observed under conditions of depressed hormonal degradation is intracellular, and is not seen under normal conditions because of rapid destruction of the hormone. A similar mechanism has been proposed for insulin action upon glucose utilization by liver(3). Another possibility is that the insulinase system of the kidney, like that of liver and pancreatic beta cells, involves the splitting of the molecule into A and B chains, and that one or both chains serve metabolic functions generally attributed to insulin (*e.g.*, protein synthesis, fatty acid esterification, K^+ flux, etc.), while other effects require the presence of the intact hormone. In any case the general concept of insulin sensitivity would require reevaluation.

Summary. Retardation of the rate of insulin degradation results in a highly significant insulin effect upon glucose utilization by the rat kidney. Insulin inactivation may be inhibited by lowered incubation temperature or the *in vivo* and *in vitro* administration of alloxan. These observations suggest that under appropriate conditions, tissues heretofore believed to be insulin-insensitive may be shown to respond to insulin.

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Received February 28, 1967. P.S.E.B.M., 1967, v125.

Reduced Immune Potential of Aged Mice: Significance of Morphologic Changes in Lymphatic Tissue.* (32230)

M. G. HANNA, JR., P. NETTESHEIM, L. OGDEN,† AND T. MAKINODAN
Biology Division and NCI-AEC Carcinogenesis Program, Oak Ridge National Laboratory, Oak Ridge, Tenn.

A number of investigations have suggested that immune competence decreases during aging(1-7). Recent studies of the immunological potential of aged (*i.e.*, ~2.5 to 3-year-old) mice by Makinodan and Peterson(5,6) have shown (a) that primary antibody-forming potential decreases with age and (b) that this decline is mainly due to a reduction in the number of potential antibody-forming cells. These results were obtained both (a) from intact animals and (b) by the *in vivo* culture method in which a given number of spleen cells from donors of different ages are cultured together with the test antigen in heavily X-irradiated young adult (*i.e.*, 12- to 14-week-old) isologous recipients.

Any study of the immune reaction in lymphatic tissue of aged animals is complicated by a number of specific and unspecific pathologic variables associated with aging.

* Research jointly sponsored by Nat. Cancer Inst., and U. S. Atomic Energy Commission under contract with Union Carbide Corp.

† Dept. of Pathology, Medical College of Georgia, Augusta.

In connection with the studies of Makinodan and Peterson(5,6), it was important to determine whether the observed reduction in the number of immunocompetent cells is caused by specific pathologic changes in lymphatic tissue such as lymphoma, or by more general changes associated with aging such as atrophy. It was also necessary to find out to what extent the spleen is representative of the immunologic capacity in the intact aged animal.

There is considerable literature concerning the pathology of aging(2,8-11). In general, these reports have been excellent descriptive studies which have not been primarily concerned with loss of function in the systems possessing pathologic disorders or in related systems. It was not the intent of the present studies to give detailed pathology of aged mice but rather to correlate the physiologic reduction in the immune response, a proliferating and differentiating system, with specific histopathologic changes and/or, more generally, with atrophy and unspecific degenerative changes in the lymphatic tissue.

Materials and methods. Animals. Male and female $BC3F_1$ [(C57BL/$_6$♀) × (C3H/An ♂)F_1 cum.] mice, ~ 3 years of age and 12 to 14 weeks of age, were used. The median life-span for both sexes is known to be ~ 138 weeks(1). The mice were caged in groups of 5 to 10 and allowed free access to food and water.

Antigen and serology. Sheep blood was obtained fresh in modified Alsever's solution and washed in phosphate-buffered isotonic saline (pH 7.1) by alternate suspension and centrifugation at 700 × *g*. Either 1 ml of a 1.0% or 1 ml of a 10.0% suspension of erythrocytes (SRBC) ~ 2.5×10^8 and ~ 2.5×10^9 cells (in phosphate-buffered isotonic saline) was injected intravenously (*i.v.*).

Individual serum samples were obtained and titrated with a Cooke microtitrator by a standard 2-fold dilution technique.

Procedure. Three studies were conducted. The first one was carried out to obtain preliminary information on the types and frequency of lesions likely to be encountered in 3-year-old $BC3F_1$ mice. Thirty-one uninjected mice (17 females and 14 males) were used. All animals were 3 years old in the month they were killed. Autopsies were performed; body, spleen, and liver weights as well as pooled weights of 2 brachial and 2 inguinal lymph nodes were recorded for each mouse. Blood was taken for red and white cell counts as well as serum for serology. All tissue samples were fixed in Bouin's fluid and stained with hematoxylin and eosin (H&E).

The purpose of the second study was to determine the hemagglutinin response in young adult (12 to 14 weeks of age) and in aged (3-year-old) $BC3F_1$ mice. Mice in each age group were injected *i.v.* with 1 ml 1% SRBC, and groups of mice (between 5 and 10) were killed at days 1, 2, 3, 4, 6, 10, and 20 after injection. Along with serology, autopsies were performed on each animal.

The third study was undertaken to determine the response of splenectomized young adult and aged mice to 1 ml of 1% and 10% SRBC, respectively. Blood was obtained by orbital bleeding 1 week prior to splenectomy to determine the level of natural SRBC hemagglutinin titer. Seven days after splenectomy, groups of young adult and aged mice were injected *i.v.* with 1 ml of 1% and 10% SRBC, respectively. On days 6, 10, and 15 after antigen injection, groups of splenectomized and nonsplenectomized young adult and aged mice were killed. Blood was taken for serology, and autopsy was performed on each mouse. All spleens were fixed for histology at the time of splenectomy.

Results. Histopathology of 3-year-old mice. The frequencies with which major histopathologic changes occurred in all aged mice used in this study are listed in Table I. Many animals possessed more than one of the categorized disorders.

Forty-six percent of the 3-year-old mice had neoplasms involving hematopoietic or lymphopoietic tissue. No significant difference could be determined when comparing incidence between male and female in this strain. The most characteristic lymphoma was a non-thymic reticulum cell sarcoma of

TABLE I. Incidence of Lesions of Major Categories in 3-Year-Old Male and Female $BC3F_1$ Mice.

Site	Incidence (%) Primary neoplasms	Other lesions
Reticular tissues		
Lymphoma Lymphosarcoma	46	—
Mesenteric lymph node disease	—	95
Lung	6	8
Liver	4	4
Reproductive organs	1	6
Kidney	1	4
Other sites	5	12

spleen and lymph nodes often involving the liver and intestinal tract. The other lymphomas were a heterogeneous group. Mesenteric lymph node disease(12,13) was a characteristic finding in the aged mice. This was characterized by enlargement of the node up to 4 to 10 times the normal size. The mesenteric lymph nodes showed wide, blood-filled spaces with incomplete endothelial linings which disrupted the normal morphology of the nodes. The nature of the condition could not be determined.

Hemosiderosis was a striking histologic feature in the spleen and occasionally in the medulla of superficial lymph nodes. It was assumed that the hemosiderosis was a result of hemolytic anemia since the average red cell count in the aged mice was 5.0×10^6 RBC/mm^3 compared with 9.5×10^6/mm^3 in the young adults.

The superficial lymph nodes (brachial and inguinal) in those mice which showed no lymphatic tissue neoplasms were also morphologically altered. A distinguishable cortex was infrequent in these nodes, and lack of germinal centers was characteristic. A prominence and hypertrophy of reticular cells in the cortical region could be observed. This reticuloendothelial hyperplasia in the lymph nodes has previously been described by other investigators(12). The medulla of the superficial nodes contained abnormally large numbers of plasma cells, macrophages (many of which contained pigment), and mature lymphocytes.

The spleen in the 3-year-old mice free of lymphomas contained in many cases intact lymphatic nodules in which germinal centers could be identified. However, in general, the spleens showed atrophic white pulp. The red pulp had granulocytic infiltration and large numbers of megakaryocytes. Occasionally hemosiderosis was observed, but no conspicuous amyloidosis was present in any of these animals.

A variety of other pathologic changes other than those mentioned above was detected in these mice (Table I).

Hemagglutinin response of young and aged mice. The primary antibody-forming capacity of young adult and aged mice (whose lymphatic tissue showed no sign of malignant disease), as measured by hemagglutinin production against 2.5×10^8 SRBC injected *i.v.*, is shown in Fig. 1. The young adult mice showed a latent phase of about 2 days, with a 48-hour log phase between days 2 and 4. The titer was sustained through day 20. In the intact aged mice, the latent phase was 2 days, with an approximate 6-day log phase reaching a peak titer on day 10. The hemagglutinin titer then decreased between day 10 and day 20.

The hemagglutinin response in aged mice with malignant diseases of lymphatic tissue was very erratic. The natural hemagglutinin titer of the aged mice with lymphatic tissue neoplasia (Exp. 1) was 2.6 $\log_2$ units with a standard deviation of 2.3 (range 0-8). At day 3 after antigen stimulation (2.5×10^8 SRBC), in a sample of 5 mice the mean titer was 2.6 $\log_2$ units, with a range from 0 to 8.

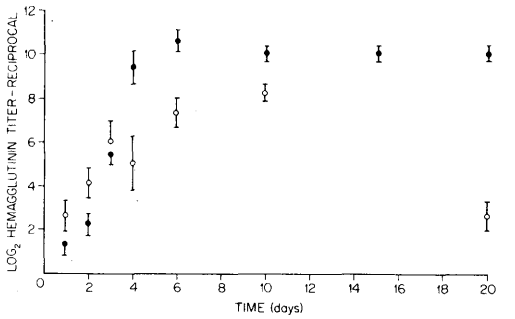

FIG. 1. Serum hemagglutinin production in young adult and aged mice injected with 1 ml of 1.0% SRBC (2.5×10^8 cells). Each point is the mean of 4 to 5 mice. |-●-|, young adult mice; |-○-|, 3-year-old mice (free of malignant diseases of lymphatic tissue); vertical bars are standard errors.

At day 6 in a sample of 4 mice the mean hemagglutinin titer was 5.5 $\log_2$ units, with a range from 0 to 9.0.

Effect of splenectomy and antigen dose on hemagglutinin response of young and aged mice. The histopathology of lymphatic tissue of aged mice free of lymphomas showed that the degenerative and atrophic changes were much more severe in non-splenic lymphatic tissue than in the spleen. This suggested that the decrease in capacity to respond to a test antigen might be greater in the non-splenic lymphatic tissue. Therefore, the hemagglutinin response to 2 different antigen doses was studied in intact and splenectomized young adult and aged male mice. The results are shown in Table II.

The mean background hemagglutinin titers of young and aged mice were determined prior to splenectomy and antigen injection, and were found to be 1.2 and 1.7 $\log_2$ units, respectively. The variation, however, was greater in the aged than in the young mice.

The relative hemagglutinin responses of the intact young adult mice to the 2 antigen doses were not noticeably different, whereas the responses of the intact aged mice seemed to differ mainly in that higher titers were sustained through day 15 with the larger antigen dose. The overall response in aged mice was about 4 $\log_2$ units below that of young adult mice. Thus, aged mice have approximately 10% of the hemagglutinin-forming capacity of young adult.

The aged splenectomized mice showed no response significantly above background when injected with the lower antigen dose, but produced 1 to 2 $\log_2$ units above background when stimulated with the higher dose. The young adult splenectomized mice produced 4 to 5 $\log_2$ units above background at both dose levels. Thus, the non-splenic lymphatic tissue in the aged mice has about 12% of the antibody-forming capacity of that of young adults which was detectable only at high antigen dose levels.

These experiments therefore confirm the previous findings by Makinodan and Peterson (5,6) that the aged animals have only about 10% of the antibody-forming capacity of the young adults. In addition, they demonstrate that most of this remaining activity resides in the spleen.

TABLE II. Hemagglutinin Production to Sheep Erythrocytes in Splenectomized Young Adult and 3-Year-Old BC3F$_1$ Male Mice.*

Treatment	Background $\log_2$ titer	1 ml 1% SRBC			1 ml 10% SRBC		
		$\log_2$ titer			$\log_2$ titer		
		Day 6	Day 10	Day 15	Day 6	Day 10	Day 15
Young adult mice							
Non-splenectomized	1.2 ± .2	—	10.0 ± .1 (10)	10.0 ± .2 (10)	10.0 ± .1 (10)	9.5 ± .1 (10)	9.5 ± .2 (10)
Splenectomized		—	5.0 ± .5 (10)	5.3 ± .5 (10)	5.1 ± .6 (9)	6.3 ± .8 (10)	5.6 ± .4 (9)
Aged mice							
Non-splenectomized	1.7 ± .7	—	8.3 ± .3 (6)	3.3 ± .4 (7)	6.3 ±1.0 (9)	6.0 ±1.0 (8)	6.3 ± .9 (9)
Splenectomized		—	1.6 ± .5 (7)	2.3 ± .3 (2)	2.3 ± .4 (8)	2.9 ± .6 (7)	3.7 ± .6 (7)

* Number of mice used at each point in parentheses. Each mean presented with 1 standard error.

Discussion. The hemagglutinin response to test antigen (SRBC) in intact aged mice showed a marked reduction in comparison with that of young adult mice. At both the 1% and 10% SRBC antigen doses, the overall hemagglutinin response measured in the aged intact mice was approximately 10%

of the response in young adult animals. This reduction in primary antibody-forming capacity was determined in senile animals having no specific lesion of lymphatic tissue other than atrophy, suggesting that the decline is primarily due to unspecific atrophy or degenerative changes. Thus, this reduction in immune potential could well be a result of loss of progenitor cells or a decrease in the number of uncommitted immunologically competent cells due to preoccupation with other antigens, as is also indicated by the large number of mature plasma cells in the medulla of superficial lymph nodes. Apparently diseases of the major non-lymphatic organs did not markedly affect the immune competence of these mice since the standard error was low at peak titer (10 days) in animals without malignant diseases of lymphatic tissue though many had malignant and non-malignant diseases of other organs.

The histopathology of the lymphatic tissue of "normal" non-antigen-stimulated old mice showed that atrophy and degenerative changes in splenic lymphoid tissue were less pronounced than in other non-splenic lymphatic tissue. This suggested that the spleen was the major source of immunological competence in the aged animals. Furthermore, results of splenectomy showed that with the lower antigen dose, loss of splenic lymphatic tissue completely eliminated any measurable hemagglutinin production in these aged mice; while with the higher antigen dose there was a weak hemagglutinin response from non-splenic sites, which was only 3-fold higher ($\log_2$ titer) than background and 12.0% of that achieved in the splenectomized young adult mice. This supports the idea that the spleen is representative of the immune capacity of the intact mouse of this age group, but that it is not necessarily representative of the immune potential of the other lymphoid organs.

A major limitation of this study is that we were working with a very select group of animals; that is, those that survived to $\sim$ 3 years of age. The complexities of the physiologic and pathologic states of these aged mice complicate the interpretation of the present studies. However, the importance of further systematic studies of this type in order to establish a baseline for the aged animal is clear. From these parameters, we might better understand the changing incidence of diseases which accompany old age. Also, the role of environmental factors during aging can be more readily evaluated once the physiologic and pathologic states of the aged animals are more clearly defined.

Summary. Hemagglutinin responding capacity to sheep erythrocyte antigen in 3-year-old $BC3F_1$ mice was approximately 10% of the response in normal young adult mice. The reduction in primary antibody-forming capacity was determined in aged animals that had no specific lesion of lymphatic tissue such as lymphoma, lymphosarcoma, suggesting that the reduction was primarily due to unspecific atrophy or degenerative changes. Results of splenectomy in the aged mice suggest that the spleen is the major source of immunological competence in the aged animals and that the reduced immune capacity in these mice is not due simply to failing spleen function.

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Received March 3, 1967. P.S.E.B.M., 1967, v125.