deposition of a similar quantity of triglyceride in the liver within a period of 2 hours.

It has been observed that adrenergic blocking agents, cord section, adrenalectomy, and other factors reduce the magnitude of the fatty liver following poisoning with CCl_4 *in vivo* (19, 20). It is quite possible that these factors only reduce the mobilization of free fatty acid from adipose tissue to liver without necessarily altering in any way the initial injury to the liver by the chlorinated hydrocarbon.

Summary. Livers, isolated surgically from normal animals and from rats intoxicated with CCl_4, were perfused *in vitro* with a medium into which palmitic acid was infused continuously. Livers from normal rats were also treated with CCl_4 *in vitro* by direct addition of the chlorinated hydrocarbon to the medium. Under the conditions of these experiments, poisoning with CCl_4 resulted in inhibition of net release of triglyceride by the liver into the perfusate and simultaneous accumulation of triglyceride in the liver. These observations support the hypothesis that the fatty liver of CCl_4 intoxication results primarily from interference with the biochemical mechanisms involved in formation and release of the triglyceride in the very low density lipoprotein of the serum.

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Amyloid. I. Use of Freund's Adjuvant in Experimental Amyloidosis (32821)

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Amyloidosis, a disease of humans as well as animals, is characterized by the infiltration of various organs by an as yet poorly defined protein–carbohydrate material (1). A variety of methods involving the injection of foreign proteins and other substances have been used for inducing amyloid in experimental animals (2); of these the method of daily subcutaneous injection of a solution of casein for over 6 weeks has been most commonly used. These methods, however, are characterized by prolonged treatments and variable results. Rothbard and Watson (3) induced amyloidoisis in W-Swiss, H-line mice after ten weekly sub-

TABLE I. Development of Splenic Amyloidosis in C_3H Mice on Injection of Freund's Adjuvant.

Injected material	No. of amyloidotic animals/total no. in study		Degree of amyloidosis
	ip	sc	
Complete adjuvant	11/13	1/5	2+
Complete adjuvant + casein	15/17	0/5	4+
Incomplete adjuvant	1/14	0/9	—
Incomplete adjuvant + casein	3/8	0/10	2+

cutaneous or intramuscular injections of Freund's complete adjuvant. The presence of *M. butyricum* was shown to be indispensable, although, this could be substituted by *N. asteroides*, and to a lesser extent by Group A streptococci. While these findings were confirmed by Tal and Laufer (4) in the same strain of mice, this method did not meet with equal success in studies by Kennedy, (5) Fruhling *et al.* (6) and Christensen (7) all of whom used C_3H mice. Fruhling *et al.* (6) and Christensen (7) combined caseinate and Freund's adjuvant injections. In Christensen's studies, a mixture of 0.05 ml of Freund's adjuvant and 0.2 ml of 5% casein, was injected subcutaneously 7 to 10 days apart. Four to six such injections were necessary before splenic amyloidosis was noted. In more recent studies, however, Letterer and Kretschmer (8) failed to produce amyloidosis in noninbred NMRI mice with weekly subcutaneous injections of Freund's complete adjuvant alone. On the other hand 66% of animals treated with casein–adjuvant mixture developed amyloidosis. In view of these apparently divergent findings, the effect of injecting mice with Freund's adjuvant with and without added *M. butyricum* or other protein antigens was reinvestigated.

Materials and Methods. Freund's complete and incomplete adjuvants were the products of Difco Laboratories, Detroit, Michigan. The sources of protein antigens were as follows: Hammerstein casein (Nutritional Biochemicals Corporation, Cleveland, Ohio), lysozyme (Worthington Biochemical Corporation, Freehold, New Jersey), bovine serum albumin (Pentex Inc., Kankakee, Illinois). Inbred C_3H and BALB/c strains of mice (8 weeks old) (C_3H/HeN, BALB/c AnN) were obtained locally from the N.I.H. farm. For some experiments C_3H mice were obtained from Jackson Laboratories, Bar Harbor, Maine (C_3H/Jax). They were found to be indistinguishable from N.I.H. C_3H strain. Freund's incomplete and complete adjuvants with or without added protein antigens were prepared as follows: 1.5 ml of adjuvant + 1.5 ml of phosphate buffered saline + (where indicated) 25 mg of casein or other antigens, were emulsified in a Vir-Tis homogenizer. Each animal received two 0.25 ml injections of the emulsion 2 weeks apart intraperitoneally or subcutaneously. Two weeks after the second injection, the animals were sacrificed, tissues were fixed in formalin, embedded in paraffin and stained with hematoxylin and eosin, Congo red and periodic acid–Schiff reagents by conventional procedures and examined for amyloid.

Results and Discussion. Studies on the degree of amyloidosis produced in C_3H mice on injection of Freund's adjuvant in a variety of combinations are summarized in Table I. It is clear that two intraperitoneal injections of complete Freund's adjuvant alone produced amyloidosis in a high percentage of animals. Addition of casein increased the degree of amyloidosis as measured as per cent of tissue involved, from 25% (2+) to over 50% (4+). Incomplete adjuvant alone was ineffective; addition of casein to Freund's incomplete adjuvant resulted in amyloidosis in three out of eight animals. It is to be noted that the subcutaneous route was almost totally ineffective under the present conditions. This might explain why the previously recommended procedures required at least four to six injections, with variable results.

Not shown in Table I are some studies on the effect of other antigens and the results of studies made in BALB/c strain of mice. Bovine serum albumin, lysozyme, or dextran

were as effective as casein when injected intraperitoneally in complete Freund's adjuvant. The BALB/c strain of mice yielded qualitatively the same results as C_3H mice. A somewhat lower frequency of amyloidosis was observed in this strain, but the significance of this difference was in doubt since the number of animals in the study became rather small due to loss by death. Differences due to strain and sex, and probably diet in the susceptibility of mice to experimental amyloidosis were observed by other workers. Williams *et al.* (9) have found that inbred strains were more susceptible to amyloidosis than pure noninbred, or hybrid strains.

Comments. In the present studies, a high degree of amyloidosis was produced in mice with two intraperitoneal injections of an antigen such as casein incorporated in complete Freund's adjuvant. The dose of antigen used in these studies and the injection schedule is similar to that recommended by Munoz (10) for obtaining antibodies in mouse peritoneal fluids. In studies in progress in our laboratory on the role of immunological phenomena in the pathogenesis of amyloid, this has been our method of choice for the production of amyloidosis in mice. Other antigens such as serum albumin should prove equally satisfactory. The rapid and reproducible procedure described here for induction of experimental amyloidosis should pave the way for elucidation of the role of immune phenomena in the pathogenesis of amyloidosis. Our preliminary studies on the use of immunosuppressive agents in experimental amyloidosis seem to suggest a role for hypersensitivity reactions in the induction of amyloid, as indeed do the reports by other workers (11).

Summary. Amyloidosis was produced in C_3H mice with two, spaced intraperitoneal injections of any of several antigens incorporated in complete Freund's adjuvant. The same regimen but omitting the antigens resulted in amyloidosis of lower degree. The subcutaneous route in otherwise identical studies, proved ineffective. Freund's incomplete adjuvant alone, given intraperitoneally produced no amyloidosis. Addition of casein to the incomplete adjuvant produced amyloidosis in some animals. For rapid and reproducible production of amyloidosis in mice, a procedure involving two intraperitoneal injections, given 2 weeks apart, of 0.25 ml of an emulsion made up of 25 mg of casein, 1.5 ml of complete Freund's adjuvant and 1.5 ml of phosphate buffered saline, is suggested.

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