Table 3. Half-life determinations of ^{176}Lu.

Half-life (10^{10} yr)	Method	References
4	β counting, gas counter	(*12*)
7.3 ± 2	β counting with absorbent, gas counter	(*13*)
2.4	β counting, gas counter	(*14*)
2.15 ± 0.1	γ counting, NaI crystal	(*15*)
2.1 ± 0.2	γ counting, NaI crystal	(*16*)
2.8	Proportional β counting, gas counter	(*16*)
2.17 ± 0.35	$^{176}Lu/^{176}Hf$ determination on a dated mineral	(*1*)
3.6 ± 0.1	$\gamma\gamma$ coincidence, NaI crystal	(*9*)
3.2	Proportional β counting with absorbent, gas counter	(*9*)
2.18 ± 0.06	β counting, liquid scintillator	(*17*)
3.5 ± 0.14	γ counting, NaI crystal	(*10*)
3.68 ± 0.06	$\gamma\gamma$ coincidence, NaI crystal	(*10*)
3.56 ± 0.05	β-γ coincidence	(*10*)
5.0 ± 0.3	γ-γ coincidence, NaI crystal	(*11*)
3.3 ± 0.5	$^{176}Lu/^{176}Hf$ determination on two dated minerals	This report

of error; they indicate an age of crystallization of 900 ± 20 million years, in good agreement with ages (880 to 930 million years) of other pegmatitic minerals in the same region (*7*).

The three Pb/U ages on the priorite are also concordant, around 1080 ± 50 million years (*8*).

The values for the half-life of ^{176}Lu deduced from the two minerals agree with each other within their limits of error; thus it may reasonably be admitted that both minerals have behaved as closed systems for Lu and Hf since their crystallization. The weighted average value is $3.3 \pm 0.5 \times 10^{10}$ years. The weight of each determination is inversely proportional to its precision.

The various determinations of the half-life of ^{176}Lu given in Table 3 range from 2 to 7×10^{10} years. The physical determinations of MacNair (*9*) and of Brinkman *et al.* (*10*) are in agreement and seem to be the most reliable. Brinkman *et al.* extracted the radioactive impurities from the Lu and found concordant values close to 3.6×10^{10} years by three counting methods.

Our determination agrees with this value within the limits of error. It definitely differs, however, from a recent value of 5.0×10^{10} years (*11*).

The geological determination of Herr *et al.* ($2.17 \pm 0.35 \times 10^{10}$ years) (*1*) is significantly lower. A possible explanation is that their sample did not behave as a closed system either for Lu and Hf or for U and Pb. Losses of lead may be inferred from the admitted age of 810 million years, which is lower than the age of pegmatitic minerals from the same region, and from the discordancy of the apparent Pb/U ages.

Additional Lu-Hf determinations on other dated minerals are necessary to obtain a more precise value for the half-life of ^{176}Lu and to obtain information about the geochemical behavior of these two elements. The analytical and the isotope dilution techniques already developed permit the application of the $^{176}Lu/^{176}Hf$ dating method to 10 g of minerals that are 1000 million years old and that contain 100 parts per million of Lu.

ANDRÉ BOUDIN
SARAH DEUTSCH
Service de Géologie et Géochimie Nucléaires, Université Libre de Bruxelles, Brussels, Belgium

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Experimental Allergic Encephalomyelitis: Synthesis of Disease-Inducing Site of the Basic Protein

Abstract. *A highly encephalitogenic peptide whose structure resembles the sequence of amino acids surrounding the single tryptophan residue in the encephalitogenic A1 protein from bovine myelin was synthesized. This peptide is similar in the sequence to peptic peptide E and tryptic T27, derived directly from the A1 protein, and is as active on a molar basis as the A1 protein. The major disease-inducing site of the A1 protein resides in a linear sequence of nine amino acids: H-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-OH. This region of the A1 protein is apparently the major encephalitogenic determinant since specific modification of the tryptophan residue in the A1 protein with 2-hydroxy-5-nitrobenzyl bromide destroyed its encephalitogenic activity.*

The factor in the central nervous system responsible for experimental allergic encephalomyelitis (EAE) is a basic protein (A1 protein) present in myelin where it constitutes at least 30 percent of the total protein (*1*, *2*). At doses of 0.1 μg or greater, the A1 protein induces EAE (*1*) in guinea pigs. The pathogenesis of EAE appears to be associated with an immune response involving sensitized lymphocytes (*3*), presumably by a delayed-type hypersensitive mechanism. Thus, EAE provides a useful model for the study of autoimmune disease and may have relevance to some human demyelinating diseases such as multiple sclerosis.

The Al protein is a basic protein

and its molecular weight is 18,000 (*4*). In solution it has an open conformation, as revealed by its high intrinsic viscosity (*4*), and rapid cleavage by proteolytic enzymes (*5*) and resistance to denaturation. Biological activity remains after the protein is heated to 100°C for 1 hour or treated with 8*M* urea (*4*). Optical rotatory dispersion measurements indicate that there is neither an α helix nor a β structure in A1 protein (*6, 7*). The complete amino acid sequence of the bovine A1 protein has been determined (*8*).

The peptide E (Table 1) used in our study was derived (*9, 10*) from a pepsin digest of the bovine A1 protein. The structure of peptide E shown in Table 1 differs from that reported (*9*) by the deletion of a glycine and a serine residue, with a total of 14 residues. The correct structure for peptide E was determined from chymotryptic and tryptic peptides. After chymotrypsin digestion of the tetradecapeptide, a tripeptide Ser-Arg-Phe (*11*) and a dipeptide Ser-Trp were isolated by high-voltage electrophoresis; the Phe-Ser-Trp sequence is established by this and previous data (*9*). From the COOH-terminal end, a small quantity (20 percent) of the peptide Pro-Gly-Phe was obtained after digestion with trypsin. The COOH-terminal phenylalanine was determined by hydrazinolysis of the intact peptide. Thus, for the first nine residues the amino acid sequence of peptide E is identical with that of peptide T27 obtained from the tryptic digest (*8*). The sequence of all peptides was determined by the direct Edman degradation procedure (*8*).

The HNB-A1 protein was prepared by treating the A1 protein with 2-hydroxy-5-nitrobenzyl bromide (*12*) as described (*13*). By spectrophotometric analysis of the HNB-A1 protein it was established that one tryptophan residue had reacted per 18,000 daltons (*6, 13*), a value close to that determined by sedimentation-equilibrium for the molecular weight of the A1 protein.

The synthetic peptides used in our study are shown in Table 1; all synthetic peptides were made by the Merrifield solid-phase procedure (*14*) with the use of (*11*) *t*-BOC-CBZ-Lys, *t*-BOC-*O*-Bz-Glu, *t*-BOC-*O*-Bz-Ser, *t*-BOC-NO_2-Arg, *t*-BOC-Gly, *t*-BOC-Ala, *t*-BOC-Trp, *t*-BOC-Phe, and *t*-BOC-PNP-Gln, and chloromethylated copolystyrene cross-linked with 2 percent divinylbenzene. The *t*-BOC protecting groups were removed with a solution of trifluoroacetic acid, methylene chloride, and mercaptoethanol (50 : 45 : 5). The peptides were neutralized with 12 percent triethylamine in chloroform. The peptide bonds, except when glutamine was added, were formed with *N,N*-dicyclohexylcarbodiimide in methylene chloride; bonds with *t*-BOC-nitro-L-Arg and *t*-BOC-Trp were formed in dimethylformamide and methylene chloride (1:2). Glutamine was coupled by use of the active ester *t*-BOC-Gln-PNP in dimethylformamide and acetic acid (100:1). The protecting groups were removed from the peptides, and the peptides were removed from the resin by treating 1 g of resin-peptide complex with 5 ml of HF and 0.5 ml of anisole at 0°C for 0.5 hour (*15*).

The synthetic material was then purified by gel filtration in 0.5*N* acetic acid on a layered column of Sephadex G-10 and G-25 (4 by 100 cm). In each case the elution pattern, determined by the optical density at 280 nm, contained a single main peak which eluted prior to,

Table 1. Sequences of synthetic and derived peptides.

Peptide	Sequence
	Synthesis
S1	Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys
S2	Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln
S3	Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln
S4	Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly
S5	Ser-Arg-Phe-Ser-Trp-Gly-Ala
S6	Ser-Arg-Phe-Ser-Trp
S7	Phe-Ser-Trp-Gly-Ala
S8	Ser-Arg-Phe-Gly-Ser-Trp
S9	Ser-Arg-Phe-Gly-Ser-Trp-Gly-Ala
S10	Ser-Arg-Phe-Gly-Ser-Trp-Gly-Ala-Glu-Gly-Gln
	Peptic digest
E	Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-Pro-Gly-Phe
	Tryptic digest
T27	Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys

Table 2. Biological activities of peptides and modified A1 proteins.

Material*	Dose (μg)	No. of animals showing EAE/No. of animals tested†		Skin test‡
		Clinical	Clinical + histologic	
Peptide S1	0.33	1/4	2/4	Negative
	3.3	2/4	3/4	
	5.0	3/4		
	33.0	2/4	3/4	
	50.0	2/4		
Peptide S2	3.3	0/4	0/4	Negative
	33	0/4	0/4	
Peptide S3	3.3	0/4	0/4	Negative
	33	0/4	0/4	
Peptide T27	0.33	1/4	4/4	
	3.3	4/8	7/8	Negative
	10	4/4	4/4	
Peptide E	0.33	3/4	3/4	Negative
	3.3	2/4	4/4	
	10	3/4	3/4	
A1 protein (bovine)	3.3	0/4	2/4	Positive (19 mm)
	50	4/5	5/5	
HNB-A1 protein	3.3	0/5	0/5	Positive (17 mm)
	20	0/5	0/5	

* Other peptides which were tested at 5 and 50 μg include peptides S9, S4, S5, S6, S7, S8, and S10. None of these peptides was encephalitogenic in guinea pigs, as judged by clinical and histologic criteria, and none gave a positive skin test. † In general, the appearance of clinical signs of EAE occurred between 12 and 16 days after injection. Occasionally, signs were observed earlier or later than this period. The peptides showed the same time sequence of the A1 protein. Guinea pigs were sacrificed at 20 days for histologic examination of brain and spinal cord. The criteria for histologic lesions have been described (*1*). ‡ The skin test was carried out 8 to 9 days after injection of 33 μg of A1 protein in the usual way to induce EAE. Samples were tested from 0.1 to 50 μg in 0.05 ml of saline. Maximum response occurred after 24 hours. Positive reactions consisted of an area of erythema greater than 8 mm. The average diameters found with 20 μg of A1 protein and HNB-A1 protein were 19 and 17 mm, respectively.

and separated from, various lesser quantities of smaller peptides. High-voltage electrophoresis at *p*H 4.6 showed that this main peak was homogeneous; the trailing material often contained two to four peptides that migrated differently from the major peptide. Based on the relative areas of the major and minor peptide fractions, the yield varied from 60 to 90 percent. Approximately 200 to 400 mg of purified peptide was usually obtained. The amino acid analyses of the hydrolyzates of the synthetic peptides, determined with a Beckman amino acid analyzer (*9*), were consistent with the formulations given in Table 1; the residues were found in integral molar ratios within ± 5 percent.

The assays (*1*) for encephalitogenic activity of the peptides (Table 2) show that only peptide S1 among the synthetic peptides is encephalitogenic. Similar clinical signs were evoked by the synthetic peptide S1, peptides E and T27, and the A1 protein. The histological changes (Fig. 1) for peptide S1 included increased vascular permeability, cellular infiltration, and perivascular cuffing. The predominant cell type was mononuclear, consisting of lymphocytes and histiocytes; in several lesions a few plasma cells were identified. The lesions were present in the meninges, choroid plexus, and around small blood vessels in the brain and spinal cord. These lesions appeared similar to those induced by the A1 protein and whole spinal cord as described (*1*).

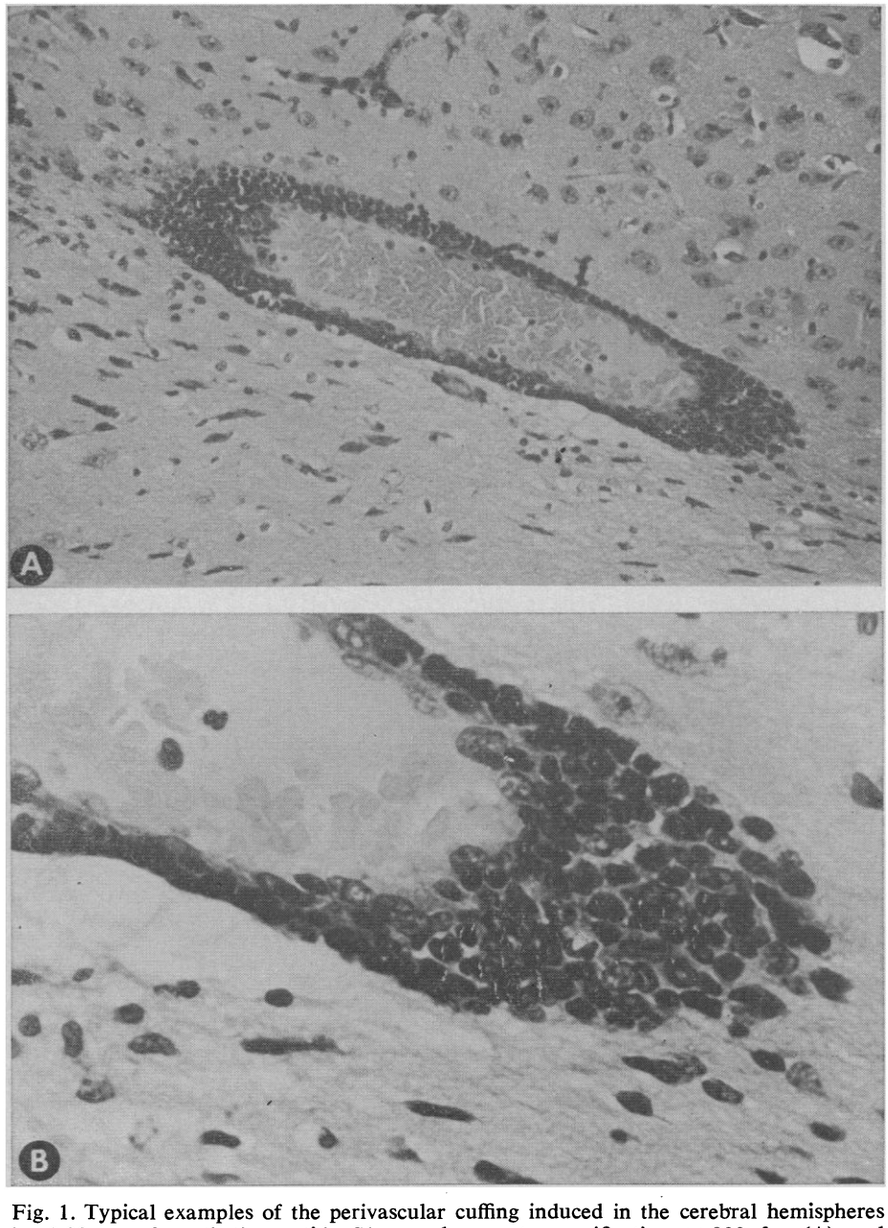

Fig. 1. Typical examples of the perivascular cuffing induced in the cerebral hemispheres by 0.33 μg of synthetic peptide S1 are shown at magnification × 200 for (A) and × 650 for (B). The sections were stained with hematoxylin and eosin.

Peptide S1, with 11 residues, is identical to the peptic peptide E through the lysine residue, and therefore additional residues (the Pro-Gly-Phe sequence) at the COOH-terminal end of peptide E are not required for encephalitogenic activity. Although the EAE assay is only partially quantitative, it appears that peptide S1 and peptide E have similar activities; both produced clinical signs and histologic lesions at 0.33 μg per animal, the lowest dose tested. In the one group of guinea pigs shown in Table 2, peptide E was highly effective clinically at 0.33 μg; the animals exhibited classical hind-leg paralysis, tremors, weight loss, and death (*1*). In the corresponding group tested with 0.33 μg of peptide S1, one animal was paralyzed; the others showed hind-leg weakness, but not a definite paralysis. All of the other synthetic peptides, such as peptide S2, gave no signs of EAE, either clinically or histologically. Peptide S2 differs from S1 only by the absence of COOH-terminal lysine, thus setting the length limit of the encephalitogenic region at the COOH-terminal end.

Thus peptide T27 is highly encephalitogenic, having activity of the same order as synthetic peptides S1 and peptic peptide E. It contains only nine residues and is identical with the other two peptides in the region from the phenylalanine to the lysine residues. Thus, the encepahlitogenic region can be reduced to a sequence of nine residues, the NH_2-terminal phenylalanine to COOH-terminal lysine. The isolation of an encephalitogenic peptide from a tryptic digest of the A1 protein is significant in that it is composed of the same sequence of amino acids as the active peptic polypeptides, namely, that surrounding the single tryptophan residue. Previously, however, when we examined the tryptic peptide fractions derived from the A1 protein, we found (*5*) a very limited activity; only 20 percent of the animals showed clinical signs. When compared with that of the peptic peptide fractions, the activity of the tryptic peptides appeared borderline (*16*). Apparently, when tested as part of the tryptic peptide fractions, other peptides interfere and mitigate its encephalitogenic activity. These data are consistent with that of Carnegie *et al.*

(*17*) who found that a tryptic peptide mixture derived from human basic protein was encephalitogenic.

Our results reveal that the encephalitogenic activity of the bovine A1 protein is primarily due to a short, linear sequence of amino acids surrounding the single tryptophan residue. The requirements for encephalitogenic activity are precisely determined within a framework of nine amino acids or less which are represented by the common sequence found within peptic peptides E and E1, tryptic peptide T27, and synthetic peptide S1, the only synthetic peptide which was active. This sequence is:

Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys

The question arises concerning the number of regions in the A1 molecule which can independently induce EAE. Is the nine-residue tryptophan region the only encephalitogenic determinant and the remaining 95 percent of the molecule superfluous? It appears that the region defined by the amino acid sequence around the tryptophan residue is the major encephalitogenic determinant because (i) specific chemical modification of the tryptophan residue with 2-hydroxy-5-nitrobenzyl bromide greatly reduces the encephalitogenic activity of the A1 protein (Table 2) and (ii) the only encephalitogenic peptides derived from the peptic and tryptic digests of the A1 protein come from the tryptophan region. Kibler *et al.* (*18*) have reported that a peptide of 45 residues, derived from bovine spinal cord, is encephalitogenic in rabbits at doses of 50 μg per animal. We have now derived (*8*) the identical peptide from a peptic digest of the A1 protein; it occupies a portion of the polypeptide chain near the NH_2-terminal region and does not overlap the tryptophan region. Therefore two independent encephalitogenic sites may exist in the A1 protein, and considerable species variability may exist in response to these regions. It is not clear why the bovine peptide of Kibler *et al.* (*18*) is not encephalitogenic in guinea pigs (*19*), whereas the bovine A1 protein is highly encephalitogenic.

The delayed skin test (Table 2) with the encephalitogenic peptides in guinea pigs sensitized with the A1 protein was negative in each case. However, the HNB-A1 protein, which is nonencephalitogenic, nonetheless gives a delayed-type skin reaction equivalent to that of the A1 protein (*1*). Thus, the skin test, which has been correlated with induction of EAE (*1, 3*), can be differentiated from the disease process; this suggests that more than one site of the A1 protein molecule may induce the delayed-skin response.

E. H. EYLAR
JUANITA CACCAM
J. J. JACKSON
Salk Institute,
San Diego, California 92112
FRED C. WESTALL
Department of Chemistry,
University of California, San Diego
ARTHUR B. ROBINSON
Department of Biology,
University of California, San Diego

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Photochemical Oxidants: Effect on Starch Hydrolysis in Leaves

Abstract. *Starch-filled leaves of plants which have been subjected to low dosages of naturally occurring photochemical oxidants, ozone, or peroxyacetyl nitrate hydrolyze their starch more slowly when placed in the dark. Delayed hydrolysis occurs irrespective of whether the oxidants were applied during the light or dark period. Occasionally this effect is evident only in the intervenal areas.*

In conjunction with research on tobacco mosaic virus (TMV) at Arcadia, California, P. C. Cheo found that starch in normal appearing, inoculated cucumber cotyledons failed to disappear as it had from those similarly treated in studies conducted at Wenatchee, Washington. The formation of starch lesions is a critical determining feature in the TMV assay (*1*). In this assay the virus concentration is proportional to the number of leaf spots which fail to translocate starch due to interference by the infecting virus. Failure of starch hydrolysis in the uninfected leaf portions made the assay useless. Because we suspected that air pollution (photochemical oxidants) was causing this problem, we installed activated carbon filters in the greenhouse. Starch disappearance, as expected, then occurred after the cotyledons were held in the dark. We conducted further studies into the effect of photochemical air pollutants on starch hydrolysis in leaves.

We observed that starch retention occurs over the entire leaf blade when plants are exposed to low dosages of naturally occurring airborne oxidants such as ozone or peroxyacetyl nitrate (PAN). Starch normally accumulates in plant leaves during the daylight hours when photosynthesis exceeds the rate of translocation of products of photosynthesis from the leaves. The following night this starch undergoes hydrolysis and is exported to areas of growth and storage. Photochemical oxidants somehow block or retard certain steps of the starch hydrolysis–translocation process.

Seeds of cucumber *Cucumis sativa*, bean *Phaseolus vulgaris* cv. "Pinto," *Cassia occidentalis*, and *Mimulus cardinalis* were grown in greenhouses equipped with activated carbon air filters to remove atmospheric oxidants. The 3-week-old seedlings were then exposed for varying lengths of time to