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# Prolonged lifespan and high incidence of neoplasms in NZB/NZW mice treated with hydrocortisone sodium succinate

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**Prolonged lifespan and high incidence of neoplasms in NZB/NZW mice treated with hydrocortisone sodium succinate.** This study investigated the effects of prolonged corticosteriod therapy on the course of spontaneous autoimmune disease and oncogenesis in NZB/NZW mice, an animal model of systemic lupus erythematosus. Twenty young female NZB/NZW mice were treated until death with low-dose hydrocortisone sodium succinate (3.3 mg/kg/day), and 21 mice received high-dose hydrocortisone (10 mg/kg/day). Fifteen control mice were injected with saline. Long-term therapy with either dose of hydrocortisone effectively prevented renal disease and prolonged lifespans in NZB/NZW mice. Fifty-six percent of low-dose treated animals developed neoplasms, and 38% of mice in this treatment group died with renal disease. Neoplasms caused death in 76% of mice receiving high-dose treatment. Long-term hydrocortisone therapy was associated with a predominance of sarcomas, which appeared in aged mice after a long period of treatment. In earlier studies conducted in this laboratory, cyclophosphamide treatment prolonged life in NZB/NZW mice. Ninety-seven percent of cyclophosphamide-treated mice developed neoplasms; most tumors were lymphomas or carcinomas. It was concluded that neoplasms occur commonly in old NZB/NZW mice with lives prolonged by immunosuppressive or antiinflammatory drugs. Nevertheless, the specific therapeutic agent used in each study influenced the types of neoplasms appearing in treated mice.

**Allongement de la durée de vie et grande fréquence des néoplasies chez les souris NZB/NZW traitées par l'hydrocortisone (succinate sodique).** Ce travail étudie les effets de la corticothérapie prolongée sur l'évolution de l'affection autoimmune spontanée et de la cancérisation chez les souris NZB/NZW, un modèle animal de lupus érythémateux. Vingt jeunes souris femelles NZB/NZW ont été traitées jusqu'à leur mort par des doses faibles de succinate sodique d'hydrocortisone (3,3 mg/kg/jour) et 21 souris ont reçu des fortes doses (10 mg/kg/jour). Quinze souris contrôles ont reçu du soluté salé. Le traitement au long cours avec l'une ou l'autre des doses a effectivement limité la maladie rénale et allongé la durée de vie. Cinquante six pour cent des animaux recevant la dose faible ont développé des cancers et 38% des souris de ce groupe sont mortes d'une atteinte rénale. Les cancers ont déterminé la mort de 76% des souris recevant une dose élevée. Le traitement au long cours a été associé à une prédominance des sarcomes, qui sont apparus chez les souris âgées. Des études antérieures de ce laboratoire avaient démontré une augmentation de la durée de vie au moyen du traitement par le cyclophosphamide des souris NZB/NZW. Quatre vingt dix sept pour cent des souris traitées par le cyclophosphamide développent des néoplasies, surtout des lymphomes ou des carcinomes. Il est conclu que les néoplasies surviennent chez les souris NZB/NZW âgées dont la vie a été prolongée par les drogues immunosuppressives ou antiinflammatoires. Le traitement spécifique employé dans chacune des études a cependant influencé le type de néoplasie.

Hybrid New Zealand Black/New Zealand White (NZB/NZW) mice spontaneously develop heterogeneous antinuclear antibodies (ANA) detected by indirect immunofluorescence [1], specific antibodies to DNA (anti-DNA) [2], hypocomplementemia [3], and immune complex nephritis [4]. Disease is accelerated in females, and 50% of female NZB/NZW mice die with renal failure at 10 months of age [5]. These animals are accepted as models of systemic lupus erythematosus (SLE) [1]. Since single-drug therapy with prednisone is used to treat most patients with SLE, it is clearly important to determine if corticosteroid therapy prevents autoimmune disease in New Zealand mice. Furthermore, the risk of oncogenesis is increased when an immunologically abnormal host is treated with immunosuppressive drugs [6, 7]. The incidence of neoplasms in NZB/NZW mice receiving long-term corticosteroid treatment has not been determined previously. This study was undertaken to evaluate the effectiveness of prolonged hydrocortisone therapy in preventing early death from renal disease in NZB/NZW mice. Unlike other experiments in which New Zealand mice were treated with corticosteriod drugs, the experimental plan was designed to assess both disease activity and oncogensis in animals receiving hydrocortisone.

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## Methods

*Treatment protocol.* Breeding and maintenance[a] of New Zealand Black (NZB), New Zealand White (NZW), and NZB/NZW mice in the Rackham Arthritis Research Unit were described in another publication [7]. Hydrocortisone sodium succinate (Upjohn Company, Kalamazoo, Mich.) was dissolved in sterile 0.15 M sodium chloride immediately before use. In the low-dose treatment group, 20 mice received daily subcutaneous injections of hydrocortisone, 3.3 mg/kg of body wt in 0.1 ml of volume. The mean age of these mice at the start of the study was 7 weeks (±4 SEM). A separate high-dose treatment group of 21 mice received injections of hydrocortisone, 10 mg/kg/day. The mean age of the high-dose treated mice when therapy began was 8 weeks (± 0.4). Fifteen control mice (mean age, 11 ± 1 weeks) received daily s.c. injections of 0.15 M sodium chloride solution, 0.1 ml. Because the supply of hybrid mice in our colony was limited, treatment was not started simultaneously in each group of animals. Nevertheless, lifespans overlapped in control and treated mice.

Mice were bled from the orbital plexus before treatment was started and after 24 and 52 weeks of treatment. Terminal blood samples were obtained from 40 animals. Sera for ANA tests, anti-DNA determinations, and complement assays were stored in sealed capillary tubes at −20° C. Mice were examined daily, and animals were killed when they appeared moribund or developed palpable masses. Complete autopsy examinations were performed following a protocol described in an earlier publication [7].

*Histological studies.* All tissue sections were examined for abnormalities which may have caused death of the animal. Selected tumor tissue was stained for collagen (van Gieson method) and for myofibrils (Mallory phosphotungstic acid hematoxylin stain). Renal tissue from each animal was stained with hematoxylin and eosin, and with Schiff sulfite leukofuchsin (PAS). Severity of renal disease was scored using an adaptation [8] of the method of Pirani, Pollak, and Schwartz [9]. Abnormalities were counted in 20 glomeruli in a 4-$\mu$ cross-section of each kidney. The glomerular lesions counted were: thickening and hypercellularity of the mesangial stalk, focal glomerular hypercellularity, thickening of the glomerular basement membrane, diffuse glomerular hypercellularity, fibrinoid degeneration, and crescent formation. In our experience, periarterial collections of lymphocytes in NZB/NZW mice coincide with development of glomerulonephritis. Although the pathological importance of these lymphocyte cuffs has not been established, they are absent in mice treated with the potent immunosuppressive drug cyclophosphamide [10]. Mononuclear cell infiltrates around renal arteries were graded on a scale of 0 to 4: 0–no lymphocytes, 1–few lymphocytes, 2–lymphocytes surrounded 25 to 50% of arterial wall, 3–lymphocytes surrounded 75% of arterial wall, 4–lymphocytes surrounded the entire artery.

*Autoantibodies.* Sera samples were tested for heterogeneous ANA on human leukocyte substrate with an indirect immunofluorescent method [10]. Each sample of mouse serum was tested undiluted and at dilutions of 1:4, 1:16, 1:64, 1:256, and 1:1024. Values were expressed as the highest tube number ($1 - \log_2$ of serum dilution) giving a positive test. DNA-binding in serum was determined using a modification of the Farr technique [10]. This assay quantitated the percent of $^{14}$C-double-stranded-DNA bound by 15 $\mu$l of heat-inactivated mouse serum. In this laboratory, values greater than 20% are positive for anti-DNA.

*Complement.* Serum levels of the third component of complement ($C_3$) were determined by single radial immunodiffusion. A pool of serum from healthy Spb:(SW)BR mice (Spartan Research Animals, Inc., Haslett, Mich.) was assigned a value of 100 $C_3$ U/2 $\mu$l. Four dilutions of this reference serum were included with unknown sera from control and treated NZB/NZW mice on each immunodiffusion plate. After incubation, diameters of precipitin rings formed by the reference serum compared to individual dilutions of the same serum plotted as a straight line on a semi-log graph. Serum $C_3$ levels were read from these standard curves and expressed in arbitrary $C_3$ units [11].

*Statistical analysis.* Chi-square analyses [12] and calculations of Student's $t$ test [12] were performed using the MIDAS software package [13] within the MTS system on the AMDAHL 470V/6 computer.

## Results

*Longevity.* Ten mice died of iatrogenic causes. These animals were excluded from studies of longevity, incidence of neoplasia, and renal histology. Cumulative deaths in all other control and treated mice are illustrated in Figure 1. The first control mouse died 15 weeks after the study began, and all mice in the control group were dead after 72 weeks of observation. The mean age at death in control mice was 45 ± (SEM) 4 weeks. Both groups of hydrocortisone-

[a] Animals used in these experiments were maintained in facilities accredited by the American Association for Accreditation of Laboratory Animal Care, and animal experiments were conducted according to the protocol of the Institute of Laboratory Animal Resources, NRC, NAS.

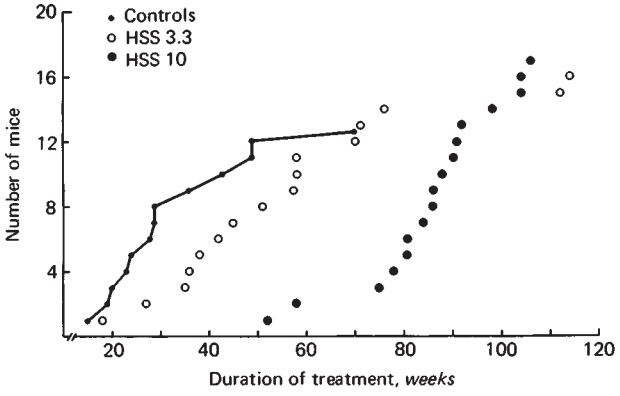


**Fig. 1.** *Cumulative deaths in 46 control and hydrocortisone-treated female NZB/NZW mice.* Ten mice dying from iatrogenic causes were excluded. Lifespans of mice receiving low-dose (HSS, 3.3 mg/kg/day) or high-dose (HSS, 10 mg/kg/day) hydrocortisone sodium succinate were prolonged significantly compared to control mice.

treated animals had significantly increased lifespans. The earliest death in a low-dose treated mouse occurred after 18 weeks of treatment, and 14 of 16 mice in this group were dead after 75 weeks of therapy. In low-dose treated mice, the mean age at death was 57 ± 7 weeks (compared to control mice, $P = 0.03$). The oldest survivors in the study received low-dose hydrocortisone (Fig. 1). One of these aged mice developed malignant lymphoma at 121 weeks of age. The other mouse died with widespread arteritis and severe renal disease at the age of 123 weeks. In mice treated with the high dose of hydrocortisone, longevity was similar to "normal" strains of mice without autoimmune disease. The first death occurred 52 weeks after the study began. Mean lifespan in high-dose treated mice was 94 ± 4 weeks; this value was significantly greater than the mean age at death in control mice ($P < 0.00001$). Two years after high-dose treatment was started, three aged survivors were killed to end the experiment.

*Causes of death.* Table 1 lists the causes of death in control and hydrocortisone-treated mice. *Control mice:* Thirteen saline-injected mice died with glomerulonephritis and widespread arteritis, and two mice died when they were anesthetized for routine bleedings. *Low-dose treated mice:* Nine animals died with neoplasms, and six animals died with renal disease and vasculitis. Pneumonia caused death in one mouse, and four mice died of iatrogenic causes. *High-dose treated mice:* Neoplasms were the most common cause of death in mice receiving prolonged therapy with high-dose hydrocortisone; 13 mice in this group died with tumors. In contrast to mice receiving low-dose therapy, only one animal in the high-dose treatment group died with severe glomerulonephritis. Another mouse receiving high-dose therapy was killed when an epigastric mass appeared after 84 weeks of treatment. On postmortem examination, the mouse had an enlarged liver with subcapsular necrosis and endothelial proliferation in hepatic sinusoids. This change was attributed to drug toxicity. One high-dose treated mouse died with purulent endometritis; another mouse hemorrhaged from dilated ovarian vessels. Four mice in this group died iatrogenic deaths.

*Neoplasms.* Twenty-four tumors arose in 22 treated mice, and two high-dose treated mice carried two neoplasms apiece. In Table 2, neoplastic lesions are separated into four categories on the basis of morphology and staining characteristics. Sarcomas, the most common malignancies, were found in 11 of 22 tumor-bearing mice. An unusual feature of these sarcomas was their tendency to involve the reproductive tract. The sarcomas identified in both groups of hydrocortisone-treated mice were mesenchymal sarcoma of uterus (4), granulosa-theca cell tumor of ovary (4), leiomyosarcoma of ovary (1), liposarcoma (1), and osteosarcoma (1). Lymphoreticular malignancies were found in 6 of 22 mice with tumors. Four mice had lymphoblastic lymphomas; two mice had disseminated lymphomas of the follicular center

**Table 1.** Causes of death in control and hydrocortisone-treated NZB/NZW mice

| | Neoplasms | Renal disease/ vasculitis | Drug toxicity | Infection | Hemorrhage | Iatrogenic[a] | Total |
|---|---|---|---|---|---|---|---|
| Controls | 0 | 13 | 0 | 0 | 0 | 2 | 15 |
| Low-dose hydrocortisone[b] | 9 | 6 | 0 | 1 | 0 | 4 | 20 |
| High-dose hydrocortisone[c] | 13 | 1 | 1 | 1 | 1 | 4 | 21 |

[a] Five mice (2 controls, 2 low-dose-treated, 1 high-dose-treated) died when they were anesthetized for orbital bleeding. Two mice in the low-dose treatment group were arbitrarily chosen to be killed for use in an electron microscope study in the 38th week of treatment. Three aged mice in the high-dose treatment group were killed to end the experiment. Longevity data and postmortem findings in these animals were excluded from this report.

[b] Therapeutic regime was hydrocortisone sodium succinate, 3.3 mg/kg/day s.c.

[c] Therapeutic regime was hydrocortisone sodium succinate, 10 mg/kg/day s.c.

**Table 2.** Neoplasms in NZB/NZW mice treated with low-dose or high-dose hydrocortisone

| | Sarcomas | Lymphomas | Carcinomas | Others | Total |
|---|---|---|---|---|---|
| Low-dose hydrocortisone | 3 | 2 | 1 | 3[a] | 9 |
| High-dose hydrocortisone | 8 | 4 | 1 | 2[b] | 15 |

[a] Benign teratoma of ovary (1), pulmonary adenoma (1), papilloma (1).
[b] Granular cell myoblastoma (1), mixed type alveolar cell tumor of lung (1).

type, previously classified as reticulum cell tumor type B of Dunn [14] but now thought to be of B cell origin [15].

*Renal abnormalities.* Glomerular lesions in control and treated mice are listed in Table 3. The mean glomerular lesion count in kidneys from control mice was 53 ± 2, reflecting severe proliferative glomerulitis. Characteristic glomerular lesions in a control mouse are shown in Figure 2. Large grade 4 periarterial infiltrates were common, and fibrinoid necrosis of renal arteries was found in 9 of 13 control mice. In hydrocortisone-treated mice, the protective effect of corticosteroid therapy was reflected in decreased number of glomerular lesions (Figs. 3 and 4) and suppressed arterial disease. The mean glomerular lesion count in low-dose treated mice was 23 ± 4, periarterial lymphocytes were suppressed, and only two of 16 mice had renal vasculitis. In the high-dose treatment groups, the mean glomerular lesion score was 23 ± 2. Periarterial lymphocytes were minimal, and 2 of 17 kidneys had arteritis. Glomerular lesions were decreased significantly in low-dose treated mice ($P<0.001$) and in high-dose treated mice ($P<0.001$) compared to control mice.

*Autoantibodies.* Treatment with hydrocortisone did not prevent the expected age-dependent appearance of ANA in NZB/NZW mice. Before treatment was started, ANA tests were positive in 33% of control mice, in 14% of low-dose treated mice, and in 24% of high-dose treated mice. After 24 weeks, all control mice were ANA-positive. ANA were present in 13 of 14 low-dose animals and in 17 of 21 high-dose animals. One year after the study began, sera samples from all control and treated mice contained ANA. Positive tests reverted to negative in three mice that received prolonged therapy with hydrocortisone. Two of these mice developed lymphoreticular malignancies, and the third mouse died with an ovarian granulosa-theca cell neoplasm.

Titered ANA values are listed in Table 4. In the first 24 weeks of the study, median ANA titers in control mice increased from 0 to 7. Low-dose hydrocortisone treatment was not associated with depressed ANA titers. Therapy with high-dose hydrocortisone transiently suppressed the expected age-dependent increase of ANA titers. After 24 weeks of treatment, mice in the high-dose group had a median titer of 3. When these mice were evaluated after 52 weeks of treatment and at death, high ANA titers reflected a vigorous autoantibody response.

Mean anti-DNA values in control mice increased from 16% to 38% in the first 24 weeks of the study

**Table 3.** Renal lesions in control and hydrocortisone-treated NZB/NZW mice

| | Glomerular lesions[a] | Periarterial lymphocytes[b] | Arteritis[c] |
|---|---|---|---|
| Controls | 53 ± 2 | 4 (0 to 4) | 9/13 |
| Low-dose hydrocortisone | 23 ± 4[d] | 2 (0 to 4) | 2/16 |
| High-dose hycrocortisone | 23 ± 2[d] | 2 (0 to 4) | 2/17 |

[a] Mean number of lesions counted in 20 glomeruli ± SEM.
[b] Median (range). Collections of lymphocytes around renal arteries were graded on a scale of 0 to 4.
[c] Number of kidneys with arteritis/number of kidneys examined.
[d] Compared to control mice, $P < 0.001$.

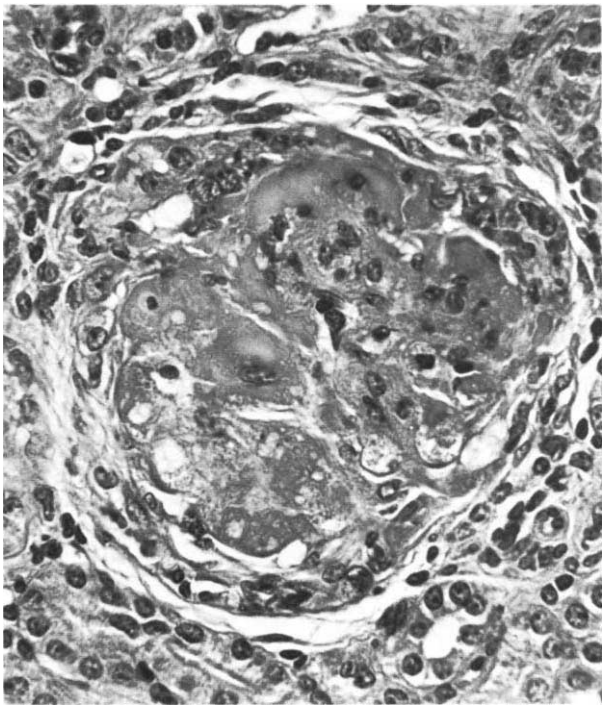

**Fig. 2.** *A severly damaged glomerulus from a 77-week-old control female NZB/NZW mouse dying with renal disease.* This shows fibrinoid necrosis and crescent formation. (Mouse #689; hematoxylin and eosin stain; magnification, ×420.)

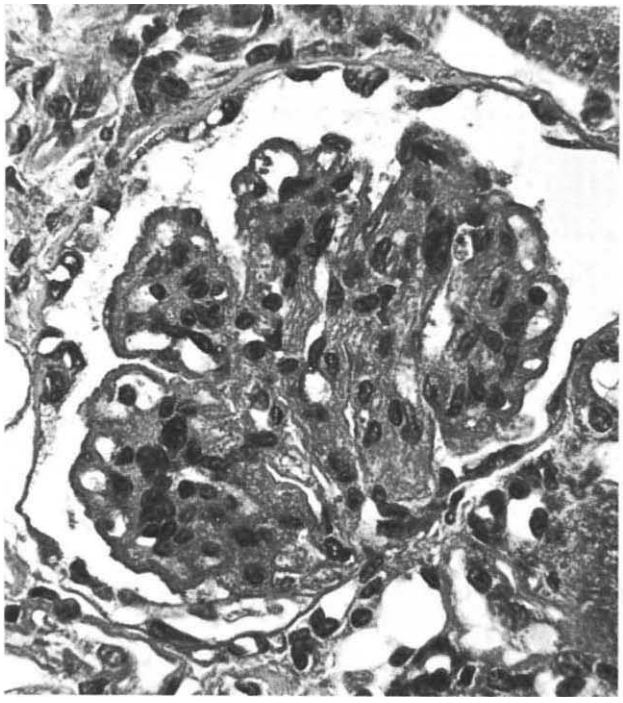

**Fig. 3.** *After 70 weeks of therapy with low-dose hydrocortisone sodium succinate (3.3 mg/kg/day).* This 77-week-old mouse died with diffuse vasculitis and sarcoma of the uterus. Renal glomerular histology did not show inflammatory changes. Thick basement membrane and an area of cellular proliferation are present in this glomerulus. This mouse died at the same age as the untreated control mouse whose renal histology is shown in Figure 1. (mouse #536, hematoxylin and eosin, ×420.)

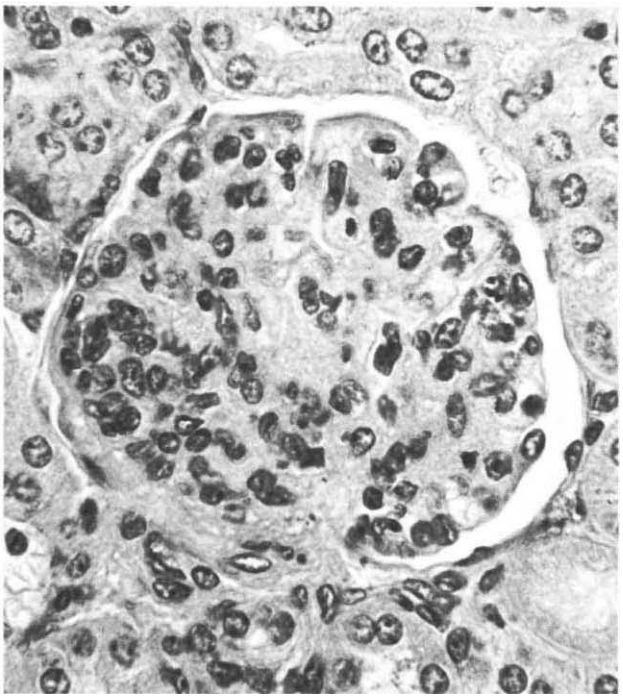

**Fig. 4.** *A 100-week-old mouse that died with widespread lymphoblastic lymphoma and alveolar cell tumor of the lung after 91 weeks of high-dose hydrocortisone therapy (10 mg/kg/day).* Glomerular abnormalities in renal tissue from this mouse were characteristic of the changes in aged mice receiving high-dose treatment. Focal hypercellularity and thick basement membrane were present in most renal glomeruli. (Mouse #804, hematoxylin and eosin, ×490.)

**Table 4.** Autoantibody levels in control and hydrocortisone-treated NZB/NZW mice

| | Weeks of treatment | | | |
|---|---|---|---|---|
| | 0 | 24 | 52 | Terminal |
| Titered ANA | | | | |
| Controls | 0 (0 to 5)[a] [15] | 7 (3 to 9) [12] | 9 [1] | 5 (1 to 9) [12] |
| Low-dose hydrocortisone | 0 (0 to 3) [14] | 7 (0 to 9) [18] | 7 (5 to 9) [10] | 5 (0 to 11) [11] |
| High-dose hydrocortisone | 0 (0 to 5) [21] | 3 (0 to 7) [21] | 7 (1 to 11) [20] | 5 (0 to 9) [17] |
| Anti-DNA | | | | |
| Controls | 16 ± 0.6[b] | 38 ± 5 | 45 | 21 ± 1 |
| Low-dose hydrocortisone | 14 ± 0.3 | 37 ± 3 | 26 ± 4[c] | 22 ± 3 |
| High-dose hydrocortisone | 14 ± 0.3 | 31 ± 3 | 41 ± 3 | 17 ± 1 |

[a] Median (range). Values are expressed as the highest tube number ($1-\log_2$ of serum dilution) giving a positive test for heterogeneous ANA with an indirect immunofluorescent technique. Brackets enclose the number of mice tested at each bleeding.

[b] Mean ± SEM. A modification of the Farr technique was used to test sera for specific antibodies to double-stranded DNA. Values are expressed as percent of $^{14}$C-DNA bound to 0.015 ml of mouse serum.

[c] Compared to high-dose treated mice, $P = 0.01$.

(Table 4). Anti-DNA levels in hydrocortisone-treated mice were similar to controls. After one year of therapy, mean anti-DNA in the low-dose group was 26%. This value was significantly smaller than the corresponding value (41%) in high-dose treated mice ($P=0.01$). Mean anti-DNA levels were low in terminal sera from all three groups of mice.

*Complement (Table 5).* $C_3$ levels were measured in sera samples collected from a limited number of animals before treatment and death. Based upon the work of others, it was anticipated that progressive immune complex disease in New Zealand mice would be associated with progressively falling $C_3$ levels [3]. In control and treated mice, pretreatment $C_3$ values were higher than $C_3$ values in terminal sera. $C_3$ levels did not change significantly, however, in any group of mice during the course of this study.

## Discussion

Disease in NZB/NZW mice is analogous to SLE in humans, and many investigators have used these animals to test the therapeutic efficacy of immunosuppressive and antiinflammatory drugs. Early studies showed that corticosteroids suppressed autoim-

Table 5. $C_3$ levels in control and hydrocortisone-treated mice[a]

| | Before treatment | Terminal |
|---|---|---|
| Controls | 120 ± 7 [13] | 104 ± 17 [7] |
| Low-dose hydrocortisone | 130 ± 27 [5] | 102 ± 14 [11] |
| High-dose hydrocortisone | 119 ± 9 [18] | 90 ± 13 [16] |

[a] Mean ± SEM. Brackets enclose the number of mice tested. Serum $C_3$ levels were determined by radial immunodiffusion. Values were expressed as arbitrary $C_3$ units, determined by comparing precipitin ring diameters produced by NZB/NZW serum with diameters produced by twofold dilutions of standard pooled mouse serum.

mune renal disease in New Zealand mice. In 1968, Casey reported that glomerulonephritis was suppressed in NZB/NZW mice receiving betamethasone, 30 μg/day, for 13 to 14 weeks [16]. Subsequently, Hahn, Bagby, and Hamilton treated female NZB/NZW mice with prednisolone, 5 mg/kg/day (given 6 days/week), from 10 weeks of age until death. Mean lifespan in treated mice was 44 weeks, compared to 35 weeks in control mice [17]. In another study, female NZB/NZW mice received daily doses of methylprednisolone, 1.5, 4.5, or 10 mg/kg, for 2 or 3 months, and the animals were observed for longevity. Lifespans were lengthened appreciably in mice treated with the highest dose of methylprednisolone [18, 19].

In the current study, lifespans were prolonged significantly in both groups of mice treated with hydrocortisone. The mean lifespan in high-dose treated mice was 94 weeks, and seven mice in this group lived longer than 2 yr. To our knowledge, this is the greatest longevity reported in any group of treated female NZB/NZW mice. In other laboratories, the superior therapeutic effectiveness of corticosteroid drugs may have been masked by premature deaths from drug toxicity or respiratory infections. In one experiment, severe hepatotoxicity developed in 15 of 37 NZB/NZW mice treated with prednisolone, 5 mg/kg/day [17]. Only one treated mouse in the current study died with liver disease. Longevity studies employing prednisolone-treated NZB/NZW mice have included treated and control animals who died with bronchopneumonia [20]. Fortunately, infections were a minor problem in hydrocortisone-treated mice in this study. Only 2 of 41 treated animals had postmortem evidence of infection.

Antibodies to double-stranded DNA participate directly in the pathogenesis of immune complex nephritis in New Zealand mice [4]. In untreated animals, increased serum anti-DNA is a marker of active autoimmune disease which precedes renal insufficiency. Nevertheless, hydrocortisone-treated mice with increased longevity produced anti-DNA throughout the first 24 weeks of treatment. The delayed onset of renal insufficiency in these animals may be attributed to the potent antiinflammatory action of hydrocortisone [21]. This impression was reinforced by the histological appearance of kidney tissue from mice in the low-dose and high-dose treatment groups. Thick basement membranes and segmental hypercellularity were common, but exudative changes were not observed in glomeruli from hydrocortisone-treated mice.

In this laboratory, lifelong therapy with immunosuppressive doses of cyclophosphamide has been associated with significant prolongation of life in two separate groups of female NZB/NZW mice. In an earlier study, neoplasms developed in nine of 10 mice receiving s.c. injections of cyclophosphamide, 8 mg/kg/day [7]. Recently, 15 female NZB/NZW mice were treated with cyclophosphamide, 5.7 mg/kg/day. These animals were littermates of the low-dose treatment mice described in this report, and detailed results of their therapy will be reported elsewhere. All 15 mice developed neoplasms, and 11 mice carried multiple neoplasms. Twenty-seven tumors arose in this treatment group; eight carcinomas, seven pulmonary adenomas, six lymphomas, four sarcomas, one histiocytoma, and one chondroma. Although a high incidence of neoplasms was a consistent finding in NZB/NZW mice treated with cyclophosphamide or hydrocortisone, patterns of neoplasia differed in the various treatment groups. In cyclophosphamide-treated mice, lymphomas, pulmonary adenomas, and carcinomas were common neoplasms. In hydrocortisone-treated mice, 53% of tumors were sarcomas.

NZB/NZW mice have two characteristics which should predispose them to develop tumors. These animals carry oncogenic C-type viruses [22], and they lose protective T-cell function after 5 months of age [23, 24]. Nevertheless, unmanipulated New Zealand mice in our colony have a low background incidence of neoplasia. Detailed autopsy examinations revealed malignancies in 3 of 46 untreated NZB/NZW mice who were observed throughout their lifespans [7] (WALKER: to be published). Although premature death from autoimmune disease may have explained small numbers of neoplasms in female mice, our young animals were not tumor-prone. In mice treated with hydrocortisone or cyclo-

phosphamide, neoplasms were the major cause of death. Most tumors appeared in aged mice after a prolonged course of therapy. It is important to note that neoplasms are a common cause of death in old mice from stocks which do not develop autoimmune disease [25]. The current study did not separate the effects of aging from possible oncogenic effects of prolonged therapy of autoimmune disease. Nevertheless, the results of these therapeutic studies support the conclusion that neoplasia is an important consequence of long-term treatment of autoimmune disease in hybrid New Zealand mice.

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