The Immune Response of the Lathyritic Rat.* (28434)

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Since the first description of connective tissue changes in lathyrism(1), investigators have turned their attention largely to morphologic and biochemical studies. One such study(2) noted a higher incidence of infection among the experimental animals than among the controls. Similarly, observations made during early studies at this laboratory also indicated an increased incidence of infection in the lathyritic animals. These observations suggested that lathyrism may have altered host resistance to infection. Though several studies have described changes in the hematopoietic system(1,3-6), none have related these changes to host defense mechanisms. Therefore, it was decided to investigate the effect of lathyrism on resistance to infection.

Since host resistance is a complex function, and since the immune mechanism is an important part of resistance, it was decided to limit the study to this one facet, the immune response. This decision was also influenced by the fact that a standardized technique had already been described using human red cells as antigens(7).

Materials and methods. One hundred and four male weanling rats of the Sprague-Dawley strain were divided into 4 equal groups of 13 experimental rats and 13 control rats each. The rats of Group I were bled and sacrificed one day before immunization. Injections of antigen were given to the rats in Groups II-IV on days 1, 3 and 5. The rats of Group II were then bled and sacrificed on day 8, those of Group III on day 12, and those of Group IV on day 16.

All of the rats were weighed, and each experimental rat was paired with a control rat of most similar weight for the purpose of pair feeding. All groups started feeding on the experimental diet 4 days before the beginning of immunization. The experimental diet consisted of ground Purina Fox Chow, to which was added di-(beta-aminopropionitrile)-fumarate (BAPN)‡ in an amount equal to 0.4% of the diet by weight. This diet was fed *ad libitum*. The control diet consisted of ground Purina Fox Chow without the added BAPN, and was given by pair feeding. Both diets were supplemented with additional vitamins in amounts adequate for antibody formation(8) by uniform admixture to the diet. Water was allowed *ad libitum*. Rats were weighed every 3-4 days and on day of sacrifice. Frequent observations were made for signs of lathyrism.

The immune response was first tested by the method of Axelrod *et al.*(7) using human red blood cells as the antigen. Human type O Rh positive blood was collected from a healthy male donor with sterile technique in acid-citrate-dextrose preservative and stored at 5°C for a period of 2-6 days until used. The cells were prepared for use by triple washing in sterile 0.85% saline, then diluting up to a 10% suspension in fresh 0.85% saline. A total of 2.5 ml was injected intraperitoneally into each rat in Groups II-IV, 0.5 ml on day 1 and 1.0 ml on both day 3 and day 5.

Sera for agglutinin titers were obtained from each rat by bleeding from the abdominal aorta under ether anesthesia just before sacrifice. Blood was allowed to clot overnight at room temperature, centrifuged, and the serum pipetted off and stored frozen at −20°C. Sera were later thawed and heated at 56°C for 3 minutes to inactivate complement, thus avoiding hemolysis. Agglutinin titers were determined by macrotechnique, using serial tube dilutions of individual rat sera against a 2% suspension (in 0.85% saline) of triple-washed (in 0.85% saline) red blood cells freshly drawn from the same donor whose blood was used for the immuniza-

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TABLE I. Numbers in Each Column Represent Average of Duplicate Agglutinin Determinations on Individual Rats. Blank spaces indicate that insufficient serum was obtained for duplicate determinations.

	Group I		Group II		Group III		Group IV	
	Lath.	Cont.	Lath.	Cont.	Lath.	Cont.	Lath.	Cont.
	0	0	960	960	480	320	480	240
	0	0	2560	640	960	1280	640	1280
	0	0	1280	1920	640	960	320	1600
	0		2560	2560	640	320	640	1440
	0	0	2560	5120	1280		480	640
		0	1280	1280	640	640	320	320
	0	0	2560	640	320		640	0
	0	0	2560	320	5120	960		
	160	0	640	480	480	640		640
		0	1280	960	640	1520	480	0
	0	0	640	1280	640	320	1280	480
	0	0	1280	2560	640	640	800	480
	0	0	640	960	640		1920	480
Mean	14	0	1686	1591	1009	800	727	717
S.D.	±46	±0	± 973	±1255	±1208	± 491	± 455	± 517

tions. Duplicate agglutinations were performed from a coded system.

Gamma globulins were determined by paper electrophoresis of selected individual rat sera. The paper strips were examined in a densitometer and comparisons made on the data obtained.

Gross examination for changes of lathyrism was made at autopsy. Microscopic study was limited to a search for abnormalities of the lymphoid organs in response to immunization. Pieces of spleen and entire para-aortic lymph nodes were fixed in 10% neutral formalin, embedded in paraffin, sectioned at 7 μ and stained with hematoxylin and eosin.

A separate attempt was made to study the immunologic aspect of host resistance to infection by using a bacterial extract as the antigen. A non-polysaccharide fraction of hemolytic *Staphylococcus aureus*(9) was injected into several rats in doses sufficient to provide 10 mg of antigen nitrogen per kg of body weight. Direct agglutination revealed titers too low for further use in this study.

Results. The immune response is measured by the height of the agglutinin titer as recorded in Table I. The titers of Group I show that, except for one rat, neither the BAPN-treated rats nor their controls had identifiable titers of agglutinins for human red cell antigens prior to immunization. The data for Groups II to IV show the presence of agglutinins in high titer by day 8 and a gradual decline in titer on days 12 and 16. Comparison of experimental and control titers shows no consistent difference between individual members of a pair, either at the time of the greatest agglutinin production, Group II, or during the decline of the agglutinin titer, Groups III and IV. These findings are also reflected in the mean values in Table I.

Results of the electrophoretic determinations of serum gamma globulins are shown in the data in Table II. The range of values is so wide that no consistent difference between BAPN fed rats and their controls is apparent.

Changes in the lymphoid organs at autopsy paralleled the agglutinin response to immunization. Grossly the para-aortic lymph nodes showed prominent enlargement equally in both experimental and control rats. However, during the decline of the agglutinin titers, the lymph nodes of the controls returned more rapidly to pre-immunization size, while those of the experimentals remained enlarged and appeared congested. The spleens were equal in size, although those of the experimentals appeared congested. Careful measurement of spleen weights in a preliminary experiment failed to show any significant difference between experimental and control rats.

Microscopically the lymph nodes showed variable follicular hyperplasia, but were otherwise unremarkable. The follicular cen-

TABLE II. Electrophoretic Analysis of Gamma Globulins.

Group	Gamma globulin of lathyritic rats		Gamma globulin of control rats	
	Quantity	% of total protein	Quantity	% of total protein
I	24.0 mm²	25.4	19.3 mm²	26.5
	21.6	31.7	12.7	27.2
	22.8	28.6	16.0	26.8
II	27.0 mm²	28.8	29.0 mm²	28.4
	23.0	23.6	21.0	24.8
	25.0	26.2	25.0	26.1
III	28.8 mm²	39.3	29.9 mm²	40.3
	18.8	20.7	17.5	26.7
	23.8	30.0	23.7	33.5
IV	25.5 mm²	26.6	16.7 mm²	16.6
	21.2	29.5	30.0	20.8
	23.4	28.1	23.4	18.7

Quantity is expressed in square millimeters representing the area under the curve obtained on the densitometer. Individual values of 2 lathyritic and 2 control rats of each group are shown above the line; their average is shown below the line.

ters of the spleens showed marked hyperplasia equally in both lathyritic and control rats. Other than moderate congestion in the spleens of most lathyritic rats, the only microscopic difference between lathyritic and control splenic tissues was a mild decrease in the numbers of reticular cells in the interfollicular areas of the lathyritic spleens.

At autopsy kyphoscoliosis and exostoses were seen in the bones of the experimental rats.

Discussion. No impairment of the immune response of the lathyritic rat was found by measuring the agglutinin titers. The reliability of this method of measuring the agglutinin titers is indicated by the reproducibility of the results. Duplicate titers determined by coding the sera were identical in 67 instances, showed a one tube dilution difference in 26 instances and a difference in titer of more than one tube dilution in only 4 instances.

The two other aspects of immunologic response studied, electrophoresis of gamma globulins and morphology of the lymphoid organs, also failed to show major differences between the responses of the lathyritic and the control rat. Minor changes in interfollicular cell distribution of lymphoid organs were not related to the agglutinin titers and appeared to involve reticular cells rather than lymphocytes. Visceral congestion was prominent in the spleens of the lathyritic rats.

Poor weight gain and exostoses characteristic of lathyrism became apparent in these animals after 7 days on the experimental diet, yet immunizations were begun after only 4 days on the diet. That these rats were indeed under the influence of BAPN after only 4 days on the experimental diet is supported by our own previous experience and by certain pieces of evidence reported in the literature. Morphologic changes of lathyrism can be seen in the periodontal membrane with the light microscope after 3 days on a 0.2% BAPN diet(10) and in the periosteum of the femur at the site of muscle attachments after one day on a 50% lathyrus pea seed diet(11).

The failure to find an altered immune response in lathyritic rats in the present study provides evidence on only one aspect of resistance. As mentioned previously, the resistance of an animal to infection is not the product solely of its immunologic capacity. It is a complex function composed of several processes, any one of which might be altered in lathyrism. For instance, the inflammatory response to certain kinds of tissue injury has been reported altered in lathyrism(12-14). Just such changes in the inflammatory response could account for alterations in an animal's ability to resist an infectious "in-

jury." However, in the reports cited, the variety of tissue injuries studied included physical and chemical injuries, but not infectious "injury" *per se,* so they provide no evidence bearing directly on the question of altered resistance to infection in lathyrism. Thus, from the results of the present experiment on the immunologic response, there is no evidence that resistance to infection is altered in lathyrism.

This last observation has led to reconsideration of the original hypothesis, that lathyrism caused decreased resistance to infection. Reexamination of one of the early reports(2) from which this hypothesis was formulated reveals that the experimental and control animals were fed different kinds of peas: lathyrus peas and edible peas. It is possible that the observed differences in the incidence of infection in the 2 groups were related to differences in the nutritional components of the 2 kinds of peas rather than to the presence of the toxic principle in the lathyrus peas. On the other hand, these differences may have been a fortuitous association, for the differences in incidence of infections originally observed in our own laboratory have disappeared. It seems reasonable, therefore, to doubt that resistance to infection is altered in lathyrism, until more evidence is available to support the proposal.

Summary. The immune response to injections of human red blood cells was studied in lathyritic weanling rats by direct agglutination and by electrophoretic analysis of serum globulins. No great differences between the response of the lathyritic and the control rats were found by either study. A minor change in the histology of the spleens of the lathyritic rats is unrelated to the immune response. No evidence is found to support the hypothesis that resistance to infection is altered in lathyrism.

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Production of Tumors in Syrian Hamsters After Oral Administration of Polyoma Virus. (28435)

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Polyoma virus has produced tumors in Syrian hamsters after inoculation by a variety of routes. Subcutaneous inoculation of newborn hamsters resulted in production of sarcomas in the kidneys, heart, liver and subcutaneous tissues(1), while subcutaneous inoculation in young adult hamsters produced only subcutaneous sarcomas(2). Meningeal sarcomas were produced after intracerebral inoculation of the virus in newborn

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