found by Zeiss *et al.* (*10*), who showed in 15 patients that a mean of 13 percent of IgE protein was antibody to ragweed antigen E. However, Zeiss *et al.* used an indirect binding method to measure IgE antibody and only dealt with one of the allergens in ragweed pollen. Even so, in one patient 41 percent of IgE protein was antibody. Earlier Ishizaka and his associates, using a similar binding method, had suggested that a high percentage of IgE protein possessed antibody activity (*11*).

Few studies of this type have been conducted in experimental animals or man. In rabbits, after repeated injections of large quantities of bacterial or protein antigens, up to 90 percent of the gamma globulin may be accounted for as antibody (*12*). Usually, however, only 10 to 20 percent of rabbit immunoglobulin G possesses antibody activity even after immunization with adjuvants (*13*). In humans, immunization with dextran results in low levels of antibody which probably accounts for no more than 2 percent of the total immunoglobulin (*14*). Bandilla *et al.* immunized humans with hemocyanin, and their results indicate that less than 2 percent of the IgG and IgA and 4 percent of IgM classes could be accounted for as antibody during a secondary response (*15*). Our finding that IgE antibody accounts for such high percentages of total IgE protein presumably reflects the local deposition of pollen in the nose and the presence in nasal tissues of relatively large numbers of IgE-producing plasma cells (*16*). Finally, the demonstration that IgE antibody accounts for such a high percentage of the total IgE protein should stimulate further studies of means to depress these levels, especially because the severity of ragweed hay fever can be related to the level of IgE antibody (*17*).

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References and Notes

1. K. Ishizaka, T. Ishizaka, M. M. Hornbrook, *J. Immunol.* **97**, 75 (1966); S. G. O. Johansson, H. Bennich, L. Wide, *Immunology* **14**, 265 (1968); D. R. Stanworth, J. H. Humphrey, H. Bennich, S. G. O. Johansson, *Lancet* **1968-II**, 17 (1968).
2. C. Prausnitz and H Küstner, *Zentr. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. 1, Orig.* **86**, 160 (1921); R. A. Cooke, J. H. Barnard, S. Hebald, A. Stull, *J. Exp. Med.* **62**, 733 (1935); A. G. Osler, L. M. Lichtenstein, D. A. Levy, *Adv. Immunol.* **8**, 183 (1968).
3. L. Wide, H. Bennich, S. G. O. Johansson, *Lancet* **1967-II**, 1105 (1967); J. Yunginger and G. J. Gleich, *J. Clin. Invest.* **52**, 1268 (1973).
4. G. J. Gleich and R. T. Jones, *J. Allergy Clin. Immunol.* **55**, 346 (1975).
5. J. W. Yunginger and G. J. Gleich, *ibid.* **51**, 174 (1973); G. J. Gleich and J. W. Yunginger, in *The Biological role of the Immunoglobulin E System*, K. Ishizaka and D. H. Dayton, Jr., Eds. (Government Printing Office, Washington, D.C., 1974), p. 221.
6. These serums were selected from among those analyzed routinely for IgE antibodies by the RAST in the clinical laboratory and contained IgE antibodies to either ragweed or grass allergens. The reactivity of the serums from the grass-sensitive patients in the RAST ranged from 6 to 25 percent and for the serums from the ragweed-sensitive subjects from 5.4 to 41.1 percent. These values are comparable to those among unselected patients with allergic rhinitis due to grass or ragweed pollen. Finally, these patients had positive skin tests to a variety of allergens suggesting that multiple sensitivities existed.
7. The immunoabsorbents were prepared by activation of Sepharose 2B (Pharmacia) with cyanogen bromide [R. Axen, J. Porath, S. Ernback, *Nature (Lond.)* **214**, 1302 (1967); J. W. Yunginger and G. J. Gleich, *J. Allergy Clin. Immunol.* **50**, 109 (1972)], and reaction with human serum albumin (HSA) (Fraction V; Sigma), partially purified ragweed extract [G. J. Gleich, J. B. Larson, R. T. Jones, H. Baer, *J. Allergy Clin. Immunol.* **53**, 158 (1974)] or rye grass extract. Three 30-ml samples of activated Sepharose 2B were reacted with 300 mg of HSA, 300 mg of rye grass extract, or 340 mg of ragweed extract at 4°C with tumbling for 24 hours. After thorough washing, 47 percent of the HSA was coupled (235 μg per milligram of Sepharose 2B), 52 percent of the rye grass extract was coupled (240 μg per milligram of Sepharose 2B), and 68 percent of the ragweed was coupled (387 μg per milligram of Sepharose 2B). The immunoabsorbents (6 mg) were added to serum samples, and 0.1*M* phosphate buffer ($K_2H_2PO_4$ and KH_2PO_4) (*p*H 7.4) containing 1 percent bovine serum albumin and 0.1 percent sodium azide was added to a volume of 2.0 ml. After rotation at room temperature for 3 days, the tubes were centrifuged, and the supernatants were analyzed for IgE protein by radioimmunoassay and for IgE antibody activity by RAST. In every case, all IgE antibody activity was removed by the immunoabsorbent containing the allergen to which the patient was sensitive.
8. L. L. Henderson, J. B. Larson, G. J. Gleich, *J. Allergy Clin. Immunol.* **55**, 10 (1975). We used serums obtained in late July as the preseasonal samples; the postseasonal serums were those showing maximal rises in IgE antibody as determined by RAST.
9. R. R. Schellenberg and N. F. Atkinson, *J. Allergy Clin. Immunol.* **55**, 83 (1975).
10. C. R. Zeiss, J. J. Pruzansky, R. Patterson, M. Roberts, *J. Immunol.* **110**, 414 (1973).
11. K. Ishizaka, T. Ishizaka, M. M. Hornbrook, *ibid.* **98**, 490 (1967).
12. A. Tiselius and E. A. Kabat, *J. Exp. Med.* **69**, 119 (1939); K. Eichmann, D. G. Braun, T. Feizi, R. M. Krause, *ibid.* **131**, 1169 (1970).
13. G. J. Gleich and R. R. Stankievic, *Immunochemistry* **6**, 85 (1969); T. L. Roszman, J. D. Folds, A. B. Stavitsky, *Immunology* **20**, 1041 (1971).
14. E. A. Kabat and D. Berg, *J. Immunol.* **70**, 514 (1953).
15. K. K. Bandilla, F. C. McDuffie, G. J. Gleich, *Clin. Exp. Immunol.* **5**, 627 (1969).
16. T. Tada and K. Ishizaka, *J. Immunol.* **104**, 377 (1970).
17. L. M. Lichtenstein, K. Ishizaka, P. S. Norman, A. K. Sobotka, B. M. Hill, *J. Clin. Invest.* **52**, 472 (1973); J. W. Yunginger and G. J. Gleich, *Ped. Clin. North Am.* **22**, 3 (1975).
18. G. J. Gleich, A. K. Averbeck, H. A. Swedlund. *J. Lab. Clin. Med.* **77**, 690 (1971).
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Infectious Etiology of Neuritic (Senile) Plaques in Mice

Abstract. *Brains of inbred female VM mice infected with scrapie agent were studied with the use of the Bodian silver impregnation method and by electron microscopy. In brains affected with scrapie, after an incubation period of between 587 and 655 days, numerous primitive, classical, and amyloid plaques were found. No plaques of any type were seen in the control mice.*

Neuritic (senile) plaques are the most conspicuous pathological changes found in people with Alzheimer's disease and senile dementia. They are also commonly found in middle-aged patients with Down's syndrome and, in smaller numbers, in a high percentage of normal old people (*1*) and have also been found in aged dogs and monkeys (*2, 3*). Morphological studies of neuritic (senile) plaques have revealed that they consist of degenerative neuronal processes, reactive cells, and amyloid, but the cause of their formation remains unknown. Here, on the basis of silver impregnation and electron microscopic studies, we report that neuritic plaques of both the classical and primitive types occur in mice in association with infectious disease.

The recent studies of Bruce and Fraser (*4, 5*) demonstrated plaques containing amyloid in the brains of mice that are infected with some strains of scrapie agent. Scrapie is a naturally occurring disease of sheep and goats that is caused by a replicating agent that can be transmitted with infected tissues to a variety of species. Morphologically, scrapie is often accompanied by vacuolar degeneration that may include a severe spongy change. Dickinson and Fraser (*6*) showed that there are different strains of scrapie agent that are distinguishable on the basis of incubation period and topography of the brain lesions in inbred mice. In the studies of Bruce and Fraser (*5*), with certain combinations of agent and mouse strains, plaques were seen in more than 70 percent of the brains of mice that were killed with the clinical disease, but not in a large number of control mice that were more than 600 days old. In Masson's trichrome preparations, the appearance of these plaques differed in certain details from neuritic (senile) plaques, although, ultrastructurally, all of the characteristic features of neuritic plaques were recognized. We report a further study of representative samples of severely affected mouse brains and control brains of comparable and greater age (some more than 1000 days) in which we used Bodian's silver impregnation technique and electron microscopy.

Four mice affected with scrapie that were known to have large numbers of amyloid plaques were examined. These were inbred female VM mice 610 to 705 days old. Two were infected with 87A scrapie agent, one with 125A, and the fourth with 51C. These agents were originally isolated from cases of natural scrapie in sheep; 87A was from a Border Leicester–Cheviot cross, 125A was from a Southdown, and 51C was

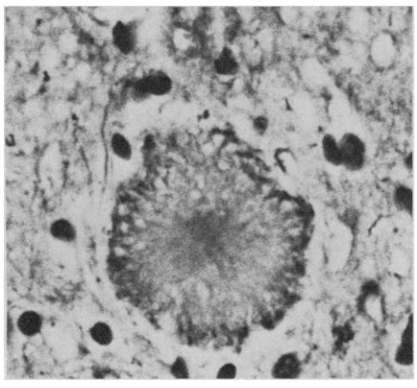

Fig. 1. Classical plaque in gray matter of VM mouse that was infected with scrapie (Bodian, × 550).

from a Suffolk. The 51C was at the second mouse passage, 87A was at the third, and 125A was at the fourth. The preparation of inoculums and the injection procedures were those used by Bruce and Fraser (*5*). The mice were killed at a defined clinical end point after incubation periods of between 587 and 655 days.

Nine control mice, unaffected with scrapie, were examined. These were all more than 600 days old, and three were more than 1000 days old. They were either uninjected or long-term survivors from scrapie-titration experiments. Their brains were examined by careful serial sectioning, and stained by the Bodian silver impregnation method.

Brains were fixed immediately by immersion in 10 percent formol saline, processed, and embedded in paraffin wax. Paraffin sections were stained by the Bodian silver impregnation method for neural processes. In addition, blocks to be used for electron microscopy were taken from the brain of the mouse infected with 125A before formalin fixation. These were fixed in 1 percent glutaraldehyde in phosphate buffer, postfixed in osmium tetroxide, and embedded in Araldite. Thick sections (1 and 5 μm) were cut and stained with toluidine blue or Congo red (*7*) and were examined by regular, phase, and polarized light microscopy. Selected areas were recut for grids, stained with lead citrate and uranyl acetate, and examined in a Phillips 300 electron microscope.

In Bodian preparations of the brains of the mice affected with scrapie, numerous plaques could be seen that clearly showed the typical profiles of degenerating processes that are the essential feature of neuritic (senile) plaques. Three types could be identified: (i) classical plaques with a central core of lightly argyrophilic material [previously identified as amyloid by the Congo red method (*5*)], surrounded by reactive cells and heavily impregnated rods and dots (Fig. 1); (ii) primitive plaques consisting of argyrophilic rods and dots without the central amyloid core (Fig. 2); and (iii) plaques made up almost exclusively of amyloid. No plaques of any type were seen in the control mice. Araldite sections that were stained with toluidine blue and Congo red also revealed three types of neuritic plaques. Primitive plaques, because they had few positively stained neuritic profiles, were difficult to identify in 1-μm sections stained with toluidine blue but were readily seen in 5-μm sections.

Electron microscopic studies confirmed the observation of Bruce and Fraser (*5*) that the plaques in scrapie consist of amyloid, degenerating neuronal processes, and reactive cells (Fig. 3). The amyloid fibrils were 9 to 11 nm in diameter and appeared to be both intra- and extracellular. Most of the degenerating neuronal processes were small and had a watery appearance with clusters of low electron opaque material, altered mitochondria, and laminated opaque bodies. Some processes contained randomly arranged microtubules and neurofilaments. Large neurites with accumulations of degenerating mitochondria, opaque bodies, and fibrillar material were occasionally seen. Particles of the "tubulofilament" type described by Narang (*8*) were commonly observed in cell processes at the periphery of the neuritic plaques. These plaques in mice are similar to the classical and primitive plaques seen in man (*9*) in the arrangement of the amyloid and neurites. In all areas studied, plaques were seen that were associated with blood vessels; vascular amyloidosis was common.

Our studies establish that some of the plaques produced in mice with certain strains of scrapie agent are of the classical

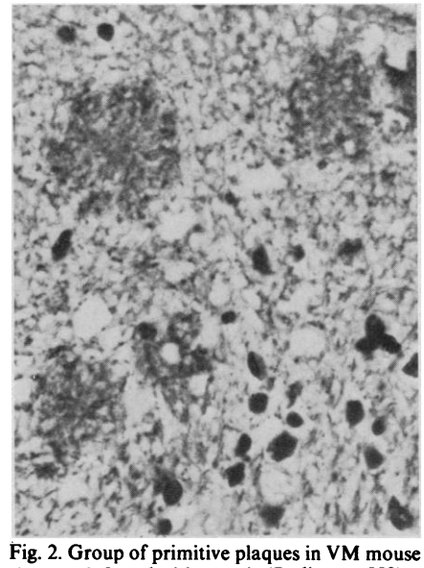

Fig. 2. Group of primitive plaques in VM mouse that was infected with scrapie (Bodian, × 550).

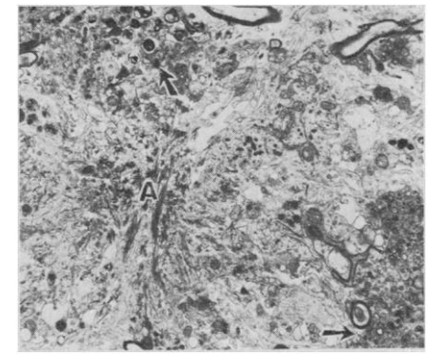

Fig. 3. Electron micrograph of part of neuritic (senile) plaque that shows wisps of amyloid (*A*) surrounded by degenerating neurites (arrows) (× 10,000).

and primitive type of neuritic (senile) plaque. To the best of our knowledge, this is the first recognition of an association between neuritic plaques and an infectious agent. Neuritic plaques are characterized by the presence of degenerating neuronal processes and amyloid and reactive cells, irrespective of pathological condition and species, although some variation has been identified in the proportion of each component in different biological situations (*2, 3, 10*). By light microscopy the plaques in scrapie are more compact and more easily identified with routine histological methods than are neuritic plaques in other conditions, and are generally richer in the amyloid component. However, the ultrastructural characteristics of the amyloid cores, altered neuronal processes, and reactive cells in these plaques are indistinguishable from those of neuritic plaques in humans and other animals (*5*). We believe that the variation in the appearance of plaques in different biological situations, as observed with a light microscope, is only a reflection of detailed differences in the amounts or distributions of their components.

On the basis of morphology observed by light microscopy, the plaques in scrapie have been subdivided on a discretionary basis into several merging categories (*5*), from shadowy plaques coinciding with the primitive type described here with silver staining, through amorphous and stellate plaques which may loosely correspond to the classical type, to plaques that consist largely of amyloid.

The primary mechanism that leads to the formation of neuritic plaques and the significance of cerebral amyloid deposition remain unclear. Some investigators (*11*) think that the amyloid itself is a direct toxic cause of neuritic degeneration and senile plaque formation, while others (*3, 10*) point out that amyloid does not act as a neurotoxic substance. The latter group

suggests that whatever causes amyloid formation may also cause terminal degeneration. It is possible that amyloid precursors leak from the bloodstream into the neuropil and cause both neurite degeneration and local amyloid formation. Most proposed mechanisms of amyloidosis involve the immune system in the production of precursor proteins, and recently Glenner *et al.* (*12*) have suggested that amyloid may be formed by phagocytes from the lysosomal degradation of antigen-antibody complexes. Even though the involvement of antigen-antibody complexes forms an attractive hypothesis for understanding the pathogenesis of plaque formation, we are not entitled to assume that this applies in scrapie since no specific humoral antibody responses to scrapie agents have been identified. Direct evidence on the chemical composition of the amyloid is therefore essential to understanding its origin. However, the absence of conventional immunological responses in scrapie is founded on work with only two agents, ME7 and Chandler (*13*), and neither produces a high frequency of plaques. Work is therefore needed on conventional immune responses with high plaque-producing agents.

This model offers possibilities for fundamental studies of the pathogenesis of senile plaques. Our findings should also encourage others to search for possible infectious agents in patients with presenile and senile dementia.

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References and Notes

1. B. E. Tomlinson, G. Blessed, M. Roth, *J. Neurol. Sci*, 7, 331 (1968).
2. H. M. Wisniewski, A. B. Johnson, C. S. Raine, W. J. Kay, R. D. Terry, *Lab. Invest.* **23**, 287 (1970).
3. H. M. Wisniewski, B. Ghetti, R. D. Terry, *J. Neuropathol. Exp. Neurol.* **32**, 566 (1973).
4. H. Fraser and M. E. Bruce, *Lancet* **1973-I**, 617 (1973).
5. M. E. Bruce and H. Fraser, *Neuropathol. Appl. Neurobiol.* **1**, 189 (1975).
6. H. Fraser and A. G. Dickinson, *J. Comp. Pathol.* **83**, 29 (1973); A. G. Dickinson, *Genetics*, in press.
7. T. Shirahama and A. S. Cohen, *J. Histochem. Cytochem.* **14**, 725 (1966).
8. H. K. Narang, *Neurobiology* **4**, 349 (1974).
9. R. D. Terry and H. M. Wisniewski, in *Ciba Foundation Symposium on Alzheimer's Disease and Related Conditions*, G. E. W. Wolstenholme and M. O'Connor, Eds. (Churchill, London, 1970), pp. 145–168.
10. H. M. Wisniewski and R. D. Terry, *Prog. Brain Res.* **40**, 167 (1973).
11. P. Schwartz, *Amyloidosis: Cause and Manifestation of Senile Deterioration* (Thomas, Springfield, Ill., 1970).
12. G. G. Glenner, D. Ein, R. D. Terry, *Am. J. Med.* **52**, 141 (1972).
13. A. C. Gardiner, *Res. Vet. Sci.* **7**, 190 (1965); D. D. Porter *et al.*, *J. Immunol.* **111**, 1407 (1973).
14. We thank H. K. Narang and A. Oakley for their assistance.

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Adenosine 3′,5′-Monophosphate in Reproducing and Differentiated Trypanosomes

Abstract. Trypanosoma lewisi, *a blood protozoan of rats, undergoes differentiation from a rapidly reproducing form to a nonreproducing form in response to the host antibody ablastin. Intracellular concentrations of adenosine 3′,5′-monophosphate (cyclic AMP), which has been implicated in controlling reproduction in cultured mammalian cells, was measured in the two developmental forms of* T. lewisi. *The concentrations were significantly different, and the results support a hypothesis under which ablastin stimulates an increase in intracellular cyclic AMP.*

Trypanosomes are protozoan parasites of the bloodstream and tissues of a large number of vertebrates including man. The pathogenic trypanosomes of the *brucei* group, responsible for nagana in cattle or African sleeping sickness in man, and the nonpathogenic rat parasite *Trypanosoma lewisi* have been widely studied in laboratory rodents. During residence in mammalian hosts and insect vectors, they undergo the equivalent of differentiation. The transformation of *T. lewisi* in the rat progresses from a rapidly reproducing population to a nonreproducing population. However, in pleomorphic strains of *T. brucei* subspecies, differentiation in the mammalian host is incomplete, and a pool of reproducing forms is always retained. These reproducing forms vary antigenically and cause relapsing infections and severe disease in the host. Although the antigenic variants arise in response to humoral antibody (*1*), the factors that induce differentiation of part of the population to nonreproducing forms are not known, but apparently they are not a result of the host immune system (*2*). The transformation in *T. lewisi*, in contrast, is induced by an antibody called ablastin. This antibody inhibits reproduction of the parasites without otherwise damaging them (*3*). The above-mentioned transformation of *T. lewisi* is complete in the presence of ablastin, but is reversed on its removal (*4, 5*). Therefore, the host's immune system first controls this infection by inhibiting parasite reproduction and eventually terminates it by mounting a trypanocidal response. In the *brucei* complex and in *T. lewisi* the reproducing and nonreproducing forms have distinguishable morphological, biochemical, and physiological features (*6*).

Because of its benign nature and the relative simplicity of its blood phase, *T. lewisi* has been studied as a model of controlled trypanosomiasis. Ablastin halts reproduction in *T. lewisi*, and morphological changes in the parasites appear (*3*). The reproducing forms are a heterogeneous population, varying in length and width (Fig. 1A). The nonreproducing (Fig. 1B) or adult forms are uniformly long and slender (*3, 7*). The coefficient of variation (CV) is a statistical measure that can be used to distinguish the populations; a reproducing population has a CV of about 25 percent while an adult population has a CV of about 3 to 5 percent (*7*). In adults, the morphological alterations are accompanied by decreased glucose and increased oxygen consumption with more oxygen consumption per mole of glucose (*7, 8*). Protein synthesis (*9*) and nucleic acid synthesis (*9, 10*) are virtually halted in adult forms. In addi-

Table 1. Cyclic AMP content of reproducing and adult forms of *Trypanosoma lewisi*. After separation from blood cells and platelets, trypanosomes were placed in a mixture of serum and Hanks balanced salt solution with glucose (50 mg/100 ml). The organisms were removed from the serum by centrifugation at 12,000*g* for 5 minutes. The supernatant was discarded, and cells were resuspended in 5 ml of HBSS. The cells were again centrifuged at 12,000*g*, and the supernatant was removed. A portion of this final supernatant washing was assayed for exogenous cyclic AMP. The cells were homogenized by high-speed vortexing in 5 percent trichloroacetic acid (0°C) (4×10^9 cell/ml). The insoluble material was removed by centrifugation at 27,000*g* for 10 minutes, and the supernatant was extracted with three volumes of water-saturated diethyl ether. The aqueous layer was removed and diluted with three volumes of distilled water; the *p*H of the water extract was adjusted to 7.1 to 7.4. The extract was then stored at −20°C until assayed. The data represent 95 percent confidence intervals around the pooled results for two extracts, each assayed in sextuplicate. The values for exogenous cyclic AMP represent the largest obtained in a given set of extracts and therefore are maximum values. Generally two-thirds of these values were negative. The limit of error of the assay was 0.2 pmole. The coefficient of variation (CV) was calculated by the formula $(\hat{s}/\bar{x}) \times 100$, where $\bar{x}$ is the mean length of a population and $\hat{s}$ is the standard deviation (*23*).

Cyclic AMP	Developmental form	
	Reproducing CV = 30.7%	Nonreproducing CV = 8.4%
Intracellular (pmole/10^9 cell)	13.2 ± 2.47	25.9 ± 1.91
Intracellular (μM)	0.167 ± 0.031	0.328 ± 0.024
Last washing supernatant (μM)	0.0024	0.0034