

## THE COURSE OF AUTOIMMUNE DISEASE IN PAROUS NZB/NZW MICE

By

REBECCA CASTOR and SARA ELLEN WALKER

*Rackham Arthritis Research Unit, Department of Internal Medicine,  
The University of Michigan Medical School, Ann Arbor, Michigan 48109, U.S.A.*

### INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease that characteristically affects young women in their reproductive years (Estes and Christian, 1971). Many women with SLE want to have children but because a number of reports have described the adverse influence of pregnancy and parturition on the course of SLE, many clinicians advise patients with active disease to avoid childbearing. Estes and Larson (1965) reported that exacerbations of SLE during pregnancy occurred more than twice as often as remissions and concluded that active lupus nephritis was a contraindication to pregnancy. In a retrospective study of 79 patients with SLE, Fraga, Mintz, Orozco and Orozco (1974) reported the onset of disease in 11 patients during pregnancy or in the immediate postpartum period. Flares of SLE occurred in seven cases. Many studies of pregnancy in women with SLE have been criticized (Fraga, Mintz, Orozco and Orozco, 1974). Small numbers of patients were described, and in some instances articles were written by obstetricians who failed to document the diagnosis of SLE in every patient.

To gain additional insight into the influence of pregnancy on SLE in women, we studied autoimmune disease in parous and nonparous mice which are animal models of SLE. New Zealand Black (NZB) mice develop autoimmune haemolytic anaemia and membranous glomerulonephritis (Howie and Helyer, 1968). When NZB mice are crossed with New Zealand White (NZW) mice, the first-generation NZB/NZW offspring spontaneously develop a disease which is analogous to SLE. Female mice develop autoantibodies and proteinuria at an early age (5 to 6 months), and 50 per cent of virgin female mice are dead of renal failure by 10 months of age. In contrast, male mice develop renal disease 3 to 4 months later than female mice; their mean survival time is 15 months (Howie and Helyer, 1968). Positive tests for lupus erythematosus (LE) cells (Howie and Helyer, 1968), heterogeneous antinuclear antibodies detected by indirect immunofluorescence (ANA) (Lambert and Dixon, 1968) and antibodies directed specifically against DNA (anti-DNA) (Steinberg, Pincus and Talal, 1969) are found in 50 to 100 per cent of NZB/NZW mice. These serological abnormalities are characteristically associated with SLE in man (Dubois, 1974). Anti-DNA and a DNA-like antigen have been eluted from renal tissues of sick NZB/NZW mice (Lambert and Dixon, 1968) and from kidneys of patients dying with SLE (Koffler, Schur and Kunkel, 1967). Immune complex deposition in the glomeruli, therefore, appears to cause renal disease in New Zealand mouse disease and in human SLE.

In the current study, autoantibodies and severity of renal disease were quantified in parous and virgin female NZB/NZW mice.

\* Reprint requests to: Sara Ellen Walker, M.D., R4633 Kresge I, 1405 East Ann Street, Ann Arbor, Michigan 48109, U.S.A.

## MATERIALS AND METHODS

*Breeding protocol.* Colonies of New Zealand Black, New Zealand White and NZB/NZW mice were maintained as described by Walker and Bole (1972). Twenty-four female NZB/NZW mice aged 1 to 3 months were mated with NZB/NZW males and allowed to breed continuously for 7 months. Two females were housed with one male in each breeding cage. Twenty-two littermate control females were housed without males, 5 or 6 to a cage. Each female breeder produced 1 to 9 litters (mean = 5 litters). Offspring were examined, counted and removed from breeding pens when they were about 1 week old. Five parous and 2 control mice died during the study. The surviving 39 mice were killed and necropsied 7 months after the beginning of the study, when they were 8 to 10 months of age. Mice were bled from the orbital plexus at the time of death, and serum was stored at  $-20^{\circ}\text{C}$ .

*Anti-DNA.* DNA binding in terminal serum was determined by a modification of the Farr technique described by Steinberg *et al.* (1969) and adapted by Walker and Bole (1975). This assay measured the per cent  $^{14}\text{C}$ -labelled double-stranded DNA bound by 0.015 ml of heat-inactivated mouse serum. A value greater than 20 per cent binding was considered to be positive (Walker and Bole, 1976).

*ANA.* Sera obtained at death were tested for heterogeneous ANA on human leucocyte substrate using the indirect immunofluorescent procedure described by Friou (1967) adapted by Walker and Bole (1975). Each sample of mouse serum was tested undiluted and at dilutions of 4, 16, 64, 256 and 1024. Test results were expressed as the highest dilution giving a positive result. Zero indicated negative fluorescence. Three morphological patterns of nuclear fluorescence were identified in positive tests. Descriptions of rim, homogeneous and coarse speckled patterns have been described by Walker and Bole (1973).

*Renal histology.* Four- $\mu\text{m}$  sections of renal tissue embedded in paraffin were stained with haematoxylin and eosin (HE) and examined by light microscopy. An adaptation of the method of Pirani, Pollak and Schwartz (1964) was used to quantify severity of renal damage in parous and control mice. Abnormalities were counted in 20 glomeruli in each section of kidney. Lesions counted were: thickening or hypercellularity of the mesangial stalk; focal glomerular hypercellularity; basement membrane thickening; diffuse glomerular hypercellularity; fibrinoid change; crescent formation. Counts of glomerular lesions were reproducible after repeated examinations by the same investigator (R. C.).

We have observed lymphocytes and plasma cells surrounding renal arteries and invading renal pelvic fat in female NZB/NZW mice aged 6 months and older. Although the pathological significance of these cells is not established, their appearance coincides with early development of glomerulonephritis. These infiltrating cells were absent in mice treated with a high dose of the immunosuppressive drug, cyclophosphamide (Walker and Bole, 1975). An observer (R. C.) who was unaware of the breeding record of each mouse graded lymphocytic infiltration around renal arteries and collections of lymphocytes in the renal pelvis in each renal section using scales of 0 to 4+.

*Statistical analysis.* Student's *t*-test was employed to compare mean values derived from 2 groups of unequal sizes (Snedecor and Cochran, 1967).

## RESULTS

*Anti-DNA*

Anti-DNA values for individual parous and control mice are shown in Fig. 1. In parous mice, mean anti-DNA was 31 per cent [ $\pm 2.5$  standard error of the mean (s.e.m.)]. In control mice anti-DNA was 31 per cent ( $\pm 2.6$ ). There was no correlation between DNA binding values and numbers of litters or numbers of offspring produced by individual females in the breeder group.

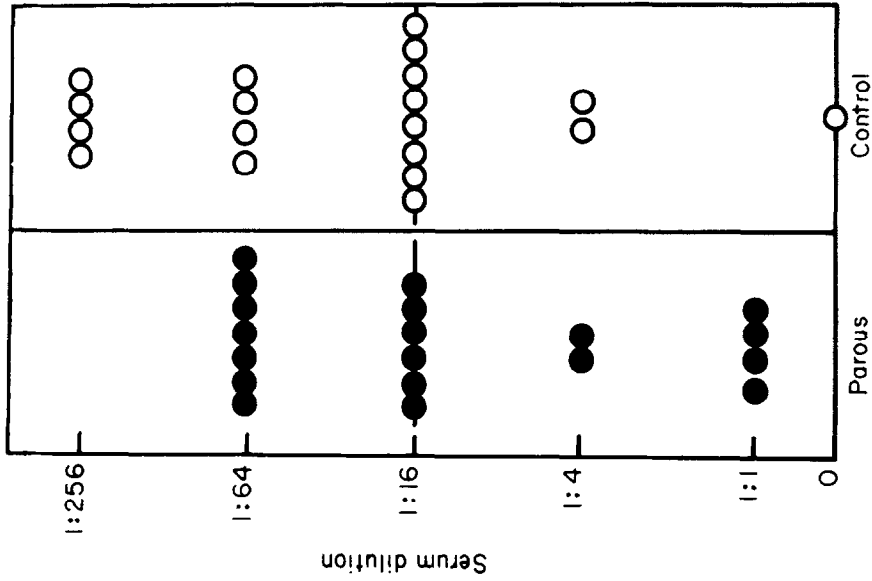
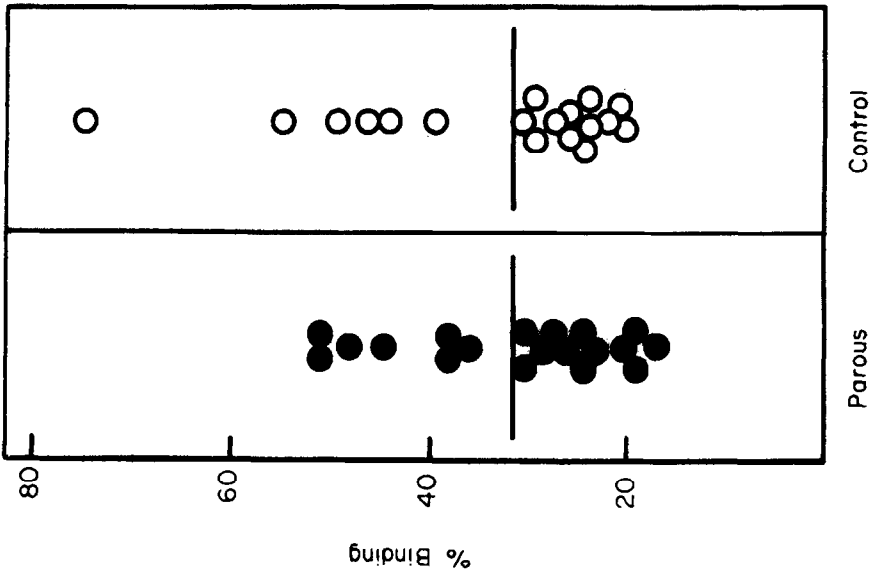


Fig. 1. Anti-DNA values in individual parous and control female NZB/NZW mice are expressed as per cent  $^{14}\text{C}$ -labelled DNA bound by 0.015 ml mouse serum. Horizontal bars indicate mean values which were the same in both groups of mice.

Fig. 2. Heterogeneous antinuclear antibodies were determined by an indirect immunofluorescent technique with mouse sera in dilutions ranging from 1 to 1024. Test results are expressed as the highest dilution giving a positive result. In the parous group and the control group, median ANA titres (marked by horizontal bars) were 1:16. One control mouse had a negative test for ANA.



### ANA

The median ANA titre in parous animals was 1:16 (range 1:1 to 1:64) compared with a median titre of 1:16 (range 0 to 1:256) in control mice (Fig. 2). No sera diluted 1:1024 were positive. There was no linear relationship between ANA titres and numbers of litters or total offspring produced by individual parous mice. When positive slides were examined for patterns of fluorescence, it was noted that patterns produced by sera from parous mice were similar to patterns produced by sera from control mice.

### Renal Histology

Glomerulonephritis was identified in every animal. Figure 3 illustrates characteristic involvement of a renal glomerulus from a parous mouse killed at 10 months of age. Figure 4 illustrates numbers of lesions counted in 20 glomeruli in renal tissue from each animal. The mean number of glomerular

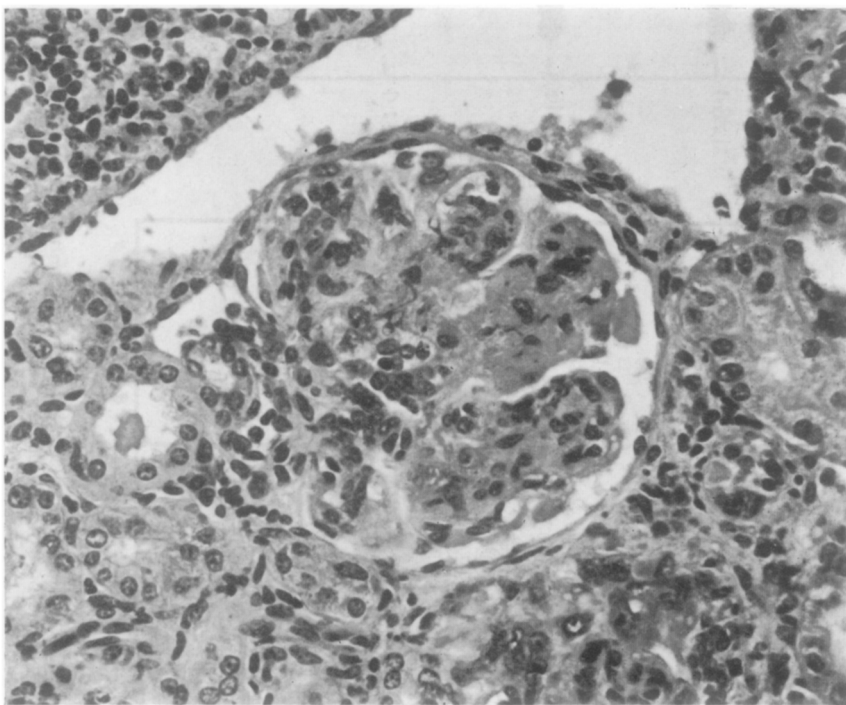


Fig. 3. A renal glomerulus from a 10-week-old parous NZB/NZW mouse shows cellular proliferation, basement membrane thickening, and early fibrinoid change. Similar abnormalities were found in renal tissue from virgin females. HE.  $\times 250$ .

abnormalities among breeder mice was 39 ( $\pm 2.1$ ), and the mean number of abnormalities in control mice was 44 ( $\pm 0.7$ ); ( $P > 0.1$ ). Lymphocytic infiltration in the kidneys in parous and control mice were similar. Parous mice had a median score of 3 (range 0 to 4) for periarterial lymphocytes; the

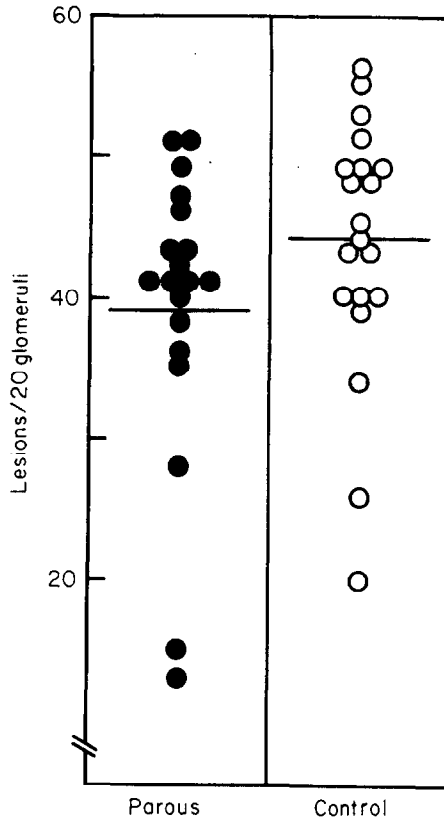


Fig. 4. Numbers of histological lesions counted in 20 glomeruli on each section of renal tissue are illustrated for individual parous and control mice. In both groups, mean values were similar.

median score in control mice was 3 (range 0 to 4). The median score for renal pelvic lymphocytes was 2 (range 0 to 4) in parous mice and 3 (range 1 to 4) in control mice. Numbers of glomerular lesions did not correlate with litters or offspring produced by parous mice.

DISCUSSION

This report describes the first study designed to measure influences of parity and parturition on the course of autoimmune disease in NZB/NZW mice. The young female breeder mice in this experiment were fertile, and produced large litters without difficulty. After 7 months of unrestricted breeding, mean anti-DNA values in parous mice and control mice were identical. The median ANA titres were the same (1:16) in each group, and the range of ANA titres was similar. In addition, there was no significant difference between mean numbers of renal lesions in breeder females and control females. It was concluded that there was very little difference between disease activity in parous females compared to virgin females at the end of the experiment.

The experimental design did not differentiate between possible hormonal effects accompanying mating without conception and hormonal effects produced by pregnancy. The influence of mating alone could be studied by housing

a third group of female mice with vasectomized male mice. Another interesting aspect of this study that deserves further investigation is the effect of parity and parturition on survival in NZB/NZW mice. Howie and Helyer (1968) found that the mean lifespan of breeders was extended 2 months longer than the lifespan of virgin mice in a study of 500 NZB/NZW females. This information suggested that parturition delayed the onset of autoimmune disease in NZB/NZW mice. Since animals were not examined for autoantibodies early in the current study, it is possible that time of onset of disease was different in the two experimental groups. If our animals had been followed until death, differences in survival times of breeder and nonbreeder mice might have been noted.

Therapeutic studies using other experimental animals have provided evidence that large doses of female hormones suppress humoral and cell-mediated immune mechanisms in rodents. Toivanen (1967) reported that guinea-pigs treated with estrone or estradiol (2.5 or 0.25 mg per animal, given twice a week) had impaired production of antibodies against *Escherichia coli*. In CBA/J mice, administration of high-dose estradiol (1.0 mg given twice a week, three times a week, or daily to each mouse) delayed rejection of first- and second-set skin allografts (Simmons, Price and Ozerkis, 1968). Arnason and Richman (1969) injected rats with spinal cord homogenates and treated the animals with ethinyl estradiol, 0.005 mg/day. The appearance and severity of experimental allergic encephalitis was suppressed in treated animals. The results of the current study suggest that flooding with female hormones during pregnancy was not adequate to prevent autoantibody production and immune complex glomerulonephritis in female NZB/NZW mice.

The current study is an extension of investigations of the interrelationships between female sex hormones and autoimmune disease in hybrid New Zealand mice. Earlier therapeutic studies in this laboratory showed that therapy with the artificial female hormone, Mestranol, induced early appearance of ANA in NZB/NZW mice (Walker and Bole, 1973). In mice treated with a naturally-occurring estrogen, 17- $\beta$ -estradiol (Walker and Bole, 1972), or oophorectomy (Walker and Bole, 1973), the incidence of positive tests for ANA and severity of renal lesions were similar to values in untreated control animals. Information gained from this parity study reaffirmed our earlier conclusions that addition or deletion of naturally-occurring female hormones failed to influence the progressive course of autoimmune disease in an animal model of systemic lupus erythematosus.

Our findings in parous NZB/NZW mice are at variance with the widely accepted opinion that pregnancy is harmful to women with active SLE. If the results of this experiment are applied to human disease, it may be suggested that the progress of autoimmune disease in certain hosts is not affected by events such as pregnancy and parturition.

#### SUMMARY

In a study designed to investigate the effects of pregnancy and parturition on spontaneous lupus-like disease in NZB/NZW mice, 24 female mice were allowed to breed repeatedly with male NZB/NZW mice. Control female mice

were caged separately without male mice. Seven months after the study began, the extent of autoimmune disease in parous and control animals was determined by assessing anti-DNA antinuclear antibodies, and the severity of renal lesions. Disease activity in both groups of mice was the same, indicating that pregnancy and parturition had no influence on autoantibody levels or severity of renal disease. It was concluded that bearing young had no effect on the course of autoimmune disease in NZB/NZW mice.

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