months ago intracerebrally with 0.2 ml of human brain from a patient who had kuru (Sepe), and the other, S-4, was inoculated 16 months ago intracerebrally with 0.2 ml of a 10 percent suspension of brain from chimpanzee A-16 (*2*), affected with experimental kuru. No illness has yet been observed in either of these animals. When the first two animals (S-1 and S-2) developed unmistakable signs of kuru, human brain tissue from six other patients with kuru, and brain tissue from three kuru-affected chimpanzees (including chimpanzee A-1) were inoculated into 12 additional spider monkeys.

The common term spider monkey refers to animals of some six species in two families of New World monkeys. The two animals used were apparently of the species *Ateles geoffreyi*, of which there are over a dozen varieties. Spider monkeys of other species and varieties are also being used in order to determine the host range of susceptibility within the group of New World monkeys. In addition, over a dozen other species of monkeys (including four New World) and one chimpanzee have been inoculated with the brain of S-1.

Since the evolution of disease after primary inoculation required more than 2 years, there may be considerable delay before additional cases of kuru in the spider monkey can be reported, unless, as in the chimpanzee, there is a significant shortening of incubation period on secondary passage. Spider monkeys are considerably less expensive and easier to obtain in large numbers than are chimpanzees, which have been the only known susceptible host for study of kuru. Their use should considerably facilitate characterization of the virus and study of the pathogenesis of the disease.

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Amyloidosis Induced in Mice by Escherichia coli Endotoxin

Abstract. *Amyloidosis was produced in mice by repeated subcutaneous injections of 0.5- or 0.005-milligram amounts of* Escherichia coli *endotoxin. Of the two strains of mice examined, amyloidosis was induced more readily in one than in the other. The ability of endotoxin to induce amyloidosis lends support to the view that stimulation of reticuloendothelial cells leads to amyloid formation.*

Amyloid is a homogeneous, eosinophilic, fibrillar glycoprotein with characteristic morphological and tinctorial properties (*1*). It is deposited in the tissues of man and animals under a variety of clinical and laboratory conditions and has been induced experimentally with multiple agents and methods varying from the administration of live or killed bacteria to repeated injections of proteins, such as casein (*1*). Superficially, these procedures have little in common and make most difficult any unifying hypothesis regarding pathogenesis. This preliminary report describes the successful induction of amyloidosis in two strains of mice by repeated administration of *Escherichia coli* endotoxin.

Escherichia coli (O127:B8) endotoxin was obtained from Difco Laboratories (*2*). The endotoxin was dissolved in phosphate-buffered saline at a concentration of 2.0 mg/ml and frozen at −20°C until it was used. Six-week-old male mice, C_3H/Hen and White Swiss (G.P.), obtained from the animal production section, National Institutes of Health, were used for the experiments.

Table 1. Splenic amyloid in C_3H/Hen and G.P. mice after administration of *Escherichia coli* endotoxin.

Endotoxin	Strain	No of mice	Daily dose (mg)	No. of injections	No. of mice with splenic amyloid*
Lot 1	C_3H/Hen	10	0.005	15	0/10
	C_3H/Hen	6	.005	30	0/6
	C_3H/Hen	10	.5	15	0/10
	C_3H/Hen	17	.5	30	2/17
	G.P.	11	.005	25	4/11
	G.P.	10	.5	25	10/10
Lot 2	G.P.	6	.5	15	6/6
	G.P.	16	.5	20	16/16
Phosphate-buffered saline	G.P.	10	.25 ml	25	0/10

* Results are given as No. mice with amyloid/No. injected.

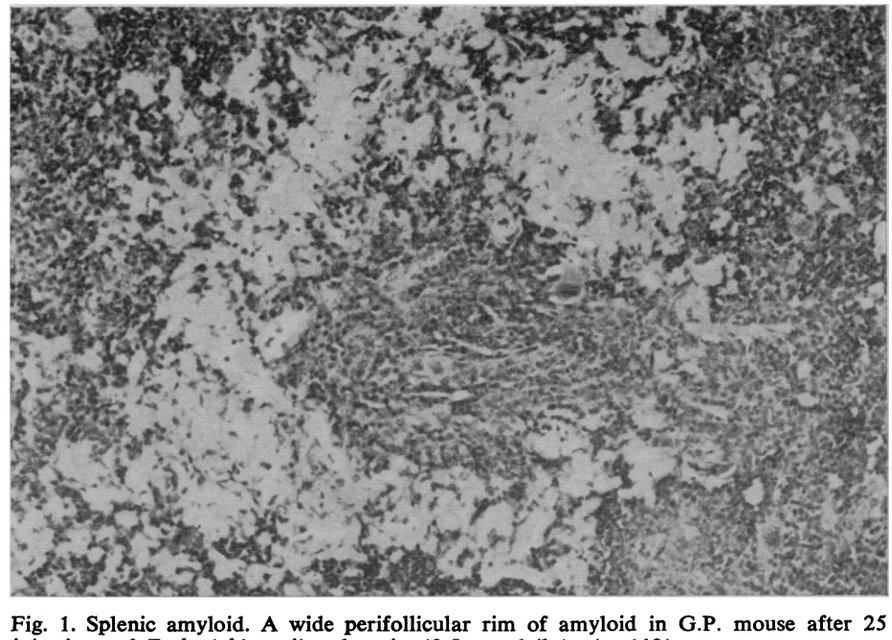

Fig. 1. Splenic amyloid. A wide perifollicular rim of amyloid in G.P. mouse after 25 injections of *Escherichia coli* endotoxin (0.5 mg daily). (× 112)

In the initial experiment, C_3H/Hen mice were injected subcutaneously with *E. coli* endotoxin in doses of 0.5 and 0.005 mg daily, five times per week. Animals were killed after 15 and 30 injections, and histologic sections of the spleen, liver, and kidney were examined. Amyloid was present in the spleen in 2 of 17 mice examined after 30 injections (Table 1).

White Swiss mice (G.P.) were selected for the next experiment, because they develop amyloidosis more readily and in greater amounts after casein than the C_3H mice do (*3*). These G.P. mice were similarly treated with daily doses (0.25 ml) containing 0.5 or 0.005 mg of lot 1 endotoxin administered subcutaneously five times a week. Control mice were injected with phosphate-buffered saline (0.25 ml) on an identical schedule.

Every G.P. mouse examined after 25 injections of the high dose of endotoxin had moderate to extensive deposits of amyloid in the spleen (Fig. 1). Four of 11 mice receiving the lower dose also had splenic amyloid. In three of these mice, deposits were focal and limited, but in the fourth there was perifollicular deposition around almost every follicle. The amyloid in all cases had the characteristic green birefringence after polarization microscopy of sections stained with Congo red. None of the animals receiving buffered saline developed amyloidosis.

Using lot 2 *E. coli* endotoxin, we found splenic amyloid in every one of the 22 G.P. mice examined after 15 or 20 injections (Table 1). In the mice with extensive splenic amyloid, hepatic and renal amyloid was also detected.

Although relatively small numbers of animals have been used, the findings reported here are with two separate lots of endotoxin and in two strains of mice. In studies to be reported (*4*), we have seen splenic amyloid in G.P. mice as early as 1 week (after five injections) and in most animals within 2 weeks of starting administration of endotoxin.

Many of the features of amyloidosis induced by endotoxin are similar to those seen following repeated casein administration (*3*); histologic involvement of the spleen, liver, and kidney are comparable. The higher incidence of amyloid in the White Swiss (G.P.) mouse after injection of endotoxin as compared to the C_3H/Hen mouse parallels the different susceptibility of the two strains to amyloid induced by administration of casein (*3*). In preliminary studies we found that repeated injections of endotoxin reproduce the abnormality in ribosome function (*5*) found after casein administration.

Endotoxin is a lipopolysaccharide derived from the cell walls of Gram-negative, as well as of some other, bacteria (*6*). This material is potent at very low doses and has many biological effects, including metabolic, circulatory, and immunological (*6*). The ability of endotoxin to induce amyloidosis can now be added to this list. Because endotoxin is a ubiquitous substance, inactivated only after autoclaving at a high temperature for several hours and because as little as 100 μg was able to induce amyloid, it is possible that endotoxin may also be involved in other types of experimental amyloidosis. Furthermore, endotoxin may play a role in human secondary amyloidosis, particularly those forms associated with chronic infection.

Endotoxin is rapidly localized in the reticuloendothelial system after parenteral administration, as has been demonstrated with labeled endotoxin (*7*) and with fluorescent antibody studies (*8*). Cohen's concept of the pathogenesis of amyloid suggests that chronic stimulation of the reticuloendothelial cell leads to amyloid production (*1*). The ability of endotoxin to induce amyloid lends support to this hypothesis.

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Lactate Dehydrogenase Isozymes: Dissociation and Denaturation by Dilution

Abstract. *The tetrameric enzyme lactate dehydrogenase dissociates into its constituent monomeric subunits in a high ion to protein concentration ratio, in certain ionic environments when frozen, and at extreme dilution. Dissociation by dilution involves changes in tertiary conformation which inactivate the enzyme. The dissociation is strongly inhibited by homologous native proteins, but only slightly by denatured or unrelated proteins.*

The enzyme lactate dehydrogenase (LDH) exists in many organisms in isozymic (multiple molecular) forms (*1, 2*). It is a tetramer with a molecular weight of approximately 140,000 and a sedimentation coefficient, at infinite dilution, of approximately 7.50*S* (*3*). This enzyme can be induced in vitro to undergo subunit reassociation, that is, molecular hybridization (*4*). Two different subunits, A and B, associate to yield five tetramers, conveniently designated LDH-1 (B_4), LDH-2 (A_1B_3), LDH-3 (A_2B_2), LDH-4 (A_3B_1), and LDH-5 (A_4). Dissociation and recombination of these subunits can be accomplished via reversible denaturation of the LDH tetramer at low *p*H, or by the use of urea, guanidine-hydrochloride, or LiCl at supraeutectic temperatures (*5, 6*), or by freezing and thawing isozymes in the presence of specific hybridization promoting ions (*4, 5, 7, 8, 9*). In addition, prolonged dialysis of certain LDH isozyme combinations against saturated NaCl solutions produces molecular hybridization (*2, 10*). These results suggest that a high ratio of ion to protein is a fundamental requirement for reversible subunit dissociation.

It has also been reported that, at protein concentrations less than 0.5 mg/ml in NaCl solutions, the enzyme dissociates into a dimer with a sedimentation coefficient of 5.5*S* and a molecular weight of about 72,000 (*11*). These results suggest that the enzyme