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Received April 11, 1966. P.S.E.B.M., 1966, v122.

Serum Proteins in NZB/Bl and Related Strains of Mice.* (31285)

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(Introduced by C. L. Pirani)
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Since autoimmune hemolytic anemia was first demonstrated in NZB/Bl mice, these animals have been investigated in detail by many workers(1,2,3), and the role of heredity has been demonstrated clearly(4,5,6,7). In patients with Systemic Lupus Erythematosus (SLE) serum gamma globulin levels are significantly elevated(8), and agammaglobulinemia may occur in association with connective tissue diseases including SLE(9,10). Some authors(11) reported elevation of serum gamma globulin levels in relatives of patients with SLE; others did not(12,13).

In view of these observations in human SLE, we studied the serum protein levels of NZB/Bl mice and related pure and hybrid strains in order to determine whether or not hypergammaglobulinemia occurred in these mice and its possible relationship to heredity and to disease activity.

Material and methods. The inbred strains used in this study were NZB, NZW, NZC and the F_1 hybrids NZB-NZW and NZB-NZC. Animals were grouped at 3, 9, and about 18-20 months of age. Where possible, equal numbers of each sex were studied, but breeding difficulties with the colony inevitably resulted in unequal numbers in the various groups.

All 3- and 9-month mice were clinically healthy when blood was drawn. Many of the 18-month-old mice of the NZB and NZB-NZW strains were ill. In our colony of NZB mice the Coombs test was positive in none of the 3-month animals, in 55% of the 9-month animals, and 100% of the 18-month animals (14). In the NZB-NZW colony the test for antinuclear antibodies was positive in about 12% at 3 months, 75% at 9 months, and 100% at 18 months; the test for LE cells was positive in none at 3 months; 35% at 9 months; and 100% at 18 months(15).

Controls used were 3- and 9-month-old mice of the C_3H, NHA, Cb, and Strong A strains. All were clinically healthy, were housed in the same laboratory and received the same diet and care.

Blood samples were drawn in capillary tubes from the retro-orbital venous plexus under light ether anesthesia. Sera were frozen if not used immediately, and were thoroughly mixed in siliconized wells prior to analysis. Total protein was determined in duplicate by a minor modification of the method of Lowry and his colleagues(16). Freshly dissolved crystalline bovine albumin‡ was used as the standard, and was checked by micro-Kjeldahl, using a conversion factor of 6.54(17). Electrophoresis was done in duplicate on cellulose acetate strips(18).

Results. The results are summarized in Tables I and II. There were no significant differences among the 4 control strains and the results therefore have been pooled in Ta-

* These studies were supported by research grants AM05374 and AM10281 from Nat. Inst. of Arthritis and Metab. Dis., USPHS.

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ble I. In both the control and New Zealand strains no differences were found between mice of 3 and 9 months. The 3- and 9-month data have also been pooled in Table I. The distribution of serum albumin and gamma globulin is shown in Fig. 1 and 2.

Total serum protein was significantly higher than in the controls in the NZB, NZW and NZC strains, but not in the two F_1 hybrids. It was higher at 16 to 19 months than at 3 and 9 months in all but the NZC strain. In the NZB strain total protein was elevated at 18 months only in females, whereas a significant elevation in total protein was found in both sexes in the NZB-NZW strain.

The mean serum albumin level was higher

TABLE I. Summary of Serum Protein Levels in 3- and 9-Month-Old Mice.*

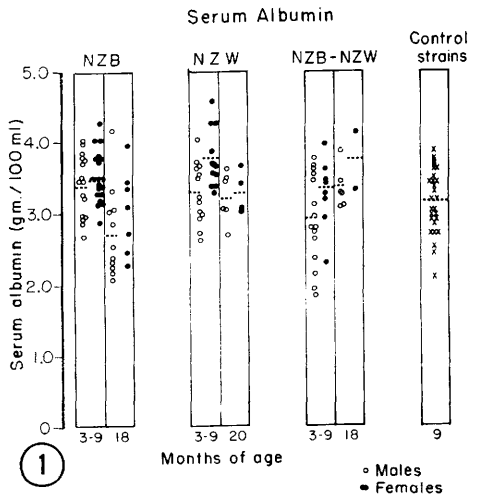
Strain	No. of animals	Total protein	Albumin	Globulins			
				α_1	α_2	β	γ
Controls	31	5.97 ± .69	3.14 ± .44	.63 ± .18	.58 ± .16	1.01 ± .17	.60 ± .17
NZB	36	6.75 ± .75[3]	3.43 ± .43[2]	.73 ± .34	.61 ± .17	1.16 ± .19[2]	.84 ± .20[3]
NZW	24	6.42 ± .38[2]	3.52 ± .47[2]	.70 ± .19	.46 ± .13[2]	1.09 ± .16	.63 ± .19
NZC†	8	6.74 ± .95[1]	3.30 ± .41	.84 ± .23[1]	.65 ± .27	1.22 ± .16[2]	.73 ± .15[1]
NZB-NZW	24	6.22 ± .54	3.08 ± .61	.70 ± .22	.61 ± .18	1.12 ± .18[1]	.75 ± .25[1]
NZB-NZC	24	6.09 ± .77	3.20 ± .73	.68 ± .24	.55 ± .18	1.06 ± .19	.65 ± .24

* The results in g/100 ml are expressed as mean ± 1 S.D.
† Only 9-month animals were available for study.
The significance of the difference from the controls is expressed as follows: [1] $p < .05$, [2] $p < .01$, [3] $p < .001$.

TABLE II. Summary of Serum Protein Levels in Older Mice.*

Strain	Age (mo)	No. of animals	Total protein	Albumin	Globulins			
					α_1	α_2	β	γ
NZB	18	18	7.31 ± .99[1]	2.87 ± .62[2]	.90 ± .27	.82 ± .23[3]	1.53 ± .36[3]	1.19 ± .28[3]
NZW	20	10	7.65 ± .70[3]	3.23 ± .30[1]	.87 ± .23[1]	.79 ± .22[3]	1.59 ± .20[3]	1.16 ± .15[3]
NZC	20	9	7.85 ± .67	3.33 ± .28	.95 ± .12	.84 ± .09	1.67 ± .30[2]	1.05 ± .31[1]
NZB-NZW	18	8	8.29 ± .65[3]	3.46 ± .37	.88 ± .22	1.01 ± .22[3]	1.80 ± .35[3]	1.27 ± .26[3]
NZB-NZC	16	10	8.23 ± .44[3]	3.79 ± .30[2]	1.04 ± .25[3]	.90 ± .12[3]	1.81 ± .20[3]	1.27 ± .23[3]

* The results in g/100 ml are expressed as mean ± 1 S.D.
The significance of the difference from the 3- and 9-month animals of the same strain is expressed as follows: [1] $p < .05$, [2] $p < .01$, [3] $p < .001$.



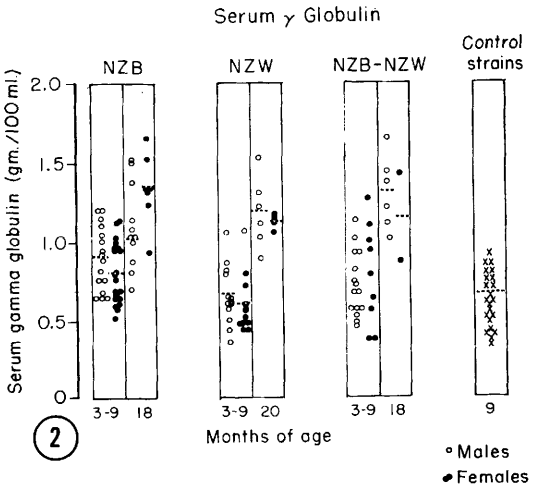


FIG. 1. Serum albumin levels in several strains of mice.
FIG. 2. Serum gamma globulin levels in several strains of mice.

than that in the controls only in the NZB and NZW strains. Changes with age varied from one strain to another.

The *serum beta globulin* level of the NZB, NZC and NZB-NZW strains was significantly higher than that of the controls. The mean level increased with age in all groups, but in the NZB strain this increase was significant only in the females.

Serum gamma globulin levels were increased significantly above the levels of controls in the NZB, NZC and NZB-NZW strains. The level increased significantly with age in all strains, but in the NZB animals the significant increase occurred only in females. Among the older NZB mice the gamma globulin levels were scattered widely, and it appeared that there might be some relationship of the gamma globulin level to the degree of proteinuria. Seven of nine NZB mice with gamma globulin levels less than the mean at 18 months of age had significant proteinuria (300 mg/100 ml or greater), whereas only 2 of the 9 with gamma globulin levels less than the mean had proteinuria of 300 mg/100 ml.

Discussion. The results indicate that the levels of the several plasma protein fractions varied considerably from strain to strain. When compared with the controls NZB mice appear to have elevated serum gamma globulin levels at 3 and 9 months of age, and the gamma globulin level increased further by 18 months. The serum beta and gamma globulin levels in the NZB-NZW F_1 strain were intermediate between those of the 2 parent strains, but the gamma globulin level of the NZB-NZC F_1 hybrid was lower than that in either parent strain.

A definite increase in beta and gamma globulin levels was observed in the oldest mice of all strains. We could find no data on the effect of ageing on serum proteins in mice, but Leonhardt(11) and Das and Bhattacharya(19) noted that serum gamma globulin levels increased with age in man. Woodford-Williams and his colleagues(20) also found that gamma globulin levels tended to increase with age but were significantly elevated only if the subjects were immobile or were not well. A higher incidence of positive tests associated with immune globulins, such as antinuclear factors(21), rheumatoid factor(22), and antithyroid antibodies(23), has also been observed with ageing. Older mice of the NZB and the 2 hybrid strains had definite abnormalities (*e.g.*, anemia, positive Coombs test, or positive tests for antinuclear factors, and LE cells) and many were ill, suggesting that the increased beta and gamma globulin levels might be associated with their disease. The fact that equally significant elevations were found in healthy animals of the NZC and NZW strains is consistent with a change related to ageing *per se*.

Summary. Serum gamma globulin levels of young NZW and NZB-NZC mice were found to be similar to those of 4 control inbred strains. Serum gamma globulin levels of young NZC and NZB-NZW mice were somewhat higher than those of the controls, whereas the levels were significantly higher in young NZB mice. In all strains studied the gamma and beta globulin levels were significantly higher at 16 to 19 months than at 3 or 9 months.

We wish to thank Mrs. Susan Vanek and Miss Mary Ellen Wilsterman for technical assistance.

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Received April 11, 1966. P.S.E.B.M., 1966, v122.

Cytotoxicity and Cell Cycle Studied with a Combined Tetrazolium-Feulgen Reaction.* (31286)

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The response of mammalian cells to irritants such as chemicals, X-irradiation, virus infection, change in temperature, etc., has been found to vary in relation to certain phases of the cellular life cycle(1). When studying the effect of cytotoxic antibodies on cultivated mammalian cells we noticed that within a non-synchronized population paired daughter cells regularly showed the same degree of cytotoxicity whereas marked variability was observed between different pairs. This observation seemed to indicate that the effect of cytotoxic antibodies was likewise influenced by the cellular life cycle. Experiments to substantiate this assumption are described here.

As a more precise tool for morphological evaluation and quantitation of cytotoxicity, we developed a combined tetrazolium-Feulgen stain. This procedure makes it possible to observe simultaneously in the same cell specific nuclear as well as cytoplasmic changes. It is assumed that this staining procedure might have wider applicability. Therefore, a description of the technique is included in this paper.

Materials and methods. Cultured mammalian cells. Chang's human conjunctiva cells were used in these studies. The strain has been continuously maintained in our laboratory for the past 4 years on Eagle's medium without antibiotics supplemented with 17% newborn calf serum. Periodic examinations assured absence of contaminants, particularly mycoplasma.

Synchronization of cell cultures to a degree of 80% was achieved by employing the technique of Terasima and Tolmach(2) with minor modifications.

Preparation of cytotoxic antiserum. Monolayer cultures of Chang's conjunctiva cells were kept on serum-free medium for 24 hours prior to harvest. The monolayers were washed with balanced salt solution (BSS) and scraped from the culture vessel with a rubber scraper. The cell sediment was washed again repeatedly and homogenized in an ordinary culture tube by means of a high speed teflon pestle in the presence of alundum (60 mesh). The cell homogenate, an equivalent of three to five million cells per dose, was repeatedly injected intraperitoneally, subcutaneously and intramuscularly (with Freund's complete adjuvant) into guinea pigs over a period of up to 10 weeks. Sera were collected intermittently by heart puncture and inactivated at 56°C for 30 minutes before use.

Cytotoxicity tests. Coverslip cultures were prepared from synchronized and asynchronous cells. Synchronized cultures were sub-

* Supported by grants E-289 from American Cancer Soc., Inc., and HD-00146 from U.S.P.H.S.