Inhibition of Experimental Amyloidosis in Alloxan Diabetic Mice (35790)

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It has been found that experimental and human amyloid-containing tissues have significantly more mucopolysaccharides (MPS) than comparable nonamyloidotic organs (1–3). Experiments with labeled sulfate indicate incorporation of sulfate in MPS during amyloid formation (4). Some authors (5, 6) reported depletion and diminished synthesis of MPS in various organs of animals with experimental diabetes.

This report deals with experimental amyloidosis in alloxan diabetic mice exposed to amyloidogenic stimuli.

Materials and Methods. Alloxan diabetes was produced in 40 healthy male Swiss white mice, 4 weeks of age, by injection into the tail vein of a freshly prepared alloxan solution, 75 mg/kg of body weight. Three days after the injection urine samples from the mice were analyzed quantitatively for glucose with glucose oxidate strips (Clinistix, Ames). Glycosuria was taken as an indication of alloxan diabetes. Three weeks after the onset of glycosuria amyloidogenic treatment was begun. The interval was intended to allow depletion of the body MPS, as in Schiller's experiments (5) a similar period was shown to result in depletion of the MPS stores. The amyloidogenic treatment consisted of heterologous transfer of amyloid as described by Shirahama *et al.* (7).

Spleen of a patient suffering from chronic lung disease and secondary amyloidosis was obtained at autopsy and homogenized (1 g/2 ml of cold saline) with a Virtis homogenizer. Hammerstein casein (Merck) was slowly dissolved in 0.05 *N* NaOH at 60° to a final concentration of 13%. The amyloidogenic treatment was given to three groups of mice: the experimental group of 40 diabetic mice, a control group of 30 nondiabetic mice treated with one/ip injection of alloxan, and a second control group of 31 untreated mice of the same age and sex (Table I). The animals were injected ip with 1 ml of the spleen homogenate. From that day, each mouse also received eight daily sc injections of 0.5 ml of casein solution. All mice also received daily ip injections of a freshly prepared ampicillin solution (100–300 mg/kg body weight) to inhibit the tendency of diabetic animals to develop skin abscesses and generalized sepsis. Ten days after the first spleen homogenate injection all the mice were killed by exsanguination. Urine samples were tested for glucose with Clinistix. Blood glucose was measured in all mice including a group of normal mice of the same age and sex, using the method of Hoffman (8).

Pieces of spleen, liver, kidney, and heart from all mice were fixed in 10% formalin and stained with Congo red and examined by ordinary and polarized light microscopy. Amyloid deposits were identified by their green birefringence when examined under crossed polars.

Results. The blood sugar levels of the glycosuric mice ranged from 100 to 500 mg/100 ml (Table II). Blood glucose levels of all the other mice ranged from 80 to 110 mg/100 ml.

Splenic amyloidosis was found in 30 of the 31 mice which were given only the casein injections. Heavy perifollicular deposits were seen in 27 of these mice and small amounts of amyloid were detected in the other three. No amyloid deposits were found in kidneys, livers, or hearts. One mouse did not develop amyloidosis.

All of the 30 animals given amyloidogenic

TABLE I. Effect of Injection of Alloxan and Casein on Blood Sugar Levels and the Development of Amyloidosis.

No. of mice	Alloxan (iv)	Alloxan (ip)	Casein (sc)	Blood sugar (mg/100 ml)	Splenic amyloidosis
40	+	—	+	100–460[a]	4[b]/22[c]
30	—	+	+	80–110	30 /30
31	—	—	+	80–110	30 /31

[a] See Table II for details.
[b] Two animals were not diabetic terminally.
[c] Eighteen animals died during the course of the experiment.

treatment and ip alloxan developed splenic amyloidosis. In the group of glycosuric mice only 22 animals survived until the end of the treatment. The others died with multiple abscesses. In 18 of the surviving animals, no amyloidosis was found; while in two, minute deposits of amyloid were present in the spleen. These two animals had antemortem blood glucose levels of 182 and 454 mg/100 ml. In the remaining two animals heavy amyloid deposits were found in the spleen. These animals had glycosuria soon after the alloxan injection, but terminally this was not present. Their antemortem blood glucose levels were normal. No amyloid deposits were found in the 18 diabetic mice which died of sepsis during the course of casein injections (Table I).

Discussion. The MPS are considered by many authors to be essential constituents of amyloid (9–11). It is also known that MPS play an important but not yet clearly defined role in the precipitation of collagen fibrils (12). Since both collagen and amyloid are fibrous proteins it is also possible that MPS play a fundamental role in the deposition of amyloid fibrils. It is reasonable to assume therefore that MPS, whether essential constituents of the amyloid fibrils proper or not, are of primary importance in the deposition of amyloid in the tissues. Deranged formation of availability of MPS may, therefore, be expected to interfere with amyloid deposition.

TABLE II. Terminal Blood Sugar Levels in Mice Given Alloxan Intravenously and Casein Subcutaneously.

Blood sugar (mg/100)	No. of mice
100–150	2
151–250	4
251–350	4
351–450	5
451–500	7

In the present experiment, alloxan diabetes was shown to inhibit the production of an experimental amyloidosis in mice. The findings indicate that the antiamyloidogenic effect is due to the diabetic state rather than to alloxan because the animals treated with ip alloxan and those in which iv alloxan did not cause diabetes were not protected from amyloidosis. It appears, therefore, that the protection from amyloidosis is due to the alloxan–diabetes *per se*.

Summary. A group of 40 mice was rendered diabetic by iv alloxan and then given amyloidogenic treatment. Of the 22 surviving animals, two, in which the diabetes was transitory, developed heavy splenic amyloidosis. Among the 20 mice with sustained diabetes, two developed minimal deposits; whereas in 18, no amyloid deposits were detected. In 30 of 31 animals given only amyloidogenic treatment and in all 30 animals given amyloidogenic treatment and ip alloxan (which did not produce diabetes), heavy splenic amyloidosis developed. The findings indicate that the diabetic state in animals inhibits amyloid deposition.

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