results lead one to speculate that individual pigeons vary more than is desired in this area of metabolism and thus are somewhat limited as a test system for drugs or conditions that modify serum cholesterol levels.

Summary. Old White Carneau pigeons maintained under regulated light (9 hr fluorescent light, 15 hr darkness), temperature (76 ± 2°C), and humidity (about 40%) conditions and in groups of 6 in cages that do not permit flying for a period of over 18 months do not have the seasonal cyclic variations in serum cholesterol possessed by pigeons that have free access to flying areas and are exposed to yearly weather conditions.

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Reduced Antibody Forming Capacity During the Incubation Period of Passage A Leukemia in C_3H Mice.* (28720)

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The present investigation was designed to study the immunologic capacity of mice during the incubation period of Gross's passage A lymphatic leukemia. Mice infected with the virus during the newborn period remain healthy until approximately 3 months of age when an acute lymphatic leukemia develops in almost all of the animals(1). Until that time, even careful histologic examination will not disclose leukemia.

The motivation for this experiment was development of an experimental model which might clarify the clinically observed association between malignancy of the lymphocytic system and immunologic capacity. At least 4 children with the congenital sex-linked type of agammaglobulinemia have developed lymphocytic malignancies(2,3,4). In addition, several cases of acquired agammaglobulinemia have been followed, often years later, by a lymphoma(5) or reticulum cell sarcoma(6). Adults with chronic lymphatic leukemia frequently have a decreased capacity to develop circulating antibody(7,8,9). Patients with Hodgkin's disease often have impaired ability to express cellular allergy as manifested by delayed hypersensitivity(10,11,12). The proposed experimental model held promise of clarifying whether lymphocytic malignancy results in immunologic deficiency because malignant cells crowd out antibody producing cells, or whether it might do so by a more basic effect on the immunologic mechanism.

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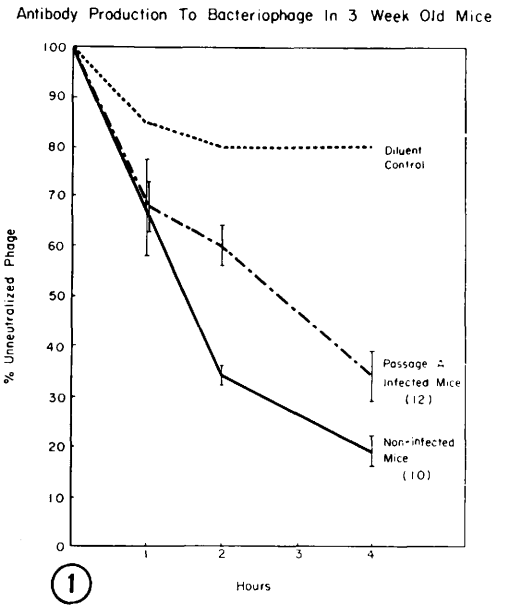
Materials and methods. Animals: C_3H_f/Bi strain mice, obtained from the original colony of Dr. J. J. Bittner, were used. They were weaned at 3 weeks of age and separately caged. Experimental and control groups were identically managed except for administration of the virus.

Passage A virus: Leukemic C_3H mice, representing the 32nd passage of Gross's passage A leukemia originating in AKR mice, were obtained from Dr. Ludwik Gross in June, 1962. The virus was passaged in C_3H/Bi mice as reported by Gross(13) until used in the 35th passage for the present experiment. The experimental mice were given an intraperitoneal injection of 0.3 ml of a cell free filtrate of the virus on the 3rd or 4th day of life.

Antigen and assay method: The antigenic stimulus was T_2 bacteriophage (*E. coli* host). The phage was grown and partially purified by standard methods(14). Each mouse received an intraperitoneal injection of 10^{10} phage particles at either 3 or 9 weeks of age, and was bled *via* the orbital sinus 14 days later. Antibody content of each serum was

assayed by the plaque neutralization technique(14). The control group of animals also received identical bacteriophage stimulation but were not infected with the leukemia virus. A diluent control was incorporated to provide an indication of the extent of the spontaneous phage neutralization which occurred during the assay procedure.



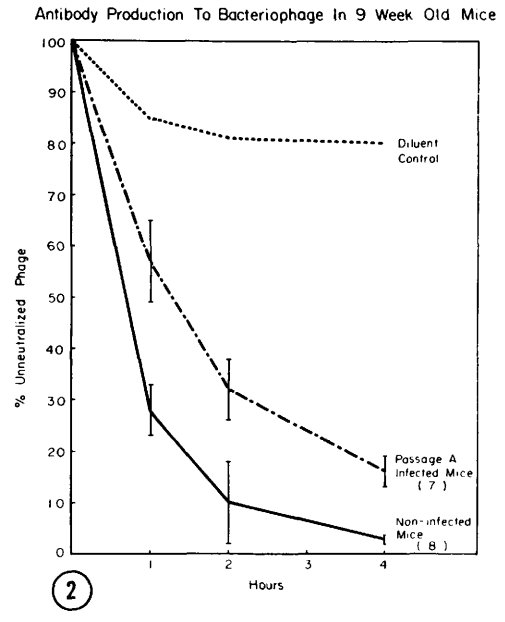


FIG. 1, 2. Each curve represents mean phage neutralizing capacity of individual serums from the designated group. Brackets denote standard error of mean.

Results. Fig. 1 graphs the rate of phage neutralization of sera from the groups stimulated with antigen at 3 weeks of age, and Fig. 2 gives comparable data for the mice stimulated at 9 weeks of age. Each point on the curve is the mean percentage of unneutralized phage remaining in the reaction tubes after incubation for the periods indicated. The vertical brackets indicate the magnitude of the standard error.

In both the 3 and 9 week old groups, the leukemia-infected mice were less able to produce phage neutralizing antibody than were the non-infected animals. The differences are significant, consistent, and apparently quantitative rather than qualitative. They are similar to those observed in comparisons of neonatally thymectomized and unthymectomized mice(15,16).

The reactivity to bacteriophage increases between 3 and 9 weeks of age in both the infected and non-infected animals but a similar difference between their responses remains. The cause for such an occurrence is unknown.

Discussion. A number of observations may bear on the interpretation of these data. Passage A virus has a tropism for thymus and lymphoid cells as demonstrated by electron microscopy(17) and by the ease of passage of the leukemia using thymus and other lymphoid organs as a source of infectious material(18). Recent studies have emphasized that the life-long immunologic capabilities of the mouse are profoundly affected by thymic function and thymus-dependent development of the other lymphoid tissues during the first few weeks of life. Certainly surgical ablation of the thymus at 1-4 days of age permanently reduces the immunologic competence of the mouse. Perhaps the virus infection, introduced in the newborn period, is another factor capable of interfering with normal thymic function and hence normal immunologic maturation during these first weeks of life. Rowe and Capps(19) have reported a virus capable of causing necrosis of the thymus in newborn mice, but have not

reported studies of the immunologic capacity of such animals. Such investigations would be most pertinent. The effect of other viral infections upon the immunologic system remains to be ascertained.

Another possible explanation for the results obtained is suggested by the observations of Wagner *et al*(20) that viral infections decreased the phagocytic activity of the reticuloendothelial system (RES). Conceivably such decreased RES activity could account for decreased antibody, especially to a particulate antigen such as bacteriophage. The viral infection might interfere with the clearance of bacteriophage and thus with its antigenicity. The experiments of Old *et al*(21) provide evidence contrary to this hypothesis. They found that RES activity was decreased immediately after infecting of mice with transplantable virus-induced tumors, but that it increased within a few days to abnormally high levels and remained elevated. If there is any analogy to the present experiment, RES activity should have been increased rather than decreased when the antigen was administered.

Demonstration of decreased immunologic function in experimental animals bearing malignancies dates back to 1913 with the original observations of Fleisher and Loeb (22). Wharton *et al*(23) suggested that this phenomenon resulted from antibody loss rather than decreased production. The earlier experiment which bears the closest resemblance to our own is that of Old and Clarke(24) utilizing a Friend leukemia model. They were studying primarily RES activity following a cell homogenate-induced leukemia, but mentioned in an abstract that such mice had decreased ability to form hemolysins to sheep red blood cells.

Our model is probably different in that obviously malignant cells were not present in the animals when the immunologic capacity was being assayed. Whatever the correct interpretation of these results, we are encouraged to pursue further the interesting relationship of immunologic competence and malignancy of the lymphocytic system. Other antigens should be used and the secondary immune response as well as the primary immune response assayed. Other viruses and substances affecting cell differentiation should also be studied for their effect on the immunologic mechanism.

Summary. Injection of Gross's passage A leukemia virus in newborn C_3H_f/Bi mice interferes with the development of their responsiveness to bacteriophage T_2. This deficiency was present in mice antigenically stimulated at 3 weeks and 9 weeks of age, and, notably, before obvious malignant cells are present.

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Endotoxin as Adjuvant in Autoimmunity to Cardiac Tissue.* (28721)

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Repeated injections of rabbits(1) and rats (2) with preparations of homologous heart tissue mixed with Freund's complete adjuvant, lead to the production of circulating antibodies specific to heart. Patients with chronic heart disease may also show anti-heart antibodies in their sera(3) and here, the antibody stimulating activity of Freund's adjuvant cannot be invoked in explanation of the auto-immune reaction. One possible mechanism is that chemical changes in, for example, an infarct, may convert it into a form antigenic for the patient(2). A supplementary mechanism may be the "adjuvant" effect of circulating endotoxin, released in patients shocked after myocardial infarction or cardiotomy.

Endotoxin has been demonstrated in the blood and myocardium of shocked animals (4) and these compounds are known to stimulate antibody production to a variety of heterologous antigens(5,6). The following experiments demonstrate the ability of endotoxin to act as adjuvant in immunity to homologous tissue antigen.

Materials and methods. Male albino rats of the Hebrew University stock, weighing 80-90 g, were given 6 weekly injections of pooled rat heart homogenate mixed with Freund's complete adjuvant (Difco) or with endotoxin. Controls received heart homogenate alone, endotoxin, or adjuvant mixed with saline.

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The preparation of the heart homogenate was as previously described(1) and an amount equivalent to 5 mg protein was given per injection.

Endotoxin (kindly provided by Prof. Moshe Shilo) was a highly purified preparation from *E. coli* having an LD_{50} of about 7 μg/kg for rabbits when given intravenously. Rats, given 100 μg/kg intraperitoneally, appeared sick for a few hours but always recovered; this dose level was used for immunization.

In further experiments (Table III) heart homogenate and endotoxin were given at different sites and at different times.

Blood samples were drawn from the tail veins at different intervals and one week after the last injection, the animals were bled out from the throat and the hearts removed, washed in saline and fixed in 10% formalin. Sections of heart tissue were stained with hematoxylin and eosin.

Sera were examined for heart-specific antibodies by the hemagglutination and hemagglutination-inhibition tests previously described(1), using tanned human erythrocytes sensitized with extracts of rabbit or rat heart. Extracts of fresh heart were poor sensitizing antigens for the tanned cells but use of extracts of rat heart homogenate that had been frozen to $-80°$ and thawed to room temperature 5 times increased the sensitivity of the test. The results cited are those with the frozen-thawed rat heart extracts unless otherwise stated.

Results. (Table I). None of the rats re-