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

hours at 4°C, followed by a 2.5 percent solution of potassium dichromate containing 1 percent sodium phosphate (0.2*M* acetate buffer, *p*H 4.1) for 4 hours at 4°C (*15*) and referred to as the glutaraldehyde-dichromate (GD) technique; and (iii) initial fixation with 8 percent formaldehyde (F) for 24 hours prior to the GD technique (*15*) and referred to as the FGD technique. Sections (1 μm) cut from the Epon embedded blocks were stained with toluidine blue. As the NEB's to be described are distinctly visible on such sections, the blocks were trimmed, and a correlated electron microscopical study (Philips EM 300; Zeiss 9A) was carried out on the immediately adjacent ultrathin sections, which were stained with uranyl acetate and lead citrate (*16*) after fixation (i), while they were first examined unstained after the GD and FGD fixation; next the GD- and FGD-fixed sections were stained with uranyl acetate only and again investigated.

On the electron micrographs of the NEB's, so-called intracytoplasmic dense-cored vesicles (DCV's) were observed. We measured their maximum length and width and established an "elongation index" representing the ratio of length to width. Next, we applied a variance analysis to the data of DCV's.

Using light optics we observed that, within the epithelium lining the bronchi, bronchioli, and even alveoli there were intercalated cell groups whose architecture and morphology are obviously different from the surrounding epithelial cells and correspond to the NEB's (Fig. 1a). They are composed of more or less parallel, nonciliated cylindrical cells with eosinophilic cytoplasm and rather oval nuclei. When

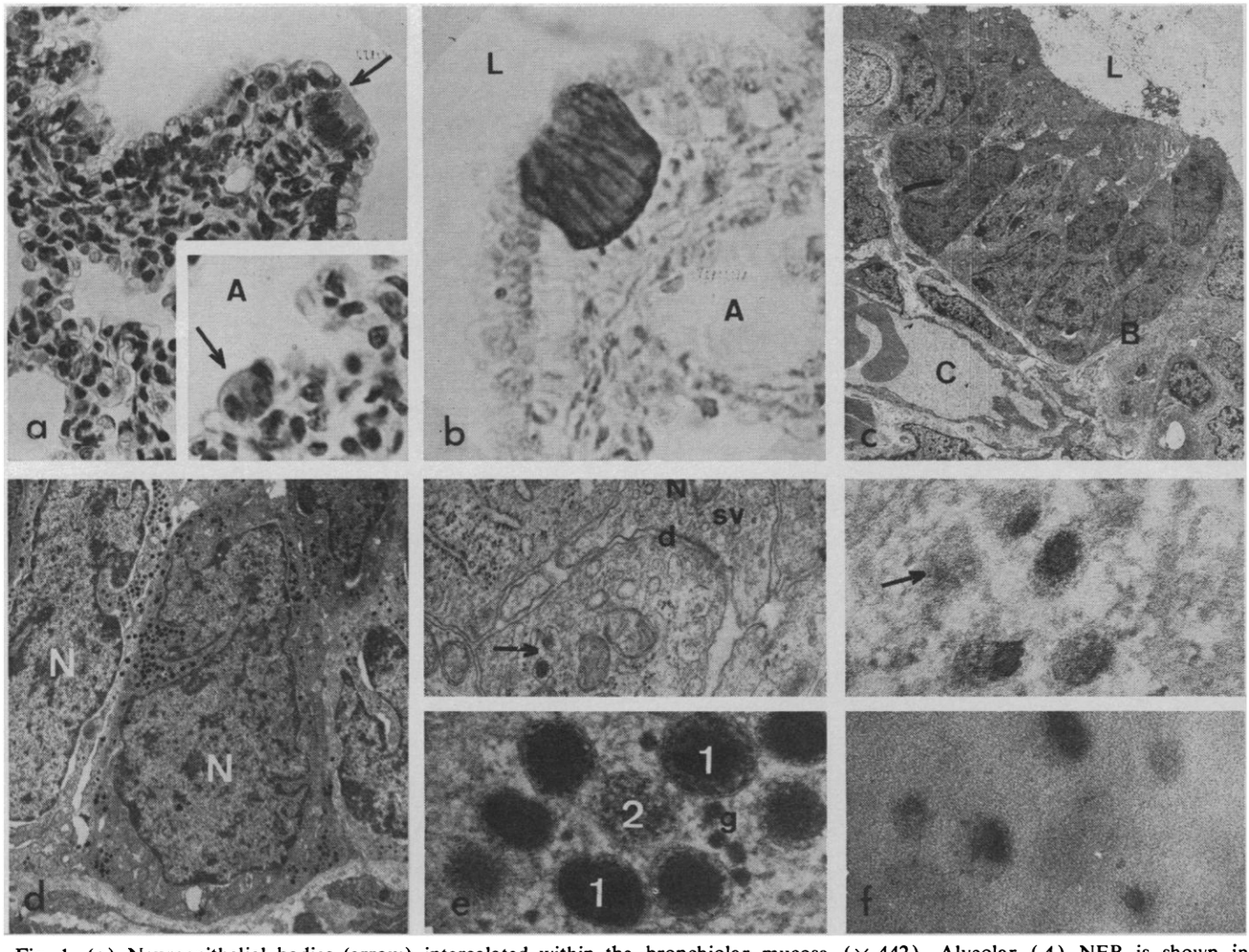

Fig. 1. (a) Neuroepithelial bodies (arrow) intercalated within the bronchiolar mucosa (× 442). Alveolar (*A*) NEB is shown in the inset; hematoxylin and eosin stain (× 702). (b) Bronchial NEB's exhibit a distinct cytoplasmic argyrophilia; *L* is the bronchial lumen and *A* is an alveolus; Van Campenhout's modification of Bodian's technique (× 977). (c) NEB's are within the bronchial mucosa and reach from the lumen (*L*) to the basement membrane (*B*); closely apposed capillary (*C*) is within the corium; glutaraldehyde fixation followed by osmic acid fixation, uranyl acetate, and lead citrate staining (× 1,641). (d) Characteristically "granulated" epithelial cells of NEB with indented nuclei (*N*) and numerous dense-cored vesicles; fixation and staining as in (c) (× 5,745). (e, bottom) Characteristic dense-cored vesicles of the first type (labeled "1") contain a dense amorphous core and some peripheral coarse granules; the second type (labeled "2") have a paler, dark-grayish core; the much smaller granules (*g*) located in between both vesicle types are glycogen granules; fixation and staining as in (c) (× 89,262). (e, top) Synaptical end formation of nerve fiber (*N*) containing many agranular synaptic vesicles (*sv*) with a granulated cell of an NEB containing dense-cored vesicles within its cytoplasm; synaptosomes are indicated by *d*; osmic acid fixation (× 28,728). (f, bottom) Dense, typically excentric deposit within dense-cored vesicles of the first type; FGD technique without staining (*15*) (× 49,248). (f, top) Cytoplasm of a corpuscular cell after FGD technique, followed by uranyl acetate staining; there are two types of dense-cored vesicles of which the first were also visible in unstained sections and contain dense deposit while the second (arrow) have not reacted (× 107,730).

followed on serial sections, these cell groups appear to be part of a truncated, conelike cellular organ with a broad base upon the basement membrane and reaching the airway lumen. In the underlying corium, one or more capillaries are usually present (Figs. 1c and 2c).

After silver impregnation (Fig. 1b) the corpuscular cells reveal a pronounced cytoplasmic argyrophilia with all the three techniques; with the Fontana-Masson technique, a slight argentaffin granulation is present as well, although it is not so pronounced as the argyrophilia. Also, positive stains are obtained by Schmorl's technique, Lison's azo reaction for serotonin, and Solcia's method.

Silver impregnation clearly outlines the nerve bundles and ganglion cells of the peribronchial and peribronchiolar plexus from which stem the nerve fibers that innervate the structures of the bronchial wall. Thus nerve endings may be traced in the immediate vicinity of the corpuscles; however, massive corpuscular argyrophilia hinders light microscopy of intracorpuscular nerve endings, which may be best demonstrated selectively with the electron microscope (Fig. 1e).

With Falck's technique, the NEB's reveal an intense yellow cytoplasmic fluorescence (Fig. 2, a and b). Its emission spectrum remains stable despite variations in relative humidity (30 to 47 percent), temperature (60° to 80°C), and duration (30, 60, and 120 minutes) of the paraformaldehyde vapor treatment. Blue-green fluorescent nerves were demonstrated in the same sections, and in a few cases thin fibers were seen in the immediate vicinity of the NEB's.

Microspectrographically (*14, 17, 18*) the fluorescence of the NEB's shows a maximum at 540 to 550 nm (corrected 530 nm), corresponding with the emission spectrum of serotonin ($9 \times 10^{-2} M$) in bovine albumin (5 percent) and of colonic enterochromaffin cells. Also the excitation spectra were identical with those of serotonin and of colonic enterochromaffin cells (maximum 460 nm uncorrected).

The light optical characteristics and intramucosal topography of the NEB's are confirmed by electron microscopy (Fig. 1c). Immediately underneath the basement membrane that lines the corpuscles, a capillary is usually apposed, its endothelial cells revealing numerous fenestrae (Fig. 2c). The corpuscular cells that exhibit usually an oval nucleus and a rather dark cytoplasm containing the classic organelles are characterized by the intracytoplasmic occurrence of numerous DCV's of two types (Fig. 1, d and e). The first type (70 percent) consists of vesicles that exhibit various wedgelike to more oval profiles (Table 1). Its dense core, in which coarse granules (90 Å) are sometimes seen, may fill up the entire vesicle or lie excentrically, the rest of the vesicle revealing a finer granulation (30 Å). The second type encompasses more rounded and smaller vesicles (Table 1), which are characterized by a large halo (150 to 200 Å) and a core constituted by the 30-Å granules (*19*). With the use of the FGD and GD fixatives (Fig. 1f), the first type of the DCV's is visualized without any contrast staining (for example, uranyl acetate) by a positive reaction in the dense core, while the second type does not react, becoming identifiable after uranyl acetate staining only.

Intracorpuscular nerve endings were traced with the electron microscope. As was anticipated, unmyelinated nerve fibers occurred in the corium and also ramified within the NEB's, establishing direct synaptic end formations (Fig. 1e) with the granulated cells. At such sites the granulated cell and the axon membrane do exhibit thickenings or synaptosomes; the axoplasm contains numerous small mitochondria and a rather homogeneous population of agranular vesicles (about 50 nm in diameter).

We did not observe any basic morphologic difference between the NEB's of fetal, neonatal, and adult rabbits. Although the architecture of the corpuscles may vary somewhat, appearing more regularly stratified in the younger animals and composed of a little more disorderly piled and more pseudostratified cells with increasing age, the other characteristics are alike. In adult animals, the NEB's are also somewhat smaller. A variance analysis performed on the dimensions of the DCV's in the different age categories revealed no significant difference.

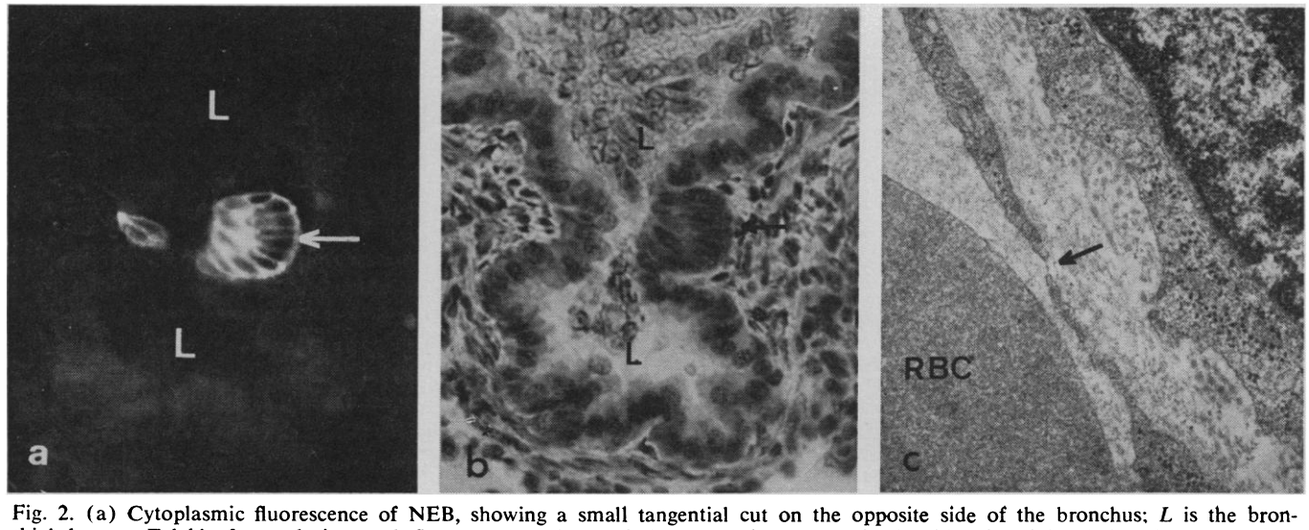

Fig. 2. (a) Cytoplasmic fluorescence of NEB, showing a small tangential cut on the opposite side of the bronchus; *L* is the bronchial lumen; Falck's freeze-drying and fluorescent amine technique; specimen examined with ultraviolet light alone (× 475). (b) Same section remounted and stained with hematoxylin and eosin, revealing the light optical characteristics of the same NEB (arrow); *L* is the bronchial lumen (× 475). (c) Electron micrograph of the endothelial cell lining of a capillary in the immediate vicinity of an NEB; the arrow indicates fenestration; *RBC*, red blood cell. Glutaraldehyde fixation with postosmification; uranyl acetate and lead citrate staining; neonatal rabbit lung (× 31,320).

These integrated light optical, histochemical, microspectrographical, and ultrastructural studies have revealed the widespread occurrence in the rabbit lung of intramucosal, innervated, dense-cored cellular corpuscles. Although these corpuscles probably have a variety of functions, they appear to be the source of an intrapulmonary production of serotonin. While the argyrophilia, argentaffinity, and constant yellow fluorescence suggest the occurrence of serotonin within the DCV's, the positive ultrastructural reaction after an FGD fixation (*15*) and the microspectrographic emission and excitation spectra indicate the high concentration of 5-hydroxytryptamine in these specialized epithelial cells. Moreover, most of the intrapulmonary bronchial capillaries are fenestrated in the vicinity of the NEB's and are drained off via the bronchopulmonary veins to the pulmonary veins (*20*). Thus the serotonin produced by the NEB's—which may be stimulated, for example, by the partial pressure of the oxygen or carbon dioxide of the intralumenal gas—may reach the pulmonary veins directly. This anatomical pathway offers an explanation for the previously obscure morphological mechanism of hypoxic pulmonary venoconstriction (*21*).

We realize that the granulated cells probably produce substances other than serotonin, whose metabolism may be combined with a variety of cellular activities in the amine and peptide spectrum. Indeed, they share many characteristics of the bronchial and bronchiolar AFG (argyrophil, fluorescent, and granulated) cells which we have identified in the human infant (*5, 22*). Although the corpuscular appearance and the obvious innervation of the NEB's are characteristics establishing a separate morphological entity, they may also exhibit various local secretory functions (*5*), modulating not only vasomotion but also other bronchial and bronchiolar functions such as mucosal secretion, smooth muscle tone, or the general integration of the activities of the pulmonary unit lobules (*6*).

The distinct innervation of the NEB's indicates their direct connection with the central nervous system, probably in an afferent as well as efferent manner. The typical synaptic end formations with a practically homogeneous population of small and agranular vesicles suggest an efferent innervation with probably a centrofugal modulating activity which may be compared to similar properties of the carotid body. The immediate contact of these NEB's with the airway lumina, their occurrence throughout the most peripheral airway branchings, their high incidence, and finally their corpuscular appearance are all indicative of a neuroreceptor function. The possibilities include a response to various stimuli and a chemo-, stretch-, baro-, or tactile-receptor function. It may be that they react to the P_{O_2}, the P_{CO_2} and/or other constituents in the airway gas, or are stimulated by inhaled pollutants or particles in the inspired air, which have passed the larger bronchi.

Additional biochemical, physiologic, and pharmacologic investigations are needed to elucidate the precise function of the identified (*23*) NEB's (*24*).

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18. The emission and excitation spectra have been measured microspectrographically on the NEB's of six rabbit fetuses, eight term rabbits, and three adult rabbits, and on the enterchromaffin cells of six adult rabbits and three human adults.
19. A variance analysis was performed on the dimensions of the DCV's. It revealed that the longitudinal axis and the elongation index of the two types of DCV's are significantly different ($P < .001$). The vertical axis does not exhibit a significant difference ($P > .05$). Hence and though both types of DCV's are morphologically, morphometrically, and histochemically different, it may not be excluded that in individual cases "rounded vesicles with dense core" represent a lateral cut of a type 1 vesicle.
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24. Since the submission of the manuscript, analogous NEB's have been identified by light optics and silver staining in the respiratory mucosa of various other mammals, that is, three adult cats, one adult lion, one adult monkey (*Ateles geoffroyi* Kuhl), one adult rock badger [*Procavia capensis* (Pallas)], one newborn pig (*Sus scrofa*), and one adult hedgehog (*Erinaceus europaeus* L.).
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Visual Discrimination in Sharks without Optic Tectum

Abstract. *After complete removal of the optic tectum, nurse sharks can learn to discriminate black versus white and horizontal versus vertical stripes. This finding is contrary to the traditional belief of exclusive tectal control over visuomotor behavior in lower vertebrates and suggests a role for the telencephalon in the vision of these primitive animals.*

The optic tectum has long been supposed to be the sole repository for the mechanisms involved in the visuomotor behavior of nonmammalian vertebrates. Indeed, Herrick referred to the tectum of lower vertebrates as the "supreme center of regulation of motor responses to the exteroceptive system of sense organs" (*1*). Based partly on this belief and related neuroanatomical data, the theory of encephalization asserts that there is a gradual shift of most visual function from midbrain optic tectum to visual cortex as one ascends the