nucleus to the level of the median eminence (Fig. 1). Of the 83 mice studied with the light microscope, this pathologic condition was found in all but three infants and two adult mice. With electron microscopy, it was possible to identify the small necrotic areas as degenerating microglial cells (*6*). At 3 hours after the MSG injection, there was a significant increase over control conditions in the number of lysosomes, myelin figures, and dense bodies within the cytoplasm of microglial cells (Fig. 2). This occurred in all mice studied within this time period. Also the lesion was very specific to these glial cells and apparently did not involve either the perikarya of neurons or the neuropil within the arcuate region. Further, the cytoplasm of astrocytes and oligodendrocytes was not affected. The possibility does exist that various changes may have been taking place within cells other than microglia, but were not made apparent at this time period (3 hours) either with light or electron microscopic study. At 24 hours after the injection there was a breakdown of the nuclear membrane with a marked increase in debris within the cytoplasm of microglial cells. Mitochondria appear markedly swollen, often with broken membranes, and the nuclei are completely pyknotic (Fig. 3). At this time after injection, myelin figures are occasionally seen in distal portions of few axons and dendrites within this area, but in general the entire neuropil appears normal. Also, there is a large increase in glycogen in normal-appearing glial processes in the lesioned area. No further spread of degeneration was seen at 72 hours after injection.

Regardless of the method of injection or the concentration of MSG given, the location of all lesions and the extent of damage was similar. Approximately 95 percent of all injected animals developed these lesions. Within the time period studied, the perikarya of all neurons within the arcuate nucleus and median eminence appeared normal. No evidence of any intracellular edema was seen. We have considered the possibility that the degenerating microglial cells were not the direct target sites for MSG or glutamic acid but were responding to other damage in the nervous system. The affected microglial cells may be invaders to the arcuate region of the hypothalamus and could be acting as phagocytes rather than being directly involved in the lesion. However, there appears to be no significant difference between the number of microglial cells in our control and MSG-treated animals (*7*). L-Glutamic acid decarboxylase has been found both in human glial cells grown in culture and in gliomas (*8*). Whether this finding can be construed as suggesting an affinity of glial cells for MSG remains to be determined. Olney (*2, 3*) and Olney and Sharpe (*4*) have shown that large, markedly swollen dendrites also were present in the area, but this may have been due to poor fixation rather than to experimental treatment. Often in electron microscopic study we have demonstrated this effect in poorly fixed samples from control animals. It seems that among other areas, dendrites and synaptic endings within the arcuate nucleus show more marked reaction to poor fixation than other regions of the nervous system. However, since MSG-treated mice showed more swollen dendritic processes within the area studied than did control mice, the possibility exists that poor fixation may have resulted from an occlusion of the blood vessels in the lesioned site due to MSG treatment. Reduction in blood flow to the ventromedial region of the hypothalamus has been demonstrated after the administration of gold thioglucose (*9*).

The doses injected in this study are large, in milligrams per gram, as compared to doses consumed by humans, and do not indicate what pathology results when comparable doses are administered by mouth either singly or in a series, or when lower amounts of MSG are consumed with food (*10*).

EDWARD A. AREES
JEAN MAYER
Department of Nutrition, Harvard University, School of Public Health, Boston, Massachusetts 02115

References and Notes

1. D. R. Lucas and J. P. Newhouse, *Arch. Opthalmol.* **58**, 193 (1957).
2. J. W. Olney, *J. Neuropathol. Exp. Neurol.* **28**, 455 (1969).
3. ———, *Science* **164**, 719 (1969).
4. ——— and L. G. Sharpe, *ibid.* **166**, 386 (1969).
5. M. M. Powers and G. Clark, *Stain Technol.* **30**, 83 (1955).
6. A. Peters, S. L. Palay, H. de F. Webster, *The Fine Structure of the Nervous System* (Harper & Row, New York, 1970), pp. 129–131.
7. J. Brawer and S. Palay, personal communication.
8. B. Haber, K. Kuriyama, E. Roberts, *Science* **168**, 598 (1970).
9. E. A. Arees and J. Mayer, *Exp. Neurol.* **25**, 410 (1969).
10. Since this paper was submitted, additional data have been obtained in our laboratory which show that adult albino rats injected with 5 mg of MSG per gram of body weight developed lesions in the arcuate nucleus of the hypothalamus similar to those described in this study. This finding is contrary to that of N. J. Adams and A. Ratner [*Science* **169**, 673 (1970)].
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Antibody to Nuclear Material Eluted from Isolated Spleen Vessels in Systemic Lupus Erythematosus

Abstract. *Splenic blood vessels were isolated from surrounding cells by treatment with trypsin and the use of ultrasound. This procedure retained vascular-bound immunoglobulins which were recovered by acid elution of vessels isolated from a patient with systemic lupus erythematosus. These eluted immunoglobulins reacted with material from cell nuclei as demonstrated by immunofluorescence.*

Inflammation of blood vessels and deposits of γ-globulin and complement in vessel walls are common pathologic features of systemic lupus (*1*). Other investigators have eluted antinuclear antibodies from isolated renal glomeruli with acid, or deoxyribonuclease, or both (*2*), but results of eluting larger blood vessels have not been reported. Comparative studies of the amounts of vascular-bound γ-globulins in various organs of lupus patients have shown that protein deposition is often most pronounced in vessels of spleen (*3*). We now report a method of isolating blood vessels from spleen with the preservation of vascular-bound proteins and the successful elution of antibodies to nuclear material from splenic vessels isolated from a systemic lupus patient.

Whole spleen tissue was obtained at autopsy from patient K.R., a 17-year-old female who died of lupus nephritis,

Table 1. Immunoglobulin composition of deposits in K.R. splenic vessels and antinuclear antibodies in serum and eluate of K.R. spleen vessels; H ab, antibody showing homogeneous pattern of staining; Sp ab, antibody giving speckled type of nuclear reactivity. Protein bound to vessels is graded 1 to 4+ on the basis of intensity of fluorescence.

Class	Protein bound to vessels	Antibodies (reciprocal of titers)		
		Eluate H ab	Serum H ab	Serum Sp ab
γG	4+	64	128	256
γM	3+	16	64	64
γA	2+	2	32	Neg.

and from a 20-year-old female control patient whose death was traumatic. Frozen sections (4 μm) of these spleens were incubated with fluorescein-labeled whole antiserum to human γ-globulin. Heavy deposits of immunoglobulins were found in the K.R. blood vessels, while no protein was detected in the vessels of the control spleen. Routine histologic examination of the K.R. spleen tissue revealed pronounced fibrinoid alteration in the media of blood vessels and marked periarterial and periarteriolar fibrosis.

To isolate the blood vessels from spleen, most of the organ was decapsulated, minced with scissors, homogenized at low speed for 2 minutes in a Waring Blendor in the presence of 5 ml of 0.01 percent trypsin contained in 0.15*M* NaCl, 0.01*M* phosphate, *p*H 7.2, per gram of spleen tissue, and then incubated for 30 minutes in a 37°C water bath. The tissue was separated with a 16 by 40 mesh strainer; nonsievable material was transferred with 50 ml of phosphate saline buffer, *p*H 7.2, into a beaker, and sonicated for 30 seconds at room temperature (*4*). The material settled by gravity, the supernatant was decanted, and 50 ml of fresh saline buffer was added. Sonication in the presence of fresh saline was repeated at least 15 times until the supernatant was clear and the tissue was white. The tissue was washed three more times with centrifugation at 2000*g* at 4°C. When samples of the final preparation were frozen and sectioned, histochemical stains revealed a network of vessels, trabeculae, and reticular fibers (*5*). Endothelium, media, and adventitia of arteries were intact. No lymphoid elements or plasma cells were identified. Immunofluorescent examination of companion sections showed the persistence of immunoglobulins in the vessel walls of patient K.R.

We eluted the preparation of vessels with saline, adjusted to *p*H 3, on a shaker at room temperature for 22 hours. The acid eluates and final washes before elution were each dialyzed overnight at 4°C against phosphate buffered saline, *p*H 7.2, and were concentrated 300 times by ultrafiltration (*6*). The concentrated K.R. acid eluate contained 3.7 mg of protein nitrogen per milliliter.

The K.R. eluate demonstrated homogeneous reactivity with nuclei when sections of normal human spleen were sequentially treated with the eluate and then fluorescein-labeled antiserum to human γ-globulin (Fig. 1). In similar tests, the control acid eluate and the final washes of both K.R. and control vessels prior to elution showed no reactivity with any tissue constituents. Complete abolition of nuclear reactivity of the K.R. eluate was effected by absorption with an equal volume of deoxyribonucleoprotein (*7*). Absorption with 2 mg of DNA per milliliter of eluate resulted in partial diminution in reactivity (*8*). The eluate showed no speckled pattern of staining either before or after nucleoprotein absorption.

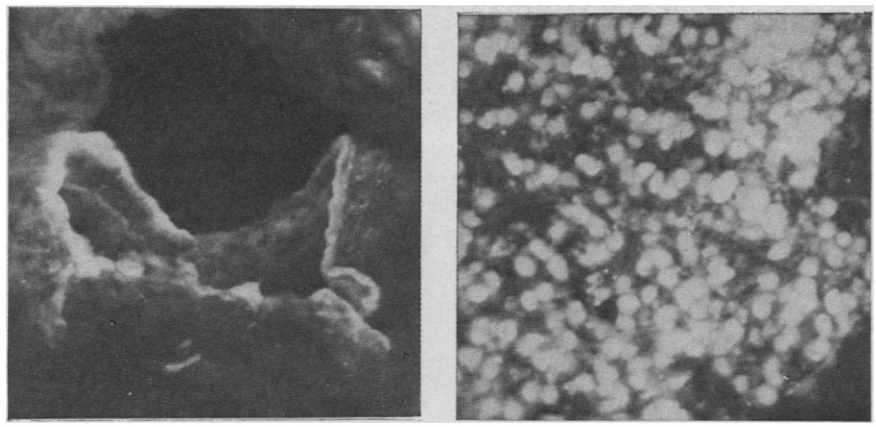

Fig. 1. Immunofluorescence showing homogeneous reaction of acid eluate of isolated lupus spleen vessels containing γ-globulin (left) with cell nuclei of normal spleen (right) (× 250).

The K.R. serum showed both homogeneous and speckled patterns of nuclear reactivity. The latter antibody was not absorbed by nucleoprotein and presumably represented antibody to buffer extract antigen (*9*). Failure to detect this antibody in the eluate was probably not related to the elution procedure because the antibody giving the speckled type of reactivity in serum withstood the same prolonged exposure to acid.

Three immunoglobulins were detected in the homogeneous antinuclear factors in serum and eluate. To determine the immunoglobulin composition of antinuclear factors, we incubated sections of normal spleen with serum or eluate and counterstained with specific antiserums to the immunoglobulins γG, γM, and γA labeled with fluorescein (*10*). The same three immunoglobulins were identified in deposits of K.R. spleen vessels by incubating sections of untreated K.R. spleen sections with the same fluorescent reagents (Table 1). These findings are consistent with the derivation of the vascular-bound deposits in spleen from circulating antibodies to nuclear material. Only γG and γM were detected in the serum antibody, giving speckled nuclear reactivity.

The identification of antibodies to nuclear material in eluates of glomeruli from patients with lupus has been cited in support of a pathogenetic role for immune complexes in lupus renal disease. The isolation of spleen vessels suitable for the elution and characterization of vascular-bound proteins has permitted extension of studies to an extrarenal organ commonly involved by vasculitis in systemic lupus.

KATHRYN H. SVEC*
SUSAN T. ALLEN
Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

References and Notes

1. J. J. Vasquez and F. J. Dixon, *Lab. Invest.* **6**, 205 (1957); P. J. Lachmann, H. J. Muller-Eberhard, H. G. Kunkel, F. Paronetto, *J. Exp. Med.* **115**, 63 (1962).
2. P. Freedman and A. S. Markowitz, *Brit. Med. J.* **1**, 1175 (1962); C. Krishnan and M. H. Kaplan, *J. Clin. Invest.* **46**, 569 (1967); D. Koffler, P. H. Schur, H. G. Kunkel, *J. Exp. Med.* **126**, 607 (1967).
3. K. H. Svec, J. D. Blair, M. H. Kaplan, *J. Clin. Invest.* **46**, 558 (1967).
4. MSE 20-kilocycle disintegrator equipped with a 1-cm end diameter titanum probe (end ratio 10:1).
5. Mallory-azan, Wright's and Weigert's elastica stains.
6. Amicon cell model 401 (100 psi) fitted with a UM-10 membrane.
7. Isolated from calf thymus at low ionic strength. E. Chargaff, in *The Nucleic Acids*, E. Chargaff and J. N. Davidson, Eds. (Academic Press, New York, 1955), vol. 1, p. 313. Final protein nitrogen was 1.75 mg/ml.
8. Worthington Biochemical Corp., Freehold, N.J.
9. J. S. Beck, *Lancet* **1961-I**, 1203 (1961), P. J. Lachmann and H. G. Kunkel, *ibid.* **1961-II**, 436 (1961); E. M. Tan and H. G. Kunkel, *J. Immunol.* **96**, 464 (1966).
10. Hyland Laboratories, Los Angeles, Calif.
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