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### Title

Effect of castration and sex hormone treatment on survival, anti-nucleic acid antibodies, and glomerulonephritis in NZB/NZW F1 mice.

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EFFECT OF CASTRATION AND SEX HORMONE  
TREATMENT ON SURVIVAL, ANTI-NUCLEIC ACID  
ANTIBODIES, AND GLOMERULONEPHRITIS IN  
NZB/NZW F<sub>1</sub> MICE\*

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NZB/NZW F<sub>1</sub> (B/W)<sup>1</sup> mice are an experimental model for systemic lupus erythematosus. They spontaneously develop an autoimmune disease characterized by the formation of antibodies to nucleic acids and the development of a fatal immune complex glomerulonephritis. There is an accelerated expression of the disorder in female animals (1). Genetic (2), immunologic (3), and viral (4) factors are involved in pathogenesis. A disorder of immunologic regulation related to abnormalities in T-cell differentiation and function may represent a fundamental derangement in these animals (5).

The accelerated disease in female B/W mice is associated with an earlier appearance of IgG antibodies to DNA and polyadenylic acid (Poly A) (6), an earlier onset and greater severity of immune complex nephritis, and a markedly decreased incidence of survival beyond 12 mo (7). Males develop these abnormalities later in life. Prepubertal castration of male B/W mice caused an essentially female pattern of disease with 100% mortality at 11 mo (8). By contrast, prepubertal castration of female B/W mice had no effect on mortality, although it markedly reduced the development of IgG antibodies to Poly A. In a preliminary experiment, the administration of androgen to castrated females resulted in prolonged survival (8).

These results suggest that sex hormones modulate the expression of autoimmunity in B/W mice, with androgens exerting a protective influence. Androgens delay the switch from IgM to IgG antibodies to nucleic acids (8), a differentiation event which is generally thymic-dependent (9). In B/W mice, the development of IgG antibodies to Poly A is abrogated by neonatal thymectomy (10). These findings suggest that sex hormones modulate autoimmunity by acting on thymic-dependent regulatory mechanisms.

In the present report, prepubertal castration of B/W mice was accompanied by the sustained administration of sex hormones. The results demonstrate that androgens and estrogens have opposing effects on disease expression and survival. The possible therapeutic use of male hormone is suggested. In addition, experiments combining castration with neonatal thymectomy suggest

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<sup>1</sup> *Abbreviations used in this paper:* B/W mice, (NZB × NZW) hybrid mice; PBS, phosphate-buffered saline; Poly A, polyadenylic acid.

that sex hormones require the thymus in order to express their immunologic effects.

### Materials and Methods

*Mice.* B/W mice were derived from our colony at the University of California Vivarium, San Francisco, Calif., and they were maintained at the Fort Miley Veterans Administration Hospital.

Four separate experimental protocols were followed: (a) prepubertal castration combined with hormone implantation at 2 wk of age, (b) retreatment with a second androgen implant at 9 mo of age, (c) androgen treatment of intact males at 8 mo of age, and (d) combined neonatal thymectomy and prepubertal castration of males.

In the first protocol, there were 11 sham-operated males, 32 receiving androgen, and 21 receiving estrogen. There were 13 sham-operated females, 14 receiving androgen, and 17 receiving estrogen. In the retreatment protocol, 10 of the original 32 castrated and androgen-treated males were used. Five mice received a second implant containing androgen, and five received an empty implant. As for the females, 6 of the original 14 that were castrated and androgen-treated at 2 wk received a second implant containing androgen at 9 mo. These were compared with five surviving females from the original sham group.

In the third protocol, 9 intact males served as shams and 14 received an androgen implant for the first time. All animals were 8 mo of age. In the fourth experiment, seven male mice were subjected to combined thymectomy and castration procedures, and 10 were sham-operated.

#### *Operative Techniques*

Animals were subjected to castration, or they were sham-operated at 2 wk of age. They were anesthetized by intraperitoneal injection of Nembutal.

**OOPHORECTOMY.** A midline incision was made over the abdomen. Ovaries were identified and removed using electrocautery. The incision was closed using 6-0 silk suture material.

**ORCHIECTOMY.** Testes were delivered through a scrotal incision. The vas deferens and spermatic vessels were transected using electrocautery, and testes and epididymis were removed. Skin was sutured using 6-0 silk suture material.

**THYMECTOMY.** (Controlled suction technique) Thymectomy was carried out at 2 days of age under a dissecting microscope at a magnification of  $1.6 \times$ . At this magnification, the entire mediastinal cavity was in the field of vision. The sternal incision was extended from the manubrium down to the sixth rib. We gently mobilized the thymic lobes by disrupting the vascular and connective tissue attachments. To achieve adequate control of suction intensity, a 3-mm hole was made in a 5-cm tuberculin syringe barrel which was interspersed between the curved pipette and the rubber tubing connected to the vacuum outlet. Each thymic lobe was engaged by the pipette at the lower pole and gently teased off. The entire removal process was visualized under the microscope. The mediastinal cavity was left empty.

*Sham Operation.* In age-matched sham-operated controls, ovaries, testes, or thymic lobes were identified, but left intact. Experimental animals were distinguished from sham-operated mice by tail clipping or ear tagging. To minimize maternal neglect, the surgical incision and maternal nasal orifices were painted with parlodion-gentian violet solution.

*Hormone Replacement.* To achieve a maintained hormone replacement in castrated animals, a 2-cm Silastic tube containing 6-7 mg of either estradiol-17- $\beta$  or 5- $\alpha$ -dihydrotestosterone powder was implanted subcutaneously immediately after the castration procedure. This treatment resulted in prompt establishment of an adequate hormone level reached within 12 h, which persisted for at least 3 mo. Sham-operated mice received empty implants.

*Observation.* Mice were observed weekly. Mortality in experimental and control groups was tabulated in  $2 \times 2$  contingency tables, and evaluated by Chi square analysis using Yates modification.

*Determination of Serum Testosterone and Dihydrotestosterone Levels.* Serum samples from sham-operated or castrated and androgen-treated male and female mice were pooled separately. Serum testosterone and dihydrotestosterone were measured using a radioimmunoassay similar to that described by Abraham (11). Both androgens were purified before assay by celite chromatography and [ $^3$ H]testosterone was added to 0.5-ml serum samples before extraction with ether to correct results for losses. The antisera used were prepared against testosterone coupled to bovine

serum albumin by the succinate ester, and they showed 66% cross-reactivity with dihydrotestosterone.

*Histopathology.* At ages ranging from 4 to 11 mo, randomly selected mice from each group were sacrificed. Their kidneys were removed and studied by light and electron microscopy, and by immunofluorescence for the presence of immune complex deposits. These examinations were performed before breaking the specimen code. Light microscopic examination was performed on 4- $\mu$ m paraffin sections stained with hematoxylin and eosin.

*Direct Immunofluorescence for Glomerular Immunoglobulin Deposition.* Cryostat sections 5- $\mu$ m thick were cut and picked up on Formolgelatin-coated slides, air-dried for 30 min, and washed twice for 15 min in phosphate-buffered saline (PBS) at pH 7.2. The slides were placed in a moist chamber, covered with antiserum, and incubated for 30 min at room temperature in the dark. Fluorescein isothiocyanate-conjugated rabbit polyvalent anti-mouse immunoglobulin (Behring Diagnostics, Sommerville, N. J.; lot 656, fluorescein:protein ratio, 2:8) was used at a titer of 1:20. Slides were rinsed quickly in PBS and washed twice for 15 min in PBS with gentle agitation, rinsed in distilled water, and mounted in glycerin-PBS. Coated sections were examined using a Wild fluorescence microscope (Wild Heerbrugg Instruments, Inc., Farmingdale, N. Y.). The brightness and extent of glomerular immunoglobulin immunofluorescence was graded on a scale of 0-4 by two observers who were calibrated against each other against sections from standard specimens used in our laboratory.

*Electron Microscopy.* Mouse kidneys were cut immediately in cold 1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3. After 1.5 h fixation at room temperature, they were fixed additionally in 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.3) for 45 min. The specimens were dehydrated with alcohol and propylene oxide, infiltrated with epon-Araldite plastic, and polymerized at 75°C. Ultra-thin sections were cut on an LKB Ultratome III (LKB Instruments, Inc., Rockville, Md.), stained with uranyl acetate and lead citrate. The sections were examined by RCA-EMU (3F and 4; RCA Solid State, Somerville, N. J.) electron microscopes.

Representative areas of 2 glomeruli from each kidney were photographed and printed at a magnification ranging from 2,500 to 15,000  $\times$ . The areas occupied by immune complexes were graded on a scale of 0-4 independently by two investigators trained in judging renal lesions as seen in electron microscopy.

*Fractionation of Serum.* Blood was collected from mice at monthly intervals, starting at the third postoperative month. The animals were bled from the orbital sinus. The blood was allowed to clot at room temperature for 1 h, and left at 4°C overnight. Serum was separated by centrifugation at 1,200 g for 10 min. Serum samples from castrated and sham-operated mice were pooled separately. 200  $\mu$ l of the pooled serum samples was subjected to ultracentrifugation in a 10-35% linear sucrose density gradient (0.15 M NaCl, pH 8.0). Bovine thyroglobulin (19S), human gammaglobulin (7S), and hemoglobin (4S) were used as sedimentation markers. 40 fractions were collected and each was analyzed for antibodies to DNA and Poly A. Peak fractions were tested for immunoglobulin content. In the 19S region (fraction 10-20) only activity against  $\mu$ -chain could be detected by Ouchterlony analysis, whereas in the 7S region (fractions 20-30), activity against  $\gamma$ -chain, but not  $\mu$ -chain, was present. To determine the specificity of binding, monospecific rabbit anti-mouse  $\mu$ -chain and anti-mouse  $\gamma$ -chain antisera were added to peak fractions from 19S and 7S regions, respectively. Anti- $\mu$  inhibited DNA binding between 77 and 100%, whereas anti- $\gamma$  inhibited binding between 66 and 78%. Addition of goat anti-mouse albumin to these gradient fractions had no inhibitory effect.

*Anti-DNA and Anti-Poly A Assays.* Antibodies to DNA and Poly A were determined separately using a cellulose ester filter radioimmunoassay. The radioactive nucleic acids were double-stranded DNA (<sup>3</sup>H]DNA from KB cells obtained from Electro-Nucleonics Inc., Fairfield, N. J.), and [<sup>3</sup>H]Polyriboadenylic acid (from Miles Laboratories Inc., Elkhart, Ind.). The radioactive antigens (750 cpm/7.74 ng Poly A; 700 cpm/3 ng DNA) were incubated with decomplexed 50- $\mu$ l aliquots of each fraction for 30 min at 37°C followed by an overnight incubation at 4°C. The antigen-antibody complexes were collected onto cellulose ester filters (Millipore Corp., Bedford, Mass.). The filters were placed in counting vials and covered with 10 ml of Liquifluor-toluene scintillation medium. Radioactivity was determined in a Packard liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). The results are expressed as corrected cpm retained on the filter, a value which is directly related to serum concentration (12).

*Calculation of 7S:19S Antibody Binding.* The radioactive binding profiles revealed clear

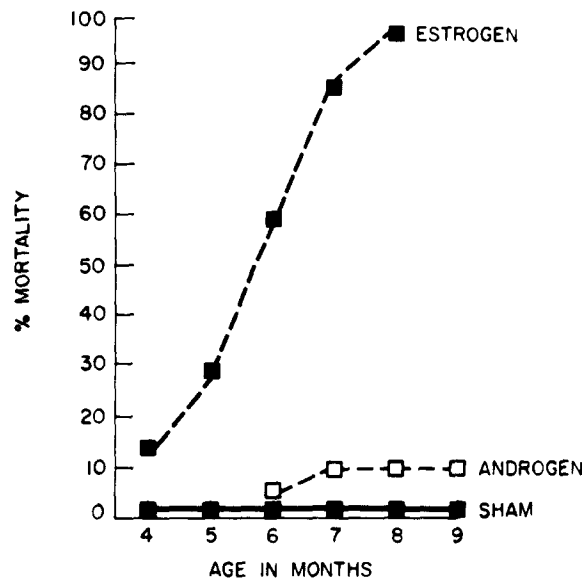


FIG. 1. Effect of prepubertal castration and sex hormone treatment on cumulative mortality in male B/W mice.

distribution into 7S and 19S peaks of activity after sucrose gradient fractionation. The radioactivity representing the fractionated 7S and 19S peaks within a single gradient were added and compared for total binding activity.

*Reproducibility and Analysis of Data.* To determine the reproducibility of our filter radioimmunoassay and the variations seen in age-matched mice, pooled serum samples from intact, sham-operated and variously treated male and female mice were subjected to sucrose gradient ultracentrifugation and assayed for anti-DNA antibodies. The ages ranged between 12 and 36 wk. The procedures were performed by the same technician. The radioactive DNA used was from the same lot. Some age-matched pooled samples were assayed on the same day, then repeated 1 mo later. The variations from the mean for the total anti-DNA binding in the 19S region ranged from 1.9 to 14.1%, whereas in the 7S region the range was between 0.2 and 7.1%. In the present communication, a minimum variation of 13% in the IgM and 8% in the IgG total binding activity from age- and sex-matched, sham-operated controls was required before a result was considered significant. These values represent 2 SD from the mean.

## Results

*Effects of Sex Hormones on Mortality of B/W Mice.* Prepubertal castration combined with maintained estrogen administration caused a greatly enhanced mortality of male B/W mice (Fig. 1). 19 of 21 mice were dead by 7 mo of age. By contrast, the cumulative mortality at 1 yr was 18% for sham-operated males and 15% for androgen-treated males ( $P < 0.05$ ). Castrated female mice given maintained estrogen also died more rapidly than sham-operated females. By contrast, castrated females given androgen had a significant decrease in mortality (Fig. 2). 8 of 14 (57%) such animals were alive at 1 yr, compared to 3 of 13 (23%) sham-operated females ( $P < 0.05$ ). Serum dihydrotestosterone concentrations in androgen-treated male and female mice were similar to the levels of testosterone observed in sham-operated males and clearly higher than those in sham-operated females (Table I).

*Effects of Sex Hormones on Antibodies to DNA and Poly A in Castrated Male*

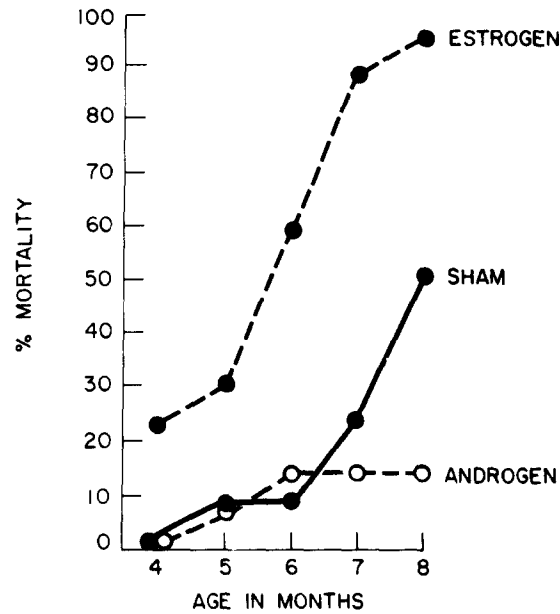


FIG. 2. Effect of prepubertal castration and sex hormone treatment on cumulative mortality in female B/W mice.

TABLE I  
*Serum Testosterone*

Group	Age in months		
	4	5	6
	<i>ng/ml</i>		
Sham male	3.04	1.26	3.52
Castrated and androgen-treated male	6.50	3.33	3.10
Sham female	1.15	0.45	0.79
Castrated and androgen-treated female	3.77	3.47	3.10

*B/W Mice.* Castrated male mice receiving estrogen had greater DNA binding activity when compared to sham and androgen-treated male mice. The increase in IgM antibodies occurred from 3 mo onward (Fig. 3A), whereas the major increase of IgG antibodies occurred at 6 mo (Fig. 3B), a time of greatly accelerating mortality.

Similar results were found for antibodies to Poly A. Estrogen-treated mice had an increase in IgM from 3 mo of age (Fig. 3C), and showed a premature appearance of IgG antibodies to Poly A at 6 mo of age (Fig. 3D). Normally, significant amounts of IgG antibodies to Poly A do not appear in male B/W mice until 11 mo of age (6, 10).

*Effects of Sex Hormones on Antibodies to DNA and Poly A in Castrated Female B/W Mice.* Castrated female mice receiving androgen had less IgM and IgG binding activity for DNA when compared to sham and estrogen-treated females (Figs. 4A and B). By contrast, mice receiving estrogen had increased DNA binding which was particularly striking at 5 mo of age. The decreased

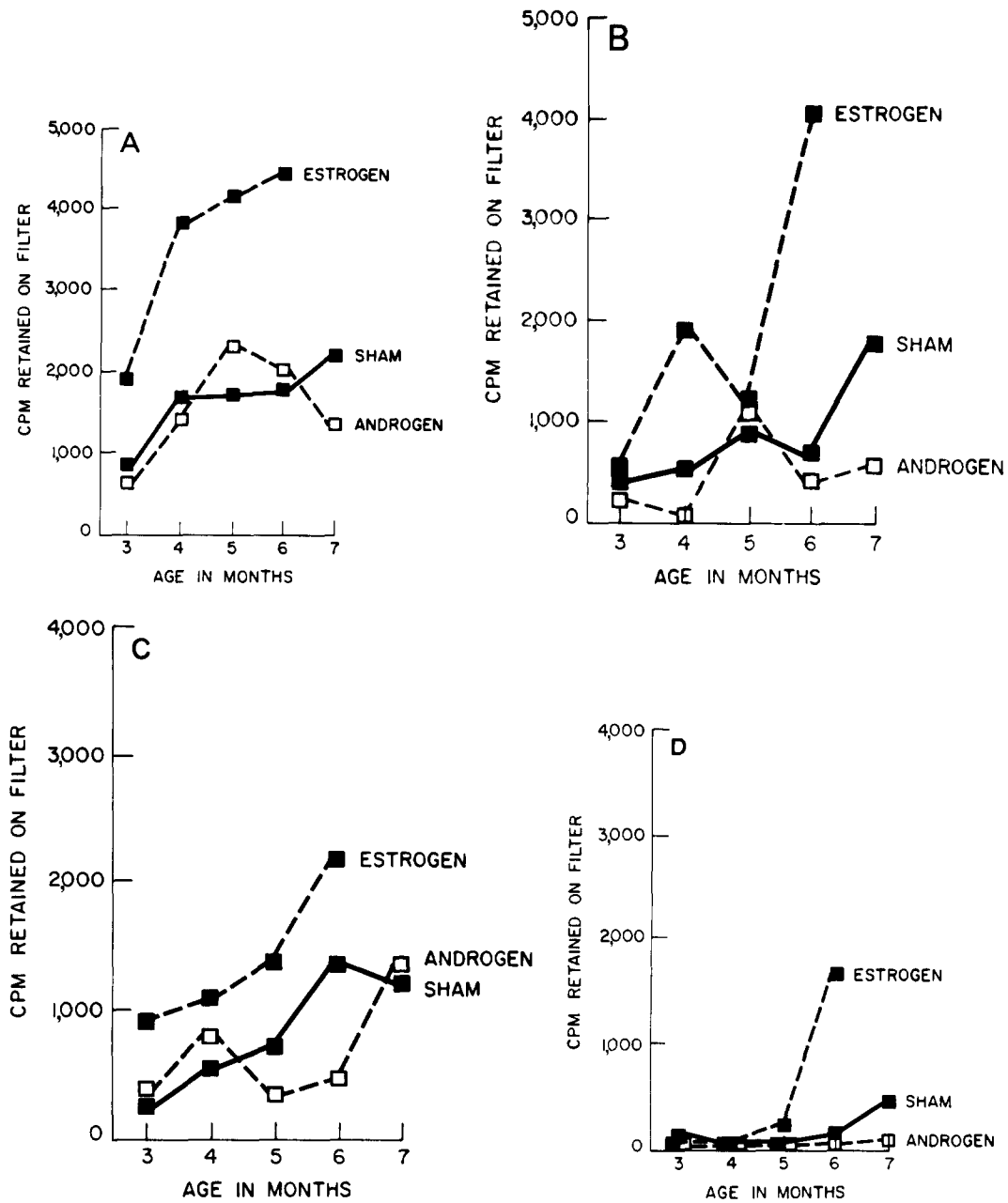


FIG. 3. Effect of prepubertal castration and sex hormone treatment of male B/W mice on (A) IgM antibodies to DNA, (B) IgG antibodies to DNA, (C) IgM antibodies to Poly A, and (D) IgG antibodies to Poly A.

binding at 6 mo may be due to the selective death of mice with the greatest amount of anti-DNA antibodies.

The effect of sex hormones on the Poly A response had the following characteristics. The augmentation due to estrogen was apparent both for IgM

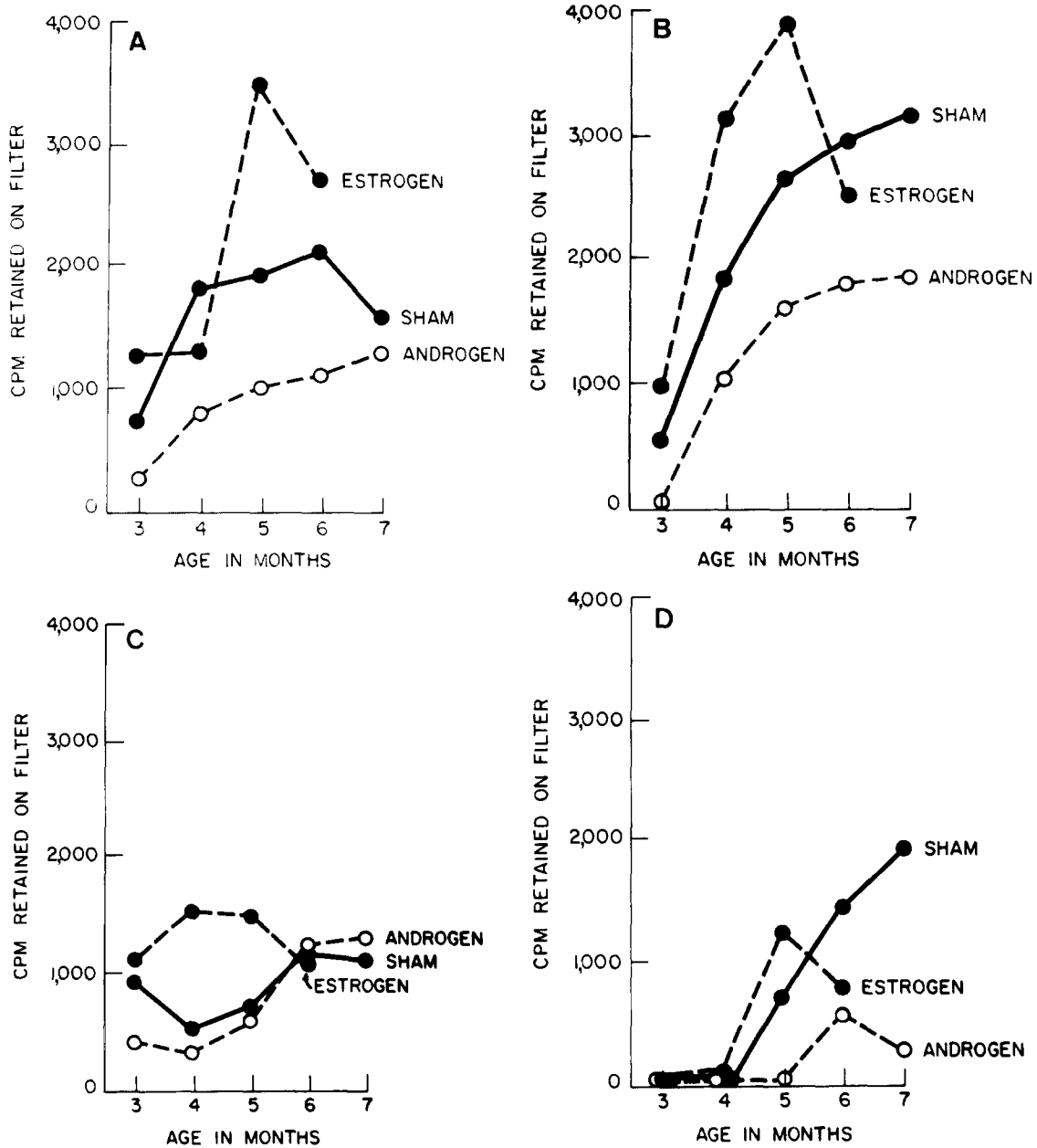


FIG. 4. Effect of prepubertal castration and sex hormone treatment of female B/W mice on (A) IgM antibodies to DNA, (B) IgG antibodies to DNA, (C) IgM antibodies to Poly A, and (D) IgG antibodies to Poly A.

(Fig. 4 C) and IgG (Fig. 4 D), and the decrease due to androgen was apparent in IgG anti-Poly A antibodies.

*Effect of Sex Hormones on Renal Pathology.* Sham-operated animals showed glomerulonephritis including progressive accumulation of lymphoid



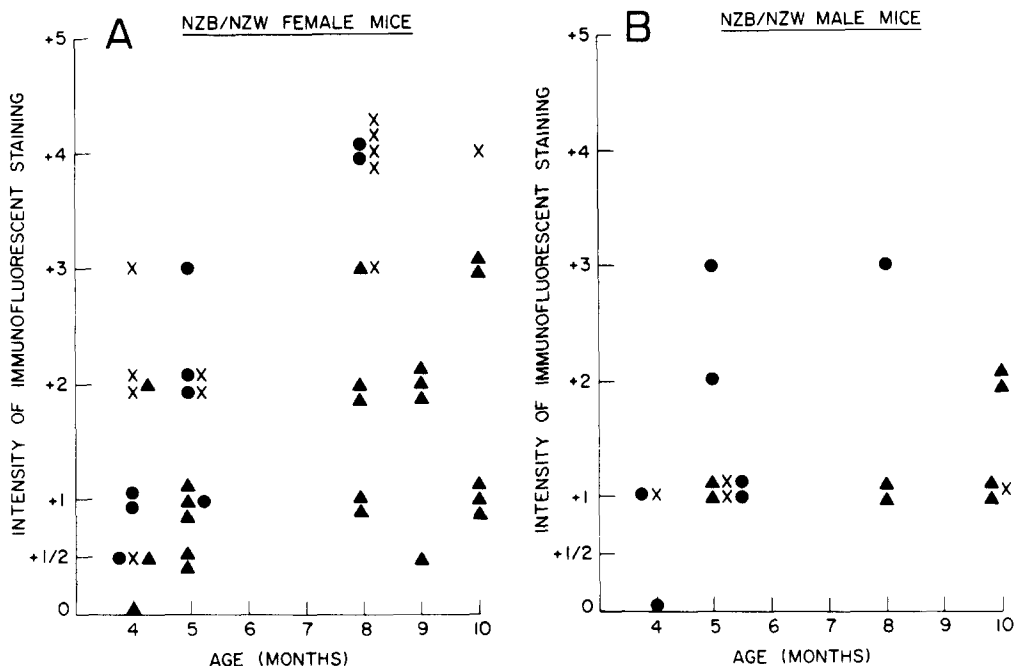


FIG. 5. Intensity of immunofluorescent staining for immunoglobulin in the glomeruli of female (A) or male (B) B/W mice at various ages. Mice were either sham-operated (X), castrated and treated with androgen (▲), or castrated and treated with estrogen (●).

cells at the calices, and subsequently around the branches of the renal artery. The glomeruli showed eosinophilic thickening of the capillary loops as early as 4 mo in sham females, with progressive glomerular enlargement and replacement by eosinophilic material. These changes were seen earlier and were more severe in sham-operated females than in sham-operated males.

At 5 and 8 mo, the lymphoid cell infiltration and glomerular abnormalities were more severe in animals which had been castrated and treated with estrogen, irrespective of sex. Furthermore, males and females castrated and given androgen showed much milder changes. This apparent exacerbation of lesions by estrogen and the converse protective effect of androgen were less apparent at very early and very late stages.

*Immunofluorescence.* Glomeruli showed significant granular staining for immunoglobulin at the earliest age examined (4 mo). Sham-operated females showed more extensive and intense immunofluorescence than sham-operated males. The exacerbation of the glomerular changes by estrogen and the protective effects of androgen were apparent in both sexes, particularly at 5 and 8 mo (Figs. 5 and 6). At 9 and 10 mo, many castrated females treated with androgen were still alive and had relatively fewer glomerular deposits of immunoglobulin compared to the few remaining sham females.

*Electron Microscopy.* The electron microscopic abnormalities present in the kidneys of B/W mice have been well described (13). In our studies, electron microscopic observation confirmed the renal involvement suggested by the mortality and immunopathologic findings discussed above.

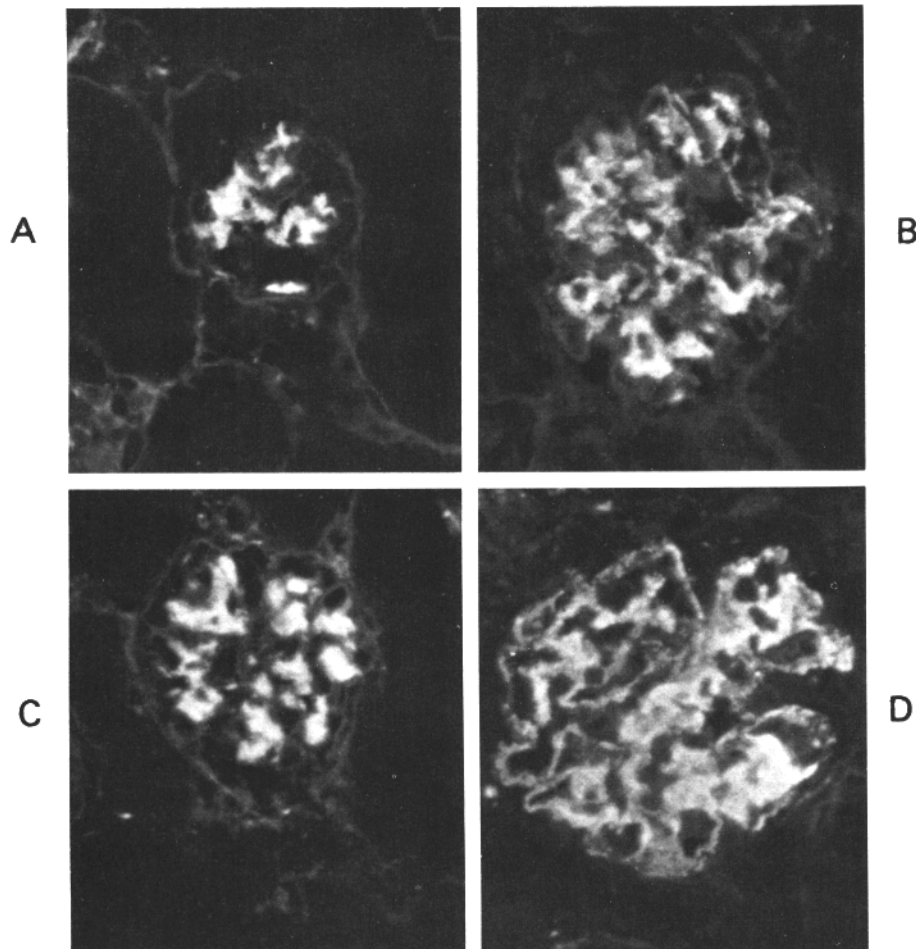


FIG. 6. Representative examples of immunofluorescent analysis of kidneys from castrated, 8-mo-old B/W mice: (A) B/W male, castrated and treated with androgen (1 plus); (B) B/W male, castrated and treated with estrogen (3 plus); (C) B/W female, castrated and treated with androgen (2 plus); and (D) B/W female, castrated and treated with estrogen (4 plus). Original magnification  $\times 312$ .

Sham-operated females at 4 mo already showed electron dense deposits in their glomeruli. Renal deposits were apparent at 5 mo in the castrated and estrogen-implanted mice, both female and male. These mice showed deposits present in the mesangial area, while castrated and androgen-implanted males at 5 mo had no deposits. At 10 mo, androgen-implanted animals still showed some protection from renal involvement (Fig. 7 A) when compared to a sham-operated female (Fig. 7 B).

*Retreatment with Androgen at 9 Mo of Age.* By 8 mo of age, antibodies to Poly A were essentially the same in sham-operated animals and in those that had received androgen once prepubertally (Table II). Without further androgen, IgG antibodies to Poly A showed the expected rise at 10 and 11 mo of age in both

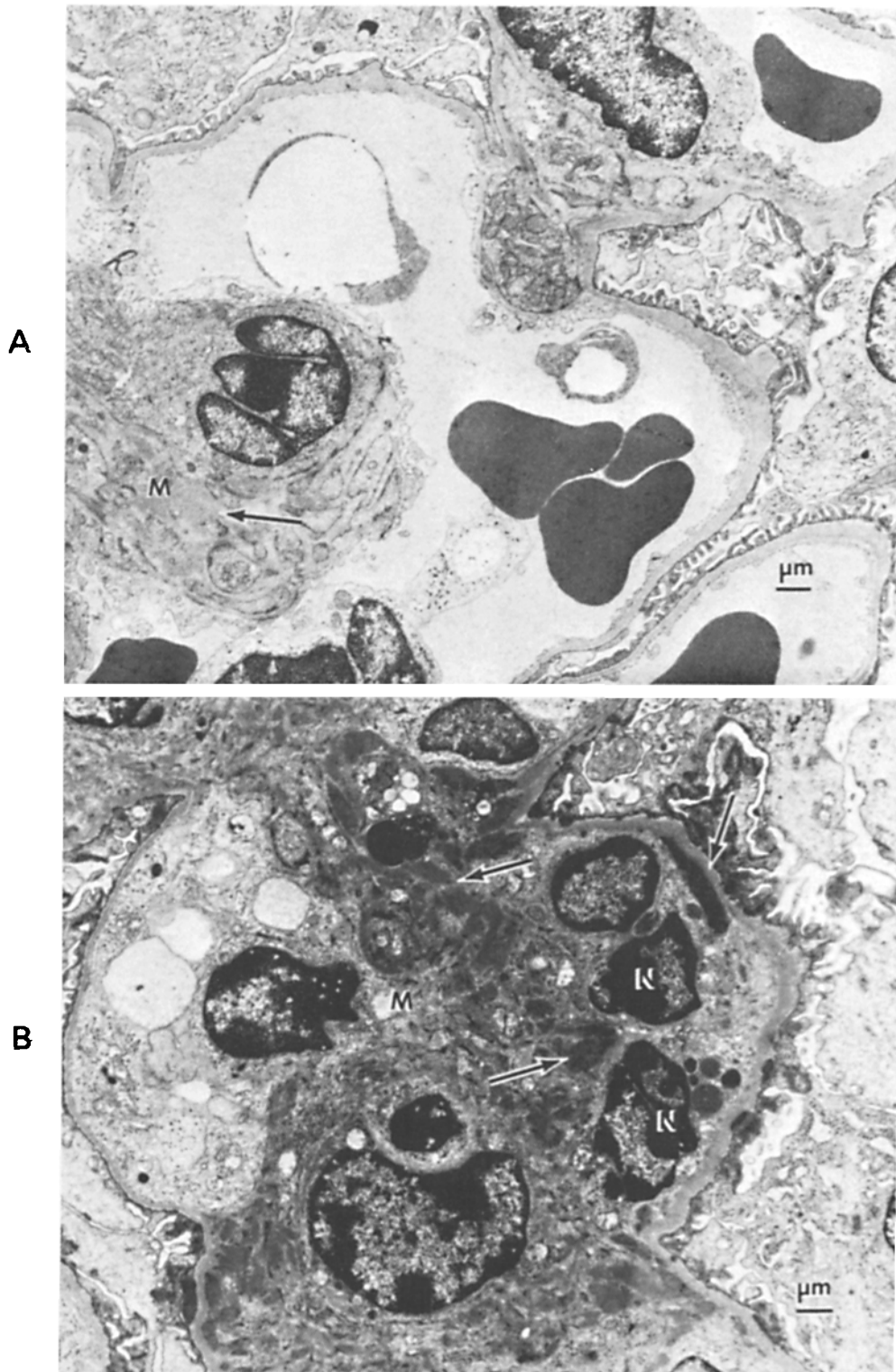


FIG. 7. (A) This is a mesangial area (M) of a glomerulus of a 10-mo-old androgen-implanted female. There are parts of three capillary loops with red blood cells present. There is a small amount of electron dense deposit (arrow) in the mesangial area. (B) This is a mesangial area (M) of a glomerulus in a 10-mo-old sham-operated female. There is considerable amount of electron dense immune complex material (arrows). There are also several mesangial cell nuclei (N) present. Magnification 5000  $\times$ .

TABLE II  
*Effect of a Second Exposure to Maintained Androgen Treatment at 9 Mo of Age*

Group	Antibodies to Poly A (ng Poly A bound/0.2 ml serum)							
	8 Mo		9 Mo		10 Mo		11 Mo	
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
<b>Male</b>								
Sham	96.6	24.6	120.1	20.5	127.8	159.8	76	92.5
Androgen once	120	27.6	131.2	21.6	130.1	130	95	87.4
Androgen twice					101.9*	49.1*	92	12.8*
<b>Female</b>								
Sham	88.9	159.4	99.1	204.3	116.5	497	90.4	375.8
Androgen once	97.8	182.3	—	—	—	—	—	—
Androgen twice	—	—	86.3	103.7*	58.9*	117.9*	48.9*	63.5*

\* A value greater than 2 SD from the mean.

sexes. This suggested that the suppressive effect of androgen had worn off by this time, and led us to attempt a second reimplantation of an androgen-containing capsule. The introduction of this additional male hormone at 9 mo of age aborted the development of IgG antibodies to Poly A in both male and female castrated mice (Table II).

*Androgen Treatment of Intact Males at 8 Mo of Age.* We have observed a spontaneous fall in serum testosterone concentration occurring in noncastrated 9-mo-old male B/W mice (from 4.7 ng/ml to 1.0 ng/ml). After 9 mo, these males develop an accelerated disease associated with high concentrations of IgG antibodies to DNA (6, 10). This temporal correlation between the decline in androgen concentration and worsening of disease led us to institute androgen treatment in intact males at 8 mo of age by the implantation of hormone-containing capsules.

The treated male B/W mice had significantly fewer IgG antibodies to DNA and Poly A when compared to the sham-treated animals. Moreover, the expected switch to IgG antibodies to Poly A was not seen in the treated mice (Figs. 8A and B). At 19 mo of age, 10 of 14 (71%) treated mice were alive, compared to 4 of 9 (44%) sham-implanted controls ( $P < 0.005$ ).

*Combined Neonatal Thymectomy and Perinatal Castration of Male B/W Mice.* We have previously reported that neonatal thymectomy of male B/W mice results in an accelerated disease associated with premature development of IgG antibodies to DNA and enhanced mortality (10). By contrast, neonatal thymectomy prevents the development of IgG antibodies to Poly A, suggesting that this IgM to IgG switch is thymic-dependent (Fig. 9).

Perinatal castration of male B/W mice also decreases survival and leads to early development of IgG antibodies to DNA (8). In these respects, castration resembles neonatal thymectomy. However, whereas the development of IgG antibodies to Poly A is virtually abolished by thymectomy (10), it is promoted by castration of males (ref. 8, Fig. 9). These contrasting effects on the Poly A response offered an opportunity to study whether or not this effect of castration required the thymus.

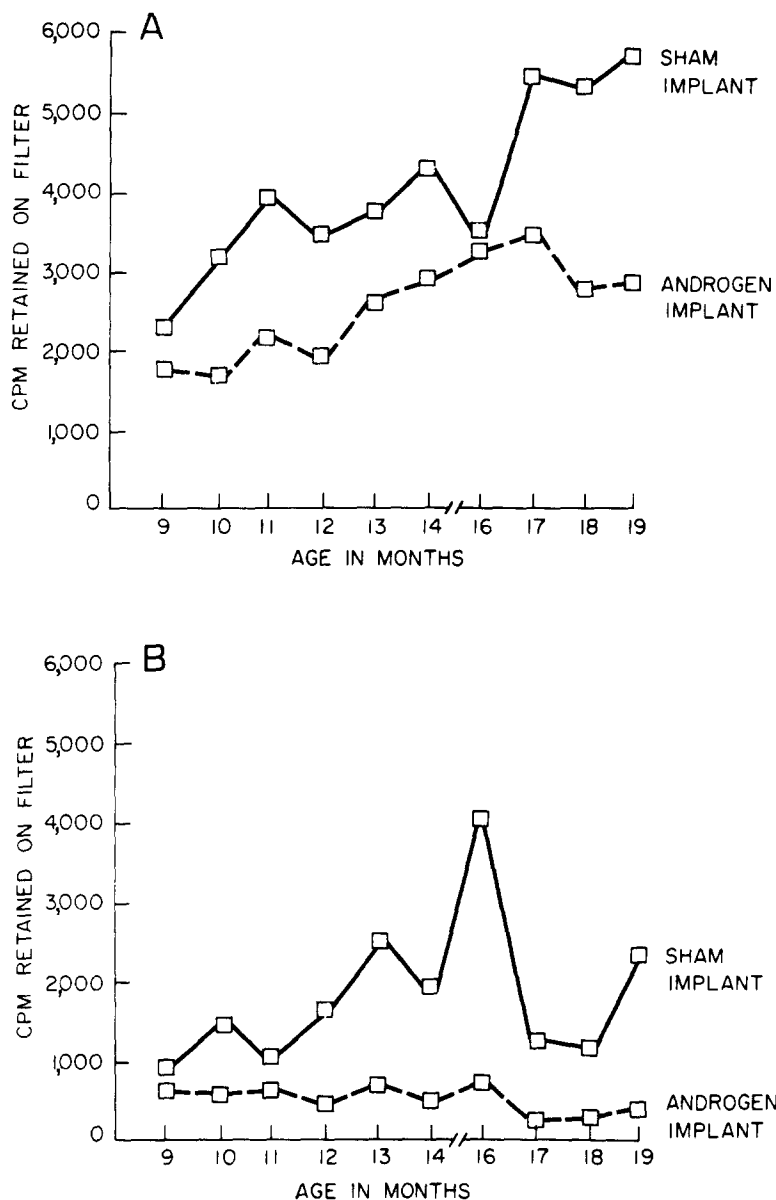


FIG. 8. Effect of androgen treatment of intact, 8-mo-old male B/W mice on (A) IgG antibodies to DNA and (B) IgG antibodies to Poly A.

Male B/W mice subjected to combined neonatal thymectomy and perinatal castration developed the expected accelerated disease but failed to develop IgG antibodies to Poly A despite the castration (Fig. 9). Therefore, the premature appearance of IgG antibodies to Poly A that usually follows castration was prevented if thymectomy was also performed. Thus, the dominant influence on the IgG Poly A response was attributable to the thymus rather than the gonads.

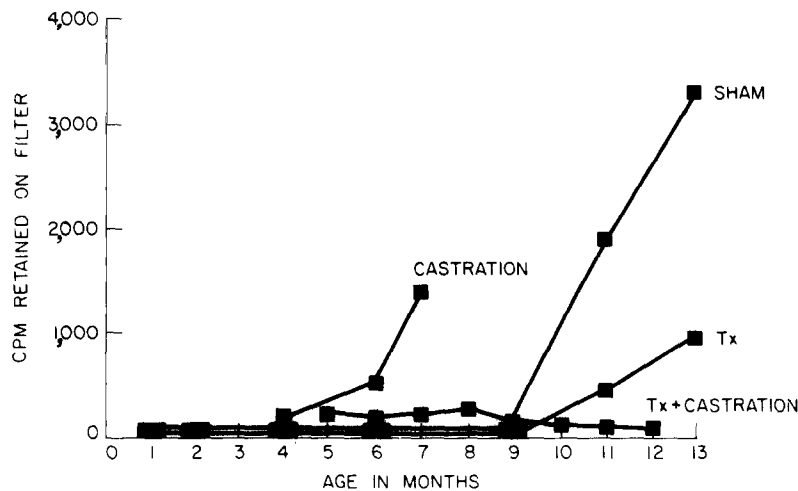


FIG. 9. Effect of prepubertal castration, neonatal thymectomy (Tx) and combined neonatal thymectomy and prepubertal castration on IgG antibodies to Poly A in male B/W mice.

### Discussion

This study demonstrates that both androgens and estrogens can modify the expression of autoimmunity in B/W mice, with male hormone suppressing and female hormone accelerating disease. The sex hormones influence survival, the formation of antibodies to DNA and Poly A, and the severity of immune complex nephritis. These results extend earlier reports that prepubertal castration alone caused a more severe disease in male B/W mice, suggesting a suppressive effect of androgen (8, 14). The combination of castration plus maintained hormone administration used in the current study showed an effect of female hormone as well.

The immunofluorescent and electron microscopic findings suggest that the effect of these hormones on antibodies to nucleic acids and on survival are mirrored in the renal histopathology. Gamma globulin deposition and electron dense deposits are reduced in androgen-treated mice, and increased in estrogen-treated animals. The mechanisms accountable for these findings might involve antibody formation, complement activation, inflammatory cell recruitment, or any combination of these or other factors. Future experiments may be able to define these mechanisms more precisely. It is of interest that certain complement components are dependent on androgen for their expression (15).

These effects of sex hormones probably explain the greater incidence of lupus and other autoimmune disorders in females (16), and the tendency to autoimmunity seen in Klinefelter's syndrome (17). It is interesting that the female:male ratio in lupus is greatest between menarche and menopause, and declines before puberty and with old age (16). An abnormality in estrogen metabolism perhaps resulting in an increased estrogen activity has recently been demonstrated in Klinefelter's syndrome (18). Moreover, it has been shown that the testes in these patients may secrete 10 times the normal amount of estrogen (19).

These opposing effects of sex hormones are not unique to B/W mice, since female mice of normal strains have greater humoral and cellular immune responses than their male littermates (20, 21). Androgens tend to cause lymphocyte depletion (22, 23) and decreased immunoglobulin concentrations (24). Orchidectomy causes an increase in immunologic reactivity (25). There is some suggestion that the suppressing effects of androgen require the presence of the thymus (21). Our studies in mice subjected to combined castration and neonatal thymectomy support this hypothesis. A simple notion consistent with current concepts of immunologic regulation would be that androgens promote the function of suppressor T cells, whereas estrogens favor the development of helper T cells.

The two experiments in which androgen treatment is instituted between 8 and 9 mo of age (either by reimplantation or treatment of intact males) suggest that male hormone may exert a suppressor effect even in older animals. Intact males treated in this manner live significantly longer than sham-implanted controls. These results suggest that androgen treatment may be beneficial in mice with already established autoimmune disease. We have also observed that female B/W mice treated with androgen starting at 3 or 6 mo of age live longer than sham-treated controls.<sup>2</sup> The therapeutic possibilities inherent in androgen administration may be worth consideration in human lupus.

### Summary

NZB/NZW F<sub>1</sub> mice of both sexes were castrated at 2 wk of age and implanted subcutaneously with silastic tubes containing either 5- $\alpha$ -dihydrotestosterone or estradiol-17- $\beta$ . Mice receiving androgen showed improved survival, reduced anti-nucleic acid antibodies, or less evidence of glomerulonephritis as determined by light, immunofluorescent, and electron microscopy. By contrast, opposite effects were observed in castrated mice receiving estrogen.

Intact male NZB/NZW F<sub>1</sub> mice received androgen implants at 8 mo, an age when they develop an accelerated autoimmune disease associated with a decline in serum testosterone concentration. Such treated mice had improved survival and reduced concentrations of antibodies to DNA and to polyadenylic acid (Poly A).

Prepubertal castration of male NZB/NZW F<sub>1</sub> mice results in an earlier appearance of IgG antibodies to Poly A. This effect of castration was prevented if neonatal thymectomy was also performed.

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