(spontaneous activity, resting metabolism, lipolysis by adipose tissue *in vitro*).

In the interpretation of these results, some caution must be exercised. Alterations in energy expenditure (spontaneous activity, resting metabolic rate) may be secondary features not having a direct cause: effect relationship in the observed increased deposition of body fat. Further, the observed increased lipogenesis *in vitro* may represent adaptation to an excessive calorie intake rather than being a causative factor. However, it would seem reasonable to conclude that the metabolic mechanisms of increased fat deposition differ in some characteristics between these two forms of hyperphagia.

Summary. Protamine zinc insulin (PZI) was administered daily to male rats by subcutaneous injection (2 units/100 gm of body weight) and comparisons were made with saline-injected control animals. As a consequence of PZI administration, the following observations were made: hyperphagia, increased body weight gain, increased lipogenesis from acetate-1-^{14}C and glucose-U-^{14}C, and no alteration in oxidation of glucose-U-^{14}C to $^{14}CO_2$, in lipolysis *in vitro*, nor in the fat concentration of adipose tissue. These results are compared with those reported previously with hypothalamic-hyperphagic rats and it is concluded that the metabolic mechanisms of increased fat deposition differ between the two forms of hyperphagia.

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Deficient Immunologic Functions of NZB Mice* (32910)

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The role of the thymus in the spontaneous autoimmune hemolytic anemia of the NZB mice is still unknown. Thymic lesions have been described in these mice (1,2) although in some cases there is no direct correlation between the appearance of signs of disease and thymic lesions. This was especially noted in animals in which the disease was transferred with spleen cells (3). The effects of thymectomy are even more contradictory since both delay (3) and more precocious development of the disease has been reported in neonatally thymectomized mice (4). The NZB thymus grafts can transfer the disease to thymectomized CBA/T6 mice that do not develop autoimmune phenomena spontaneously although thymus cells from sick NZB mice are unable to transfer the disease to normal young animals (3,4). Although in-

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creased immune responses to sheep red cells have been described early in the life of the NZB mice (5), no reports concerning the capacity of NZB mice to induce the cell mediated thymus-dependent type of reactions have been reported.

To test this function in young and old NZB mice, the Simonsen graft-versus-host assay was chosen since it can be easily quantitated. In addition, it has been repeatedly shown that this function of lymphoid cells is markedly depressed in thymectomized mice (6,7). The experiments presented here demonstrate that spleen cells from old NZB mice which show Coombs positive hemolytic anemia are incapable of inducing graft-versus-host reactions even when the number of lymphoid cells is adjusted to the numbers used from normal mice. On the contrary, young NZB mice are capable of expressing normally this type of reaction.

Material and Methods. NZB male mice of different ages were used in the experiment (Table I). Details of origin, breeding, and care have been reported in a previous paper (8). NZB mice of seventh generation, maintained in our laboratory over the past three years, Strain A mice and (NZB×A) F1 hybrids from our colony were used.

The graft-versus-host assay used in the experiments was the one described by Simonsen (9). Spleen enlargement was assessed in young (NZB×A) F1 hybrid recipients injected intraperitoneally with spleen cells from one of the parent strains. Litters of 8-day-old animals were divided in groups and prepared as: (a) experimental group receiving cells from the animals whose immunologic competence was to be assessed (in the present experiments, old NZB donors); (b) negative controls receiving syngeneic cells; and (c) positive controls receiving cells from normal young mice of NZB and A strains. Eight days after cell administration the recipient animals were sacrificed, their body and spleen weights were determined, and a relative spleen weight (mg/10 gm of body wt.) was calculated. By dividing the mean relative spleen weight of the experimental group by the mean relative weight of the negative control, the spleen index was calculated. Indices of 1.30 or less are considered negative. The number of spleen cells injected in these tests was usually 20×10^6 but in some experiments (see Table I) 50×10^6 cells were used. The details of preparation of cell suspensions were reported in a previous paper (7). The percentage of lymphocytes in the spleen suspensions was obtained with an aliquot of the suspension in lactate Ringer to which 3% bovine almumin was added, smeared on glass slides and stained with Wright's stain. Coombs tests and hematocrits were performed as described in a previous paper (8).

Results. As is seen in Table I, 480–500 day

TABLE I. Deficiency of Graft-Versus-Host Reactions in NZB Mice.

Donor strain[a]	Age of donors (days)	No. of spleen cells	Lymphocytes (%)	Spleen indices	Mean	Positive
NZB	480–500[b]	20×10^6	36–41	0.96, 1.02, 1.04, 1.06, 1.11, 1.20, 1.20, 1.26, 1.30, 1.32	1.14	1/10
NZB	480–500[b]	50×10^6	36–41	1.12, 1.12, 1.15, 1.20, 1.20, 1.25, 1.27, 1.30, 1.43, 1.50	1.25	2/10
NZB	150	20×10^6	66–69	2.06, 2.27, 2.30, 2.35, 2.42	2.28	5/5
NZB	45	20×10^6	73–76	1.64, 1.67, 1.97, 2.00, 2.04, 2.10, 2.32, 2.35, 2.47, 2.90	2.14	10/10
A	45	20×10^6	73–79	1.57, 1.90, 1.96, 2.04, 2.14, 2.56, 2.90, 2.92, 2.97, 3.50	2.44	10/10

[a] All donors were male; all test litters were 8 day old (NZB × A)F1 hybrids.
[b] Positive Coombs test; all other donors were Coombs negative.

old Coombs positive male NZB mice had a marked decreased graft-versus-host reactivity. Only 1 of 10 animals had a spleen index higher than 1.30, the higher limit for the negative assays. The same negative results were obtained when the total number of nucleated cells in the inoculum was adjusted to contain 15–20 $\times$ 10^6 lymphoid cells. This suggests that the effect was probably not related to a simple decrease of lymphoid elements in the spleens of the old NZB mice, but to some type of more specific modification of the lymphoid competence. Coombs negative NZB at 45 or 150 days of age were able to induce positive graft-versus-host reactions. As will also be seen from Table I, a decrease in percentage of lymphoid elements in the spleen of the old NZB mice was observed. The 480–500-day-old mice had 36–41% lymphoid cells in their spleens while higher values ranging from 66 to 76% were observed in younger animals. The hematocrits of the old Coombs positive animals were decreased (36–41%) as compared with the younger Coombs negative animals (46–53%).

Discussion. Several possible explanations for these findings need be considered. For example, the decreased capacity to exercise graft-versus-host activity may relate simply to the aging process as shown in CBA mice using the same type of test (10). Immunological deficiencies may occur in the aged mice in an irregular fashion, depending on the type of stimulation and the assay chosen. In addition, the aging process may selectively influence the thymus dependent type of reactions, mainly homograft rejection, graft-versus-host reactivity and delayed sensitivity. In several reports different immunological parameters have been found to be depressed in aging mice (11,12).

Another possible explanation is that the decrease in the graft-versus-host reactivity is secondary to the disease of the NZB animals since the young healthy NZB mice were capable of expressing normal reactivity. Such an effect might be mediated through alterations in the thymic function, manifested by a decreased activity of the thymus dependent population of cells. Such a deficiency could permit the full development of the autoimmune phenomenon since neonatally thymectomized mice have been shown to develop such reactions in high incidence (8). The deficiency of the graft-versus-host reactivity may also be due to an autoimmune process directed toward the lymphoid cells of the NZB mice, interfering in some way with their capacity to exercise normally their immunologic functions. A similar type of inhibition of lymphocyte function has been observed after treatment of mice with antilymphocyte serum (13). An alternate possibility is that the spleen cell preparations from autoimmune NZB mice, transferred to the F1 hybrid for the test, contain or have the potential to produce autoantibodies that protect the recipient from the graft-versus-host reaction. This possibility must be considered because Batchelor and Howard (14) have shown that antiserum prepared against one component of the F1 hybrid combination may prevent the graft-versus-host reaction produced with the opposite parent strain cells. Another possible explanation for these observations in the NZB animals is that NZB mice are regularly infected with a viral agent associated with the development of their autoimmune process (15,16) and it seems possible that graft-versus-host reactions are rendered ineffective by a direct or indirect consequence of the viral invasion. In support of this postulate are the observations (17,18) that infection with measles or rubella viruses can interfere with responsive functions of the thymus dependent population of lymphoid cells both *in vivo* and *in vitro*.

Additional experiments performed in our laboratories show that old Coombs positive NZB mice may have impairment of ability to achieve homograft rejection as demonstrated by the inability to reject normally carcinogen induced lymphomas of C3H origin. Further, young or old NZB females grafted with NZB male skin accept permanently the grafts which indicate that this strain either does not express the sex-linked histocompatibility gene prominent in other black strains (19) or that an immunological inadequacy, revealed with this weak histocompatibility barrier, exists in the NZB animals even in early life.

Summary. Spleen cells from Coombs posi-

tive 480–500-day-old male NZB mice were incapable of inducing graft-versus-host reactions when injected into (NZB×A) F1 hybrids. This occurred even when the total number of nucleated cells injected was adjusted to contain 15–20 × 10^6 lymphoid cells. Younger, 45 and 150 day old, NZB mice were capable of inducing graft-versus-host reactions in a normal fashion.

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Effects of Vitamin B_6 and B_1 Deficiencies and Restricted Caloric Intake on Tissue Zinc Levels* (32911)

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Studies from this laboratory have suggested that there is a decreased availability of insulin in vitamin B_6 deficient rats (1). Although the role of zinc on insulin metabolism has never been determined, associations of zinc and insulin have been reported for more than 30 years. Recent *in vitro* studies (2,3) using isolated epididymal adipose tissue have clearly shown that zinc in concentrations in the range of those observed in human and rat plasma and higher inhibit insulin activity. Hsu (4) has reported that vitamin B_6 deficiency in rats results in decreased zinc content of plasma, liver, pancreas, and heart tissues. Work in this laboratory using a different experimental protocol appeared in disagreement with Hsu's conclusions. This report contains a study of the effects of vitamin B_6 deficiency on tissue zinc concentrations.

Since some pyridoxal enzymes are magnesium dependent and it has been shown that magnesium supplementation of vitamin B_6 deficient rations for rats prevents at least in part some effects of vitamin B_6 deficiency, [protects against calcium oxalate urolithiasis, increases urinary citrate and decreases xanthurenic acid excretion (5)] the effects of vitamin B_6 deficiency on tissue Mg levels

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