Influence of Splenectomy on Resistance to Pneumococcal Infection in Rats. (24578)

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Recent clinical reports have suggested that there is enhanced susceptibility to infection in splenectomized infants and children. King and Shumacker(1) reported 5 grave infections among splenectomized infants. Smith *et al.* (2) described 19 cases of severe infection in children following splenectomy; however, many of their patients had Cooley's anemia, a chronic debilitating disease. In Gofstein and Gellis' analysis(3) of 107 splenectomized children, there were only 4 cases of severe infection. Furthermore, the experience of others does not support the concept of enhanced susceptibility to infection in the splenectomized child. Doan(4) noted only 3 severe infections among 87 children who had splenectomy. Likewise Pierce (personal communication) and Walter and Chaffin(5) have seen no evidence of increased susceptibility to infection in their respective series of splenectomized infants and children. It is difficult to draw definite conclusions from uncontrolled clinical experiences. In animals, it is well known that splenectomy may convert certain latent parasitic infections into manifest disease—for example, *Bartonella muris* in rats, and *Eperythrozoan coccoides* in mice(6). The recently splenectomized monkey has greatly enhanced susceptibility to malarial infection(7). But with respect to the common bacterial infections, the issue is less clear-cut. Most of the work on animals was done before the prevalence and importance of latent bartonellosis in laboratory animals was realized(6). The availability of a Bartonella-free strain of rats in our laboratory made possible an experimental approach to the problem.

Materials and methods. The plan of the study was to compare the response of intact and splenectomized rats to varying doses of intraperitoneally injected bacteria. CF Nelson rats were used. The Bartonella-free nature of the colony was first demonstrated by performing splenectomies on 30 weanling rats. A month later, all animals appeared well, and tail blood was examined. Hematocrits and reticulocyte counts remained within normal limits, and no intra-erythrocytic organisms were seen. The bacterium used was type I pneumococcus originally derived from a patient with otitis media. Lyophilized ampuls containing the organism were broken and contents grown out in trypticase soy broth at 37°C; thereafter, virulence and capsule formation were enhanced by serial intraperitoneal inoculation of RF mice and reculturing from heart blood of moribund animals. Heart blood from third passage was cultured in brain-heart infusion at 37° for 10 hours. For inoculation, serial dilutions were made in brain-heart infusion at room temperature. Assay of inoculum was made by colony count on the surface of blood agar plates. *Exp. 1.* Forty-five suckling rats, 6 to 11 days old, were splenectomized under ether anesthesia, while 51 littermate controls were subjected to laparotomy. Approximately 10 days later, when animals were 17 to 20 days old, they were challenged, all on the same day, by intraperitoneal injection of graded dilutions of the 10-hour 37° culture of pneumococci. For each dosage of inoculum, littermate controls were used. Each family including mother was housed in a separate cage; it was demonstrated that under these conditions there was no cross-infection of non-inoculated rats from animals who succumbed to infection. Animals were checked 3 times daily; those who died were autopsied and cultures were made from the heart blood. Leucocyte counts were made from tail blood by conventional methods, except that 0.6 times the usual volume of blood was used. *Exp. 2* was similar to Exp. 1. Forty-three rats were splenectomized and 42 were laparotomized. Operations were performed at age 5 to 8 days, and bacterial challenge was made on a single day approximately 2 weeks after operation when animals were 19 or 20 days old.

Results. In both splenectomized and con-

TABLE I. Mortality of Intact and Splenectomized Suckling Rats after Intraperitoneal Infection with Pneumococci.

	Dilution of inoculum	Calculated No. bact. per animal	Splenectomized Surv.	Dead	Cumulative % dead*	Control Surv.	Dead	Cumulative % dead*
Exp. 1	$10^{6.5}$	12.6	0	2	100	0	3	100
	10^{7}	4	0	8	100	0	8	100
	$10^{7.5}$	1.3	3	7	84.2	5	7	78.3
	10^{8}	.4	5	6	53	3	9	57.9
	$10^{8.5}$	.13	5	2	18.8	7	2	11.8
	10^{9}	.04	6	1	5	7	0	0
Exp. 2	10^{7}	23	0	10	100	1	8	95.8
	10^{8}	2.3	1	7	94.1	1	9	88.2
	$10^{8.5}$	.73	6	4	56.2	3	4	54.5
	10^{9}	.23	5	4	29.4	7	1	14.3
	$10^{9.5}$	.07	5	1	5.6	7	1	5

* Calculated by method of Reed and Muench.

trol groups, animals were found dead between 44 and 96 hours after inoculation. Autopsies revealed peritonitis in most and hemorrhagic pneumonitis in about half. In all animals who succumbed, pneumococci were cultured from heart blood.

Mortality data are presented in Table I. In Exp. 1, mean survival time of 26 splenectomized animals that died was 60.1 hours; that of the 29 dead controls was 58.6 hours. The LD_{50} (calculated by method of Reed and Muench) for splenectomized animals was 0.36 bacteria; for the controls, it was 0.33 bacteria. In Exp. 2, the mean survival time of the 26 dead splenectomized animals was 65.7 hours; that of the 23 dead controls was 56.3 hours. The LD_{50} for splenectomized animals was 0.64 bacteria; for controls, 0.56 bacteria.

There was no significant difference in survival time of animals given the higher and lower dilutions of bacterial inoculum. The low LD_{50} values are attributed to the combination of high virulence of the infecting organism and inefficiency of the colony counting as a measure of viable bacteria.

It is concluded that under conditions of this experiment, there is no demonstrable influence of splenectomy on resistance to pneumococcal infection in young rats.

Leucocyte counts. In *Exp. 1,* white cell (WBC) counts from tail blood were performed on survivors 2 weeks after bacterial challenge. The mean WBC of the splenectomized group was 16,500 $\pm$ 618, that of controls 12,120 $\pm$ 395 (for this difference, $p < 0.1\%$). In *Exp. 2,* WBC counts were made on all animals 3 days before and one day after bacterial inoculation; but data are analyzed only for those animals who subsequently died of infection. For the splenectomized group, mean WBC was 17,370 before infection and 13,800 after infection. For controls, mean WBC was 10,910 before infection and 8,370 after infection. Thus there was some evidence of a post-splenectomy leukocytosis, but no evidence of an extreme leukocytosis in response to infection, such as has been observed occasionally in patients after splenectomy.

Discussion. Pneumococcus was selected for these experiments because it is the organism most frequently reported in clinical post-splenectomy infections. Immature suckling animals were used because of the impression that for resistance to infection the spleen is most important in early life, and because of claims of special susceptibility to infection in the splenectomized infant. Nevertheless, these experiments fail to reveal any difference in resistance to acute infection between normal and splenectomized subjects. It is, of course, possible that such a difference might have been demonstrated with a less virulent strain of bacteria, or with another organism, host, or route of infection. However, lack of good experimental evidence, and conflicting clinical reports render the concept of enhanced susceptibility to bacterial infection in splenectomized subjects open to question.

It is of interest that convincing demonstra-

tion of a role of the spleen in resistance has thus far been demonstrated only for infections in which intra-erythrocytic parasitism is prominent(6). Conceivably this situation is related to the function of the spleen in removing abnormal red cells and their inclusion bodies(8).

Summary. No difference in susceptibility to infection by a highly virulent strain of pneumococci could be demonstrated between normal and splenectomized suckling rats. The implications of this finding are briefly discussed.

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Antimetabolite Activity of Lysine Analogues on Lysine Incorporation into Rat Bone Marrow Protein *in vitro*. (24579)

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Lysine deficiency in the young rat is attended by anemia(1) brought on by retarded development of the hematopoietic system(2). Recently, Rohdenberg(3) reported that rats fed a lysine-deficient ration accumulated abnormal levels of fat in their bone marrow. It is apparent that integrity and activity of this tissue is markedly sensitive to lysine deprivation. As part of a study on action of amino acid analogues on protein synthesis by isolated mammalian tissues, we investigated the effect of 4 lysine analogues on *in vitro* incorporation of this amino acid into proteins of bone marrow.

Materials and methods. Rats of both sexes maintained on Purina Lab. Chow and weighing 90 to 125 g were decapitated and their femurs rapidly removed and placed in chipped ice. Twenty-five rats furnished sufficient bone marrow for 12 flasks in each experiment. Both ends of bone were cut with scissors and the bone marrow removed by forcing ice cold Krebs Ringer Phosphate (KRP) buffer through the bone with #23 hypodermic needle. The buffer, which was also used as incubation medium, contained .12 M sodium chloride, 1.3 x 10^{-2} M potassium chloride, 1.8 x 10^{-3} M calcium chloride, 6.5 x 10^{-4} M magnesium sulfate, and 0.01 M sodium phosphate, pH 7.2. This procedure dissociated most of the tissue into free cells. The fraction which remained intact was passed through 60 mesh, monel metal sieve and then combined with the free cells. The bone marrow cells were washed 3 times with cold KRP by alternate suspension and sedimentation in refrigerated centrifuge at 2°C and were then resuspended in 2 volumes of buffer. S-(β-Aminoethyl)-L-cysteine hydrochloride had been synthesized by the method of Cavallini *et al.*(4) and was a gift of Dr. F. Tietze of Nat. Inst. for Arthritis and Metabolic Diseases. The ϵ-C-methyl lysine (2,6-diamino heptanoic acid) was prepared as the dihydrochloride according to the method of McLaren and Knight(5). The δ-hydroxylysine hydrochloride was purchased from Calif. Corp. for Biochemical Research, Los Angeles. The latter 2 analogues were present in their 4 diastereoisomeric forms. Hexahomoserine was prepared as described by Gaudry(6). DL-Lysine-1-C^{14} was a gift of Dr. M. Rothstein, Univ. of California, Berkeley, which had been prepared by a new method(7). Solutions of all compounds were pre-