Amyloidosis has been studied for over a century, but its etiology remains unknown. Amyloid deposits can occur spontaneously in many different species of animals or can be induced by a wide range of materials such as repeated inoculation with casein, bacteria, egg albumen, turpentine, or endotoxin from Escherichia coli (1, 2, 5, 6, 8, 10). Experimentally induced amyloidosis in laboratory animals may gradually regress or disappear from one or more organs once the causal agent is removed (5, 6, 11, 13).

Recently, Pearsall and Lagunoff (9) reported that amyloidosis occurred in the majority of treated mice of one inbred strain at 2 weeks after a single injection of Candida albicans or Saccharomyces cerevisiae cells. A lower incidence of amyloidosis occurred in several other strains, usually later than 2 weeks after treatment. The results of the present study are in agreement with these findings (9); injections of C. albicans and C. parapsilosis cells into outbred mice induced amyloidosis. Moreover, our study showed that the disease process was irreversible. A highly significant number of animals died of systemic amyloidosis within 400 days after the last treatment.

(A preliminary report of this work was presented at the First Annual Collaborative Conference, Carcinogenesis Program of the National Cancer Institute, October 1972.)

MATERIALS AND METHODS

Animals. Eight-week-old female outbred CFW mice were obtained from Carworth Farms (Charles River, Inc.), New York, N.Y. The animals, weighing between 25 to 30 g, were housed eight animals per cage and fed and watered ad libitum.

Preparation of fungal cells. A suspension of C. albicans or C. parapsilosis was inoculated into 500-ml Erlenmeyer flasks containing 200 ml of medium. The medium consisted of 5% cerelose (crude glucose), 0.5% proteose peptone (Difco), 0.25% yeast extract (Difco), 0.1% KH2PO4, and 0.5% MgSO4.7H2O. The inoculated flasks were placed on a New Brunswick gyroshaker G25 operating at 60 rpm and incubated for 24 h at 37 C. The flasks were removed from the shaker and kept for 24 h at room temperature to allow the growth to sediment. The medium was decanted, 150 ml of fresh medium was added, and the flasks were again shaken for 24 h. Fresh medium was added a third time, and the cultures were grown for 24 h. The cells were collected by centrifugation. C. parapsilosis cells were killed by a mixture of acetone and methanol, washed with distilled water, and lyophilized. C. albicans cells were washed with distilled water and lyophilized. The harvested growth consisted of yeast cells only. Growth was obtained when lyophilized C. albicans cells were placed on Sabouraud glucose agar.

The lyophilized cells were homogenized in a mortar with a pestle and suspended in tricaprylin (triolein, 99.9% pure) that was obtained from Drew Chemical Co., Boonton, N.J. The injections (0.1 ml) were given subcutaneously into the inguinal area with sterile, disposable plastic syringes fitted with 25-gauge needles.

Each of 75 CFW mice was injected with a total of 90 mg of C. parapsilosis suspended in 1.0 ml of tricaprylin given in 10 injections of 0.1 ml during a period of 27 days. Similarly, 15 mice received 90 mg and 15 received 135 mg of C. albicans per 1.0 ml; one of the mice in the former group died during the period of treatment. Seventy-five mice were maintained as untreated controls; six animals died and were not included in the study. All mice in the experimental and control groups were weighed at the start of the experiments and then monthly throughout the period of observation. Mice were observed thrice weekly.

No infections were observed at the site of injection in any of the mice, including those treated with the viable C. albicans cells.

Histopathology. Moribund animals were killed and autopsied. All surviving mice were killed at 400 days and autopsied. The spleen, kidney, liver, and other abnormally appearing tissues such as lung were removed and processed for histological examination. Blood smears, prepared from a sampling of moribund
mice, were treated with Wright stain. Histological sections were stained with hematoxylin and eosin, Congo red, and periodic acid-Schiff stain with diastase. All diagnoses of amyloidosis were based on histological examination. Animals that were not autopsied were not included in the data.

RESULTS

Most animals with amyloidosis could be recognized prior to becoming moribund; the body was often turgid and the eyes had a pale glassy appearance. Frequently, animals were either edematous or ascitic or both. Blood smears from moribund animals showed an increase in the number of leukocytes. The spleens were abnormal, the liver mottled, and the cortical surface of the kidneys of many animals was pale, blotchy, and diffusely granular. Moderate to severe adhesions often occurred between various organs or between an organ and the parietal peritoneum.

Deposits of amyloid were recognized readily in the spleens (Fig. 1) and kidneys. Most animals had deposits in the liver (Fig. 2). In severely afflicted animals, amyloid was observed in the majority of internal organs including heart and lung. Often, most of the normal structure of the spleen and liver was replaced by amyloid (Fig. 1 and 2).

Amyloidosis was diagnosed as early as 27 days after the last treatment in animals who received a total dose of 135 mg of C. albicans cells and 75 days after treatment with 90 mg of either C. albicans or C. parapsilosis cells. The results suggest that the course of the disease may depend upon the dose of the fungal cells injected into the mice.

Amyloidosis was observed in 60 to 70% of the mice treated with fungal cells, whereas 4.5 to 5.5% of the untreated and tricaprylin control animals were afflicted with amyloidosis (Table 1). The incidence of amyloidosis may be higher than that shown in Table 1.

The survival rate of the animals in the groups injected with lyophilized fungal cells was lower than that of the tricaprylin or untreated control groups. Only 17% of the animals treated with C. parapsilosis cells were alive at 400 days after treatment, which was significantly less than in the tricaprylin or untreated control groups (Fig. 3). However, significantly fewer untreated control animals survived to 400 days after treatment than tricaprylin control animals ($\chi^2 = 18.3, P < 0.05$). The reason for a difference of this magnitude between the two control groups is not known.

Similar results were obtained in the experimental groups injected with two different doses
FIG. 2. Section of a spleen from a mouse injected with C. parapsilosis cells showing heavy deposits of amyloid. Hematoxylin and eosin.

TABLE 1. Amyloidosis induced in mice with lyophilized Candida cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice injected</th>
<th>No. of mice autopsied</th>
<th>Total dose (mg)a</th>
<th>No. of mice with amyloidosis</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. parapsilosis</td>
<td>75</td>
<td>46</td>
<td>90</td>
<td>28</td>
<td>60.9</td>
</tr>
<tr>
<td>C. albicans</td>
<td>15</td>
<td>11</td>
<td>90</td>
<td>7</td>
<td>63.6</td>
</tr>
<tr>
<td>C. albicans</td>
<td>15</td>
<td>11</td>
<td>135</td>
<td>8</td>
<td>72.7</td>
</tr>
<tr>
<td>Untreated control</td>
<td>75</td>
<td>55</td>
<td></td>
<td>3</td>
<td>5.5</td>
</tr>
<tr>
<td>Tricaprylin control</td>
<td>75</td>
<td>65</td>
<td></td>
<td>3</td>
<td>4.5</td>
</tr>
</tbody>
</table>

a Total dose resulting from 10 equal treatments each suspended in 0.1 ml of tricaprylin.

of C. albicans cells. One of 15 animals injected with 90 mg in 1.0 ml of tricaprylin was alive 400 days after treatment, whereas none of 15 animals injected with 135 mg in 1.0 ml of tricaprylin survived to 400 days after treatment.

DISCUSSION

Animals afflicted with amyloidosis became moribund from 27 days after the last inoculation to the termination of the experiment at 400 days. At 400 days, 8 of the 13 surviving mice treated with C. parapsilosis cells had deposits of amyloid. Such variability may be due partly to the mixed genetic background of the outbred CFW stock of mice. Moreover, a low percentage of aged mice of the CFW stock developed "spontaneous" amyloidosis. Six of 120 untreated and tricaprylin control animals developed amyloidosis (Table 1); however, these animals were still alive 400 days after the last treatment. The frequency of amyloidosis is known to differ among inbred mouse strains inoculated with fungal cells (9).

Pearsall and Lagunoff (9) observed that viable fungal cells were more effective in producing amyloidosis than killed cells. In the present study, systemic amyloidosis did not result from a mycotic infection. Fungal elements were not observed in histological sections of any autopsied animal. In a previous study, amyloidosis
was not observed in mice injected with extracts from several fungal species (3). These extracts did not include all the components of the cell wall. Cell wall components may be necessary for the induction of amyloidosis.

Experimental amyloidosis in laboratory animals may regress or disappear from one or more organs once the causal agent is removed (5, 6, 11, 13). The course of the systemic amyloidosis described in the present study seems to parallel that described in man (4, 12). Limited deposits of amyloid are found frequently in aged humans, but the prognosis for systemic amyloidosis is unfavorable (4, 12). Part of the problem in determining or evaluating treatment for systemic amyloidosis in humans is the lack of a comparable experimental animal test system. The techniques described in the present study may therefore represent a model system for further studies of systemic amyloidosis.

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**LITERATURE CITED**


