taining uric acid and oxonic acid, a uricase inhibitor. It was characterized by hyperuricemia, hyperuricosuria, deposition of uric acid in the kidney tubules, distention of tubular lumens, and early tubular and interstitial nephritis, and thus could serve as a useful experimental model for uric acid nephropathy.

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## Heterologous Transfer of Amyloid—Human to Mouse* (33594)

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Isologous (1–4) and homologous (4) transfer of amyloid in mice was recently reported. These studies indicated that casein-sensitized donor spleen cells whether living or dead can induce amyloidosis in recipient mice within 3–7 days following intravenous or intraperitoneal injection. In addition, serum from sensitized isogenic donor mice was also reported to accelerate induction of amyloidosis in the X-irradiated recipient model (4). The sum total of the above experiments has been (a) the introduction of the concept of a transfer factor in the spleen homogenate or serum of the donor which is responsible for accelerated induction of amyloidosis, and (b) greater interest in its isolation and characterization in an attempt to understand further the pathogenetic mechanism of this disease, a disorder of increasing clinical and investigative significance (5,6). We recently confirmed many of the above facts. From our experiments, however, it was apparent that use of immunosuppressive agents may be unnecessary in this transfer system whereas both of the recipient models reported to date were treated with immunosuppressive agents, viz., X-irradiation or nitrogen mustard (1–4). This new recipient model without use of immunosuppressive agents may be more useful for the study of the nature of amyloid transfer factor, because it removes variables that were already shown to enhance amyloidosis in the induction model (6–8). This communication reports the successful heterologous transfer—human to mouse—of amyloid, again using a recipient model untreated by immunosuppresive agents.

*Materials and Methods.* Three human spleens were obtained at autopsy from patients with primary, secondary, and myeloma-associated amyloidosis respectively, and were stored at —10°. One nonamyloidotic human spleen was used as a control. 1.0 g (wet wt.) of spleen was homogenized in 2.0 ml of cold physiological saline solution with a Potter–Elvehjem homogenizer. One-ml ali-

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TABLE I. Incidence and Gradation of Splenic Amyloidosis in the Recipients Based on Light and Polarization Miscroscopic Examination of Paraffin Sections Stained with Congo Red.

| Type of donor spleen amyloid | Casein injections[a] into (group no.) | Gradation and incidence of splenic amyloidosis in recipients: Days after transfer: 3 Incidence | 3 Severity | 5 Incidence | 5 Severity | 7 Incidence | 7 Severity |
|---|---|---|---|---|---|---|---|
| Primary | 1 | 1/3[b] | 0–1+[c] | 4/4 | 1+ | 4/4 | 1–2+ |
| | 2 | 0/3 | 0 | 0/3 | 0 | 0/3 | 0 |
| Secondary | 1 | 2/3 | 0–1+ | 4/4 | 1+ | 4/4 | 1–2+ |
| | 2 | 0/3 | 0 | 0/3 | 0 | 0/3 | 0 |
| Myeloma associated | 1 | 0/2 | 0 | 3/3 | 1+ | 3/3 | 1–2+ |
| | 2 | 0/3 | 0 | 0/2 | 0 | 0/4 | 0 |
| Normal control | 1 | 0/2 | 0 | 0/4 | 0 | 0/4 | 0 |
| | 2 | 0/2 | 0 | 0/2 | 0 | 0/2 | 0 |

[a] Group 1 mice received casein injections daily; and Group 2 mice received no casein.

[b] Denominator represents total number of mice used, and numerator represents the number of mice with splenic amyloid.

[c] 0 = negative; 1+ = 1-25% replacement of tissue by amyloid; and 2+ = 26–50% replacement of tissue by amyloid.

quots of the homogenate (about 300 mg amyloid or control) were administered intraperitoneally once to the recipients (inbred male $C_3H$ mice).

The recipient mice, 7–10 weeks old, were divided into two groups. Group 1: Each mouse was given 3, 5, or 7 daily injections of 0.5 ml of 10% casein solution in 0.05 *N* NaOH, starting on the same day the spleen homogenate was administered. Mice were sacrificed on the day following the last casein injection. Group 2: Each mouse was given the amyloid spleen homogenate but no casein, and was sacrificed in a same fashion as the casein treated group. After necropsy, paraffin sections of spleen, liver, and kidney from all animals and other organs from selected animals, were stained with Congo red and examined by light and polarization microscopy. Portions of the fresh tissues were also fixed in osmium tetroxide, embedded in Epon, and examined in a Siemens Elmiskop I electron microscope after thin sectioning.

*Results and Discussion.* The mortality of the mice that received the amyloidotic spleen homogenate was relatively low; a total of 5 were lost from 62 initial animals, whereas that of the recipients of nonamyloidotic spleen homogenate was higher; 10 of 26 initial mice.

The incidence and gradation of splenic amyloidosis in the recipients was based on light and polarization microscopy as shown in Table I. This table includes only animals sacrificed at specific time intervals but not those found dead in cages. In the group that received both amyloid spleen homogenate (of any of the 3 types of amyloid) and casein injections, splenic amyloid was found in 3 of 8 recipients on day 3 after transfer, in all on day 5, and more heavily in all on day 7. In addition, slight hepatic amyloid was found in most recipients (8 out of 11) on day 7, while renal amyloid was present in 2 of 11 mice. None of the animals that received the amyloid spleen homogenate alone without successive casein injections, and none of the animals that received the nonamyloid spleen homogenate with or without casein injections, showed amyloid deposits in those organs in the above time span (up to 7 days). Histologic features of the amyloidotic organs of the recipients (Fig. 1) were generally similar to those of the murine amyloidosis induced by casein injections alone (6). Electron microscopy of these organs demonstrated the de-

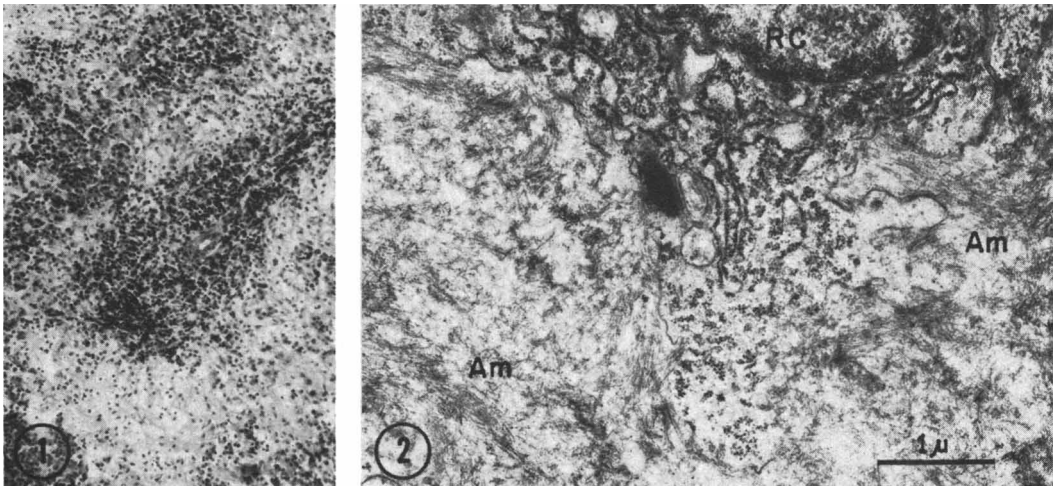


FIG. 1. Photomicrograph of the spleen obtained from a mouse on day 7 of human primary amyloid spleen homogenate and casein injections. Considerable amyloid is present in the marginal zone about the follicle and extends to the red pulp; Congo red and hematoxylin; × 80.

FIG. 2. Electron micrograph of the spleen from a mouse on day 7 of human myeloma associated amyloid spleen homogenate and casein injections. A fixed reticular cell (RC) with active cytoplasmic features (ribosomal clusters) shows a close relationship to amyloid fibrils (Am); fixed in 2% phosphate-buffered osmium tetroxide, embedded in Epon, stained with uranyl acetate and lead citrate; ×16,000.

position of amyloid fibrils (Fig. 2) whose ultrastructure and relation to cells was comparable to that seen with casein injections alone (6, 9, 10).

In our laboratory, regular daily injections of 0.5 ml of 10% casein solution in 0.05 *N* NaOH induces amyloid in some $C_3H$ mice after 12–20 injections and in all after 22 injections, but never as early as 3, 5, or 7 days. In the present system, as in the isologous and the homologous transfer of mouse amyloid, we have been able to shorten significently the duration for induction of amyloidosis to 3–5 days again without use of immunosuppressive agents. While the exact nature of the amyloid transfer or accelerating factor is not known, the present results demonstrate that the factor may be common to various types of human amyloid-laden tissues as well as amyloid tissues from different species.

From previous published data and the present results the following at least may be concluded. The transfer factor of amyloidosis (including the possibility that it is amyloid itself or its precursor) is not species specific. A single administration of the factor did not cause amyloidosis, at least under the conditions used in the present study. In addition to the factor from amyloidotic donor spleens, some other factor such as antigenic challenge with casein injections daily, may be necessary or at least helpful for accelerated induction and continuation of amyloidosis in the recipient. Success in human to mouse transfer of amyloid makes it probable that other laboratory animals in which amyloidosis has been successfully induced (rabbit, guinea pig, hamster) can now be used to elucidate further the nature of this transfer.

*Summary.* Intraperitoneal administration of human amyloid spleen homogenate together with casein caused amyloidosis in recipient $C_3H$ mice within 5 days. The results suggest the existence of a factor accelerating induction of amyloidosis that is active across histocompatibility barriers and provide a simpler model without immunosuppressive agents for further investigations of this transfer factor.

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# Effect of Cycloheximide on the Antiviral Action of Interferon (33595)

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Evidence was provided that interferon (IF) is not directly responsible for the antiviral state in IF-treated cells but rather it acts by inducing the cells to produce one or more substances, perhaps protein(s) which are able to prevent viral growth directly or indirectly. Specifically, IF activity can be prevented by pretreating the cells with actinomycin D or with inhibitors of functional protein synthesis, i.e., puromycin or fluorophenylalanin (FPA) (1–4), suggesting that the development of the antiviral state involves synthesis of messenger ribonucleic acid (mRNA) and proteins. Although this interpretation comes from indirect evidence it will be used as a working model to be critically examined in view of the present findings.

We have used cycloheximide as an inhibitor of protein synthesis to prevent IF activity in mouse embryo (ME) cells. Unexpectedly the cultures treated with IF in the presence of cycloheximide showed substantially the same level of resistance as did the control cultures treated with IF alone. In the present study an attempt was made to explain this apparently anomalous result.

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*Materials and Methods.* Cycloheximide (Sigma Chemical Co.) was used at final concentration of 10 μg/ml. Actinomycin D (Merk Sharp Dohme) was used at final concentration of 5 μg/ml unless otherwise specified. DL-*p*-fluorophenylalanine (Mann Research Lab.) was used at final concentration of 80 μg/ml when the drug was dissolved in phenylalanine (PA)–free medium or 600 μg/ml unless specified otherwise, when medium containing 50 μg/ml of PA was used. This dose of FPA has been previously shown to inhibit 70% of the incorporation of phenylalanine$^{14}$C in the system (5). The reversibility of these inhibitors was confirmed by the full yield of vesicular stomatitis virus (VSV), strain Indiana, from cultures treated with cycloheximide or FPA for 5 hr and then washed free of inhibitor before VSV challenge.

Mouse IF was prepared and assayed as previously described (5), 50 or 100 units/ml were used.

Primary ME tissue cultures were prepared from 15-day-old embryos (NIH strain) using Eagle's minimum essential medium (MEM) supplemented with 10% of fetal bovine serum and antibiotics. During the experiments the concentration of serum was 2%. The level of antiviral resistance was expressed as inhibition of yield of VSV after a