Antagonism of Vasoactive Amines in NZB/W Glomerulonephritis
I. Beneficial Effect of Methysergide (37573)

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New Zealand black by New Zealand white F_1 hybrid mice (NZB/W) spontaneously develop an auto-immune syndrome consisting of antibodies to nucleic acids (1), Gross virus antigens (2), and autologous thymocytes (3), as well as widespread lymphocyte infiltration. They die by 12 mo of age of glomerulonephritis caused by deposition of immune complexes containing both nucleoprotein and Gross virus antigen and their antibodies (4). The pathologic constellation is very similar to that seen in human systemic lupus erythematosus.

Kniker and Cochrane (5) have reported that the immune-complex glomerulonephritis associated with experimental acute serum sickness in rabbits can be largely prevented by the combined antagonists of the vasoactive amines, histamine and serotonin. A therapeutic trial of methysergide, a serotonin antagonist, has been conducted in NZB/W mice with positive results and is reported here.

Methods and Results. Methysergide (kindly provided by Sandoz Pharmaceuticals) was administered orally in the animal's drinking water which was titrated to pH 3 with hydrochloric acid for optimal stability of the drug. Controls received pH 3 water. The inhibitory effect of the drug in doses varying from 0 to 6 mg/100 ml drinking water, given for 24 hr before testing, was measured by injecting 10 μg of histamine or serotonin intradermally into the rear footpads of mice immediately after being given 0.2 ml of 0.25% Evan's blue intravenously. The resultant cutaneous bluing was graded from 0 to 4+. Each group contained five mice. Antibodies to DNA were measured by a Farr assay previously described (6). Proteinuria was quantitated by "Albutest" dipstick from Ames Co. This has been found in our laboratory to correlate well with 24 hr urine collections.

BUN was determined by the phenate-hypochlorite method (Hyland). Immunofluorescence was performed with fluorescein conjugated anti-mouse immunoglobulin obtained from Meloy Laboratory and chromatographed on DEAE-cellulose. A Reichert-Zetopan microscope with HBO 200 W mercury lamp and BG 12 exciter filter was used. Evaluation of glomerular histology included basement membrane thickening, cellular infiltration, and sclerosis. Immunofluorescence and histologic grades were scaled from 0 to 4+ and were performed blind (7).

Mice were obtained from the National Institutes of Health Animal Production Section and housed in adjacent cages.

The effect of methysergide in NZB/W mice is shown in Table I. Doses of 4 and 6 mg % in drinking water for 24 hr almost completely

TABLE I. Effect of Methysergide in NZB/W Mice.

Dose (mg %)	Footpad reaction[a] to		
	Saline	Serotonin	Histamine
0	0.5	3.2	2.2
2	0.5	1.8	NT[b]
4	0.4	0.8	NT
6	0.2	0.7	1.9

[a] Drug was administered in drinking water at concentration given. Foot pad bluing was graded 0 to 4+ 10 min after injection of 10 μg of vasoactive amine in 20 μl saline.

[b] NT = not tested.

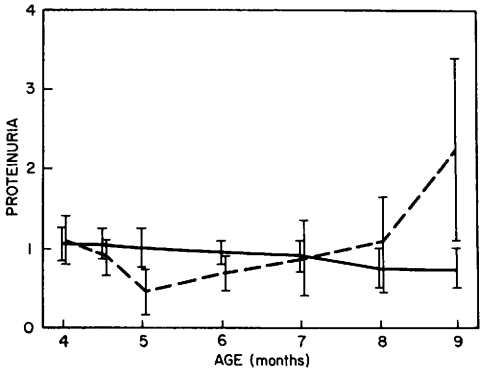

FIG. 1. Decreased proteinuria in methysergide treated NZB/W mice. Proteinuria graded 0 to 4 +. Averages ± SD plotted. (—) Methysergide treated; (- -) controls.

antagonized the effect of injected serotonin but had no significant effect on histamine. Four month old NZB/W female mice were randomly allocated into two groups of 28 each. Control mice from these groups did not have detectable immunoglobulin deposits in their glomeruli. One group was given 6 mg methysergide/100 ml pH 3 drinking water for the duration of the experiment while the controls received pH 3 drinking water. The amount of fluid taken was measured periodically and averaged 20% less in the drug treated group. Four mice were sacrificed each month and their kidneys were examined histologically.

The degree of proteinuria is shown in Fig. 1. By 9 mo of age the control group had strikingly greater proteinuria than the drug treated animals. The difference is significant with $p < 0.01$ (Student's t test). There was no difference in proteinuria before 9 mo. The controls had higher BUN levels than the drug treated animals at 9 mo of age (34 ± 14 mg % compared to 22 ± 5 mg %) but this difference was not significant. All the abnormal values (greater than 30 mg %) occurred in the control mice.

The histologic and immunofluorescence data are given in Table II. The pathologic changes at 9 mo were significantly more severe in the control group with $p < 0.01$. At 8 mo the difference was not significant. Immunofluorescent staining was also greater in the controls but was not statistically significant.

Since DNA anti-DNA immune complexes are among those deposited to produce the glomerulonephritis anti-DNA antibody levels were measured in both groups and are given in Fig. 2. There was no difference between the groups until 9 mo, when the titer decreased considerably in the control group. In individual mice this decrease was closely correlated with the degree of proteinuria.

Discussion. These data show that the serotonin antagonist, methysergide, exerts a protective effect during the course of the spontaneous immune-complex glomerulonephritis of NZB/W mice. The treated and control groups did not differ until 9 mo of age when the experiment was terminated. At this point the methysergide treated animals had significantly less proteinuria and less severe renal histology. Both these differences were significant at the $p < 0.01$ level. The most sensitive indicator of the drug action was decreased proteinuria as was observed in Kniker and Cochrane's study of acute serum sickness in the rabbit (5). Glomerular staining with anti-mouse immunoglobulin was less intense in the treated animals but the difference was not statistically significant, possibly because the discriminatory range of the technique is limited. The identical levels of anti-DNA antibodies in the two groups until the onset of significant proteinuria suggests that methysergide did not exert an immunosuppressive effect. The late fall in anti-DNA titer in the controls is presumably due to renal losses of immunoglobulin.

The beneficial action of methysergide was presumably due to the inhibition of deposition of circulating immune complex in the glomerular basement membrane by decreas-

TABLE II. Renal Pathology of NZB/W Mice.

Age (mo)	Drug	Immuno-fluorescence	Histology	
8	0	2.6 ± 0.8[a]	2.2 ± 0.9[a]	
	+	2.0 ± 0.7	1.5 ± 0.4	
9	0	3.6 ± 0.7	2.75 ± 0.5	$p < 0.01$
	+	3.0 ± 0.9	1.92 ± 0.2	

[a] Immunofluorescence and histology graded 0 to 4+ (see text). Averages ± SD given.

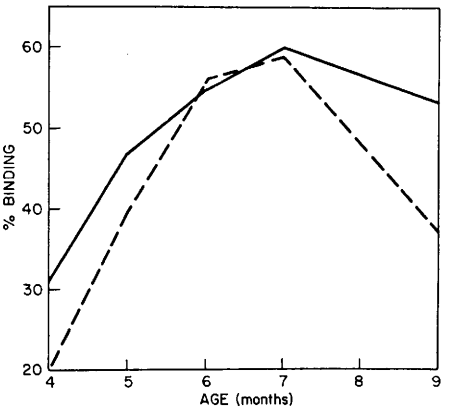

FIG. 2. Anti-DNA antibodies in methysergide treated and control NZB/W mice. Anti-DNA antibody expressed as percentage binding of tritiated DNA. (—) Methysergide treated; (- -) controls.

ing the permeability of the capillaries. Since serotonin is the predominant vasoactive amine in mice (8) such an effect of methysergide was expected. However, the absence of an antihistamine effect may have prevented the complete suppression of immune complex deposition.

This beneficial effect of methysergide in NZB/W mice has stimulated further study of this type of therapy. In particular it is important to determine whether the drug is effective in older diseased B/W mice, whether it prolongs their life, and whether it is able to synergize with other modes of therapy (9). These studies are in progress. It does appear that cyproheptadine, which antagonizes both serotonin and histamine, also delays the onset of proteinuria in NZB/W female mice.

The agents currently known to be effective in treating NZB/W glomerulonephritis or human systemic lupus erythematosus, corticosteroids and immunosuppressive drugs, are toxic. It is hoped that the vasoactive amine antagonists will be able to increase therapeutic efficacy and reduce treatment toxicity.

Summary. New Zealand black/white hybrid mice spontaneously develop many autoantibodies and a fatal immune-complex glomerulonephritis as part of an auto-immune process of unknown etiology. Amelioration of the nephritis of acute serum sickness in rabbits by antagonism of vasoactive amines prompted a therapeutic trial of the serotonin antagonist, methysergide, in NZB/W mice. When the drug was started early in life treated animals had less proteinuria and renal pathology than untreated controls.

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