scattered and small amounts of IgG deposited in the choroid plexus, we were unable to elute, recover, and immunochemically identify the antibody specificity of the deposited IgG.

Electron microscopy revealed large clumps of electron dense material beneath the endo- and epithelium as well as within the basement membrane of renal glomeruli, confirming previous observations (*2*). The choroid plexus contained patchy electron dense deposits, predominantly within extracellular, perivascular spaces, but occasionally also within or adjacent to the epithelial or vascular basement membrane (Fig. 1a). No leukocytic infiltrates or alterations of epi- or endothelial cells were apparent in the choroid plexus.

The temporal relationship between development of circulating ADNA, ANA, and IgG deposits in the choroid plexus was studied in (NZB × W)F_1 female mice. In these mice, the incidence of ADNA and ANA rose sharply at 4 to 6 months of age, whereas the incidence of IgG deposits in the choroid plexus rose 1 to 2 months later. In other experiments, repeated immunization of 6-week-old (NZB × W)F_1 female mice with DNA coupled to methylated bovine serum albumin (DNA–m-BSA) hastened the appearance and increased the titers of ADNA. Such mice had high ADNA titers at 3 months of age and an 85 percent incidence of IgG deposits in the choroid plexus. In contrast, 6-week-old littermates immunized with methylated-BSA failed to develop choroid plexus lesions at 3 months. As compared to lesions accompanying the spontaneous disease, the choroid plexus lesions in mice immunized with DNA–m-BSA occurred more often, were greater in number, and were sometimes associated with mononuclear cell infiltrates (Fig. 1c).

Our findings may have important implications concerning the central nervous system derangement accompanying SLE in many patients. Our fluorescent and electron microscopic study of autopsied tissue of two SLE patients who had suffered neurologic manifestations and had high ADNA and ANA titers showed dense IgG deposits in the choroid plexus. Atkins *et al.* also drew attention to IgG and IgM deposits in the choroid plexus of SLE patients (*5*). Once deposited, such complexes might activate the complement system, releasing vasoactive factors. These factors could cause permeability changes resulting in water, electrolyte, and *p*H shifts which might account for the diffuse and usually transient central nervous system disturbances frequently seen in these patients. Evidence for activation of the complement system with

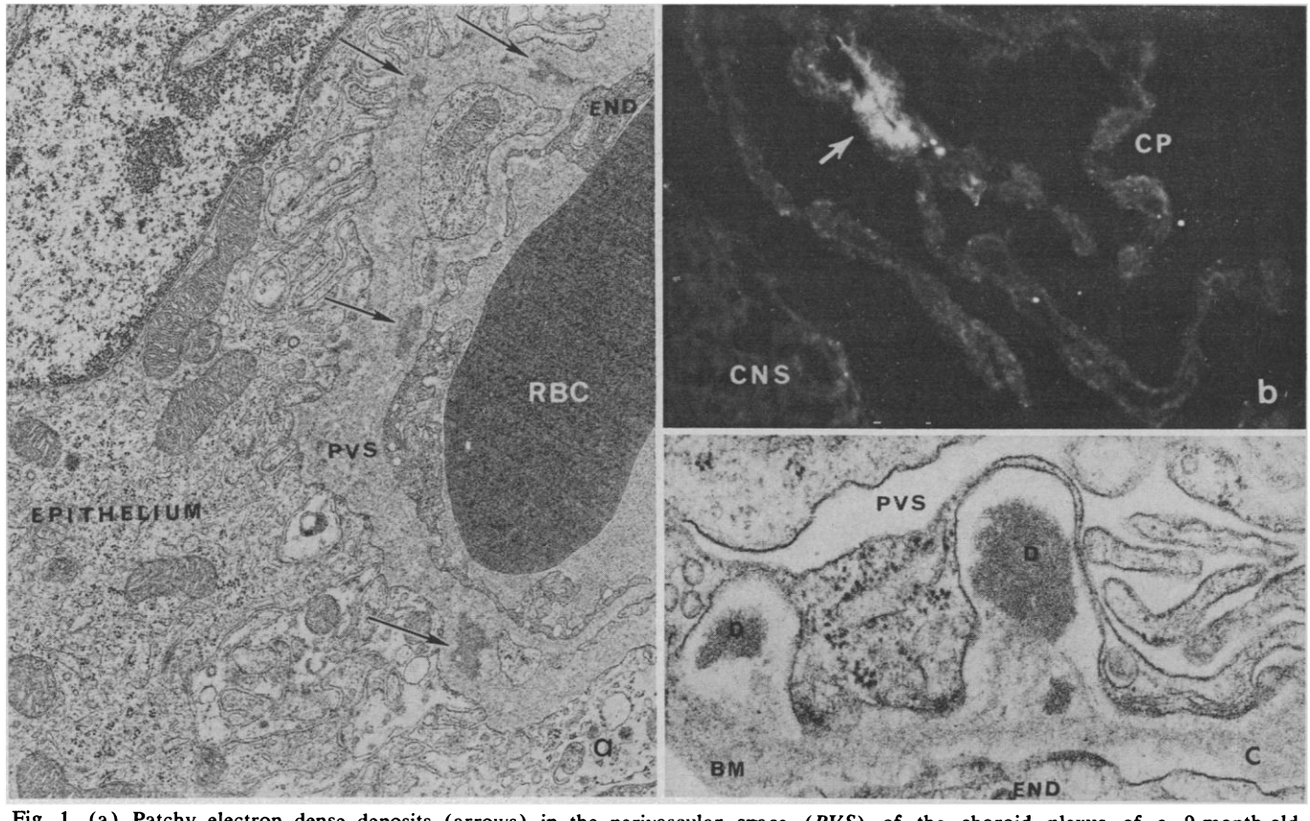

Fig. 1. (a) Patchy electron dense deposits (arrows) in the perivascular space (*PVS*) of the choroid plexus of a 9-month-old (NZB × W)F_1 female mouse; *END*, endothelial cell; *RBC*, red blood cell (× 13,500). (b) IgG deposits in the choroid plexus of a 9-month-old female (NZB × W)F_1 mouse. A frozen section (4 μm) was stained with fluoresceinated rabbit antibody to mouse IgG; *CP*, choroid plexus; *CNS*, central nervous system parenchyma (× 185). (c) Higher magnification of electron dense deposits (*D*) within and adjacent to the vascular basement membrane (*BM*) of the choroid plexus of a 3-month-old (NZB × W)F_1 female mouse. The mouse was immunized with 25 μg of DNA–m-BSA (intraperitoneally) at 6, 10, and 12 weeks of age; *PVS*, perivascular space; *END*, endothelial cell (× 30,000).

resultant lowering of C4 hemolytic activity in the cerebral spinal fluid in some patients with central nervous system SLE has been reported (*6*). Finally, the association of neuropsychiatric disorders with other acute and chronic immune complex diseases may be related to trapping of such deposits in the choroid plexus.

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Serotonin Producing Neuroepithelial Bodies in Rabbit Respiratory Mucosa

Abstract. *The intrapulmonary lining epithelium of rabbits contains newly identified corpuscles composed of argyrophil, argentaffin, yellow fluorescent, ultrastructurally granulated and innervated epithelial cellular organs. These are proved, by electron microscopic cytochemistry and microspectrography, to be a source for intrapulmonary production of serotonin. Probably they are intrapulmonary neuroreceptor organs modulated by the central nervous system which exhibit local secretory activities.*

While physiologically the occurrence of several reflexes originating in the lungs of newborns (*1*) and adults (*2*) is well documented and although the important role of the pulmonary nervous system in various diseases such as asthma and other acute or chronic respiratory or pulmonary insufficiency syndromes is well recognized, basic information is lacking about the topography and fine structure of the presumed intrapulmonary receptor structures. Earlier studies do indeed only include a rather crude study by light optics, generally based on methylene blue or silver impregnation techniques that revealed the pulmonary nerve endings as more or less complex and ramifying fibers (*3*). Recently we identified (*4*), by light optical and histochemical techniques only, the occurrence of neuroepithelial bodies in human infant bronchial and bronchiolar mucosae and proposed that the corpuscles are probably neuroreceptor or secretory organs, which modulate locally various bronchial and bronchiolar functions (*5*), such as mucosal secretion, smooth muscle tone, vasomotion, and in a more general way integrated pulmonary lobular activity (*6*).

We have attempted to obtain a comparable animal model that would elucidate their fine structure in normal and experimental conditions. We now report analogous neuroepithelial bodies (NEB's) throughout the respiratory mucosa of the rabbit lung, observed by combined light optics, cytochemistry, microspectrography, and electron microscopic cytochemistry.

We now demonstrate that these newly observed NEB's probably have an intrapulmonary receptor function which is related—as in the carotid body—to the central nervous system and which apparently liberates various substances within the lung, one being serotonin.

We took lung biopsies of 28 rabbit fetuses near term (up to 3 to 4 days before birth) delivered by cesarean section, 12 term rabbits (1 to 21 days old), and 6 adult rabbits. For light microscopy the tissues were fixed in Bouin's fluid or in formalin, embedded in paraffin, serially sectioned, and stained with the usual techniques. Argyrophilia was detected according to Bodian's silver proteinate technique as modified by Van Campenhout (*7*), Singh's technique (*8*), and Grimelius's silver nitrate technique (*9*); the sections were also subjected to the Fontana-Masson argentaffin reaction, Schmorl's technique for lipofuscin and argentaffinity (*10*), Lison's azo reaction for serotonin (*11*), and Solcia's lead hematoxylin stain (*12*).

We also investigated tissues with Falck's histochemical fluorescent amine technique (*13*), as in our earlier studies (*4*), but with an epifluorescent microscope (Leitz) (excitation filters BG 38, BG 12; dichromic mirror TK 495 and barrier filter 510, or interference filter 438 and barrier filter 470). Emission and excitation spectra were measured by means of a Leitz microspectrograph with the use of an EMI 9558 QA photomultiplier and a Hewlett-Packard 7004B-XY recorder. For emission, the light source was an HBO 100-watt mercury lamp, and for excitation an XBO 150-watt xenon lamp. Emission spectra were corrected according to Ritzen (*14*); the excitation spectra were corrected, indirectly, by comparison with pure serotonin ($9 \times 10^{-2}M$) in bovine albumin (5 percent) and colonic enterochromaffin cells studied in an identical way.

For electron microscopy, we fixed biopsies in three different ways: (i) in 2.5 percent glutaraldehyde (0.1*M* phosphate buffer, *p*H 7.2), followed by osmic acid; (ii) in 3 percent glutaraldehyde (0.2*M* cacodylate buffer, *p*H 7.4) for 4

Table 1. Measurements of the dense-cored vesicles after different electron microscopy fixation techniques; LA, longitudinal axis; VA, vertical axis; EI, elongation index; SR, staining reaction; S.D., standard deviation.

Vesicle population	Feature	Mean (Å ± S.D.)	Measurements (No.)
Glutaraldehyde (2.5 percent) followed by OsO_4			
Type 1	LA	1340 ± 196	237
	VA	1021 ± 165	237
	EI	1.32 ± 0.07	237
Type 2	LA	1121 ± 150	161
	VA	989 ± 142	161
	EI	1.12 ± 0.06	161
FGD fixation			
With SR	LA	1146 ± 163	144
	VA	890 ± 133	144
	EI	1.30 ± 0.13	144
Without SR	LA	810 ± 103	71
	VA	681 ± 96	71
	EI	1.19 ± 0.09	71