The Effect of Casein Diet on the Development of Amyloidosis in Hamsters (33663)

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The role of the diet in the pathogenesis of amyloidosis has been studied for many years. Kuczynski (1, 2) reported that casein diet as well as casein injections produced amyloidosis in mice. Jaffee (3, 4) fed casein injected mice a diet rich in fat and cholesterol. He found that a high fat diet had a delaying or protective effect on the development of casein injected amyloidosis in mice. Jaffee also found that a high beef protein diet protected against amyloid development. He proposed that the reason for an increased production of amyloid by a high casein diet was due to irritation of the intestinal mucosa. Grayzel *et al.* (5), found that powdered milk and bread produced amyloid in albino mice. Pirani *et al.* (6) reported that a chronic scorbutic diet led to the development of amyloidosis in guinea pigs. Experiments by Williams *et al.* (7) demonstrated that a low protein diet decreased the incidence of renal amyloid in CBA mice.

Leishmania donovani was found to produce amyloidosis in hamsters by Gellhorn *et al.* (8). This experimental model has been used by many investigators to study the pathogenesis of amyloidosis in hamsters. To our knowledge, there are no reports of the role of a casein diet in *Leishmania* induced amyloidosis in hamsters. The effect of low and normal casein diets on this experimental model was thus studied.

Materials and Methods. Sixty-two 4-month-old male Golden hamsters were inoculated intravenously with 0.1 ml of a calculated dose of *Leishmania donovani* from a donor animal's spleen utilizing the technique of Stauber (9). Each dose of Leishmania contained approximately five parasites. Thirty-five inoculated animals were randomly selected and placed on a low casein (8%) diet. The remaining 27 inoculated animals were placed on the normal casein (27%) diet. Fourteen control uninoculated hamsters were divided equally and started on both diets. All animals were weighed weekly.

The diets were obtained from Nutritional Biochemical Corporation, Cleveland, Ohio. The diets were exactly the same except for the normal casein diet which had 27% casein and low casein diet 8%.

Several animals on each diet were randomly selected and sacrificed on three different occasions—8, 20, and 28 weeks after inoculation. All other animals were allowed to die of disease. All animals were autopsied with gross and microscopic examination of the liver, spleen, kidney, adrenal, heart, and gastrointestinal tract. Histologic sections were stained with hematoxylin and eosin, PAS, and Congo red. Each histologic section was reviewed by two different investigators. The presence of amyloid was determined by examining sections, stained with Congo red under polarized light. Amyloid was recognized by its characteristic birefringence and dichroism according to the method of Ruch (10).

Results. Microscopic examination of the liver, spleen, heart, adrenal, and gastrointestinal tract of inoculated hamsters on both normal casein and low casein diets was performed. No amyloid was found in either group of animals sacrificed at 8 weeks. Two of five animals sacrificed at 20 weeks on the normal casein diet had amyloid in the liver, spleen, and kidneys. No amyloid was found in the low casein diet animals. Extensive amyloid was found in three of four animals sacrificed at 28 weeks on the normal casein diet. No amyloid was found in the animals on the low casein group.

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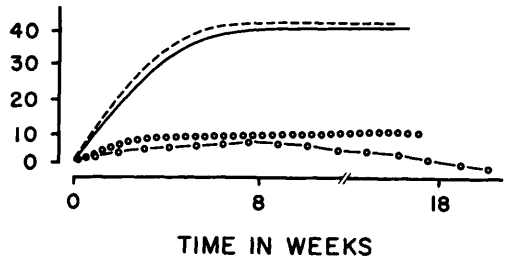

FIG. 1. Weights of hamsters: (O), low casein noninoculated control animals; (- - -), normal casein noninoculated control animals; (—), normal casein inoculated animals; (O—), low casein inoculated animals.

In comparing sacrificed normal casein diet and low casein diet inoculated hamsters, no amyloid was found in Group I sacrificed at 8 weeks. In those sacrificed at 20 weeks, two of five animals on the normal casein diet had amyloid. No amyloid was found in the low casein diet animals (Group II). Group III, sacrificed at 28 weeks had extensive amyloid found in three of four animals sacrificed on the normal casein diet. No amyloid was found in the sacrificed animals on the low casein group.

Of the nonsacrificed animals, the animals on the normal casein diet that died of natural causes had a much higher incidence of amyloidosis. nine of 12 normal casein diet animals had extensive amyloidosis while only 3 of 21 animals on the low casein diet had amyloidosis. The p value for the difference in incidence of amyloid was <0.001 using the chi square test of significance. The animals on the low casein diet as a group lived longer than the animals on the normal casein diet but this difference was not statistically significant. The average life span of the normal casein diet animals was 68 days as compared to 98 days of the low casein diet animals. The weight of the animals varied as to diet as reported in Fig. 1. The low casein animal was sluggish and had poor fur while the normal protein group appeared healthy.

Amyloid was detected at the twentieth week in the normal casein diet animals but not until the twenty-eighth week in low casein animals that were sacrificed serially. When amyloid was present it was found in equal concentrations in the liver, spleen, adrenals, and kidneys. No amyloid was found at any time in the noninoculated control animals on either diet. No amyloid was found in either the gastrointestinal tract or the heart of any animals. However, a focal myocarditis was found in five animals on the normal casein diet. Election microscopy revealed fibrils characteristic of amyloid in the kidney. Amyloid in the kidney was limited to glomeruli.

Parasite counts on smears of the spleens of inoculated animals showed little correlation with the presence of amyloid. Parasites were found in the spleens of animals in both diets regardless of the presence of amyloid. Great variability was found in duplicating the parasite counts several times in each animal. Animal weights were recorded weekly, as shown in Fig. 1.

Bacterial cultures of the stool were obtained at autopsy using blood agar and McConkey's media. There was no significant difference in the flora inoculated on control animals. *E. coli* comprised 40% of the bacteria isolated from inoculated animals on either diet. Other organisms isolated in equal proportions included *Proteus mirabilis, Aer bacter aerogenes, Pseudomonas* species, *Staphylococcus albus,* coagulase negative, and enterococci.

Discussion. There are many chronic diseases such as leprosy, chronic tuberculosis, and rheumatoid arthritis that predispose a patient to amyloidosis. Amyloidosis is much more common in American patients with leprosy than Mexican patients with the same disease (11). It has been postulated that diet may influence this difference.

From previous animal studies and our studies, diet control can influence amyloid formation. In CBA mice and now in Leishmania infected hamsters, a low protein diet delays amyloid formation.

The amyloid was deposited earlier, in greater amounts, and much more often when the animals were on a formal casein diet. Once the amyloid developed in the low casein animal, the distribution of the liver, spleen, kidney, and adrenals was the same as in the normal casein diet. The gastrointestinal tract

was always spared in both groups. Despite the extent of amyloidosis in animals on the normal casein, there was no statistically significant difference in life span. Indeed, the animals on the low casein diet had poor fur, were thinner, and much weaker, yet had less amyloid.

The result of decreased amyloid formation may not be directly attributable to the decrease of casein but due to protein depletion. It may be this general effect on protein synthesis that controls amyloid formation, rather than casein effect per sec.

The effect of the diet had no qualitative or quantitative effect of the intestinal flora. Bacterial products and bacteria can produce amyloid (12, 13), but these were not factors in the study. If the casein effect was mediated by an immunological reaction, one would expect the GI tract to be infiltrated with amyloid since the casein was given by mouth. The effect of a low casein diet in controlling amyloidosis should be studied further with paired feedings and modified diets.

Conclusion. A low casein diet decreases the incidence and delays the appearance of *Leishmania donovani*-induced amyloidosis in the golden hamster. In contrast, 75% of the animals on the normal casein diet developed extensive amyloid infiltration in the liver, spleen, adrenal, and kidney.

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