and over a 2-fold increase in urinary $H^+$ concentration. The effect on $Na^+$ excretion was variable. About half the animals responded with a 4-fold increase in $UV^{Na^+}$ and 2-fold increment in urine flow. The remainder showed no change in $Na^+$ excretion. It appears that there is a definite species difference in the response to ouabain of the rat kidney on one hand, and the chicken and dog kidney on the other. The most likely explanation of the phenomenon noted in the rat is that ouabain may have a biphasic effect whereby proximal reabsorption of $Na^+$ is inhibited and distal reabsorption, including cationic exchange for $K^+$ and $H^+$ is stimulated.

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## Response of Splenectomized Mice to Bacterial Agents.* (32429)

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Previous studies from this laboratory showed that splenectomized and normal monkeys responded similarly after infection with streptococci(1), pneumococci(2) or staphylococci(3). Results of studies on the role of the spleen in resistance to infection employing other experimental animals have varied depending on species, infective agent and route of inoculation employed(4). The present report is concerned with mortality following intravenous, intraperitoneal, intranasal or aerosol challenge of white mice with various bacteria at different intervals after splenectomy.

*Materials and methods.* White Swiss mice of both sexes, weighing 14-16 g, were splenectomized under ether anesthesia. Non-splenectomized controls were similarly treated, except that the spleen was manipulated, but not removed. *Streptococcus hemolyticus,* Group A (Stollerman strain T14), *Staphylococcus aureus,* Smith strain, *Diplococcus pneumoniae,* type III (ATCC No. 6303), and a *Pseudomonas aeruginosa* isolated from a patient at University Hospital were utilized.

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Streptococcal and staphylococcal inocula consisted of dilutions of 6-hour Trypticase Soy (BBL) broth cultures inoculated from blood agar plates incubated 24 hours. Pneumococci and the pseudomonas were grown for 18 hours on blood agar plates inoculated from 8-hour Todd-Hewitt (Oxoid) and Trypticase Soy broth, respectively. Inocula to be given intraperitoneally, intravenously and intranasally were prepared in physiologic saline. Suspensions of the pneumococcus used in aerosol challenge of mice in a Model 3 Henderson apparatus(5) were prepared in 0.1% Bacto-gelatin (Difco) in physiologic saline.

*Results.* As seen in Table I, no significant differences in mortality rates were observed among splenectomized, sham-operated and normal mice inoculated intravenously with the streptococcus 4 hours, 1, 3, 7, 14 and 21 days after operation. For example, in mice inoculated with $7.4 \times 10^4$ organisms as early as 4 hours after operation, 24 of 30 (80.0%), 21 of 30 (70.0%) and 25 of 30 (83.3%) deaths occurred in splenectomized, sham-operated and normal mice, respectively.

TABLE I. Response of Splenectomized Mice to Intravenous Streptococcal Challenge.

| Day Post-Splen | No. Organisms | Mortality Splenectomized No. | % | Sham-Operated No. | % | Normal No. | % |
|---|---|---|---|---|---|---|---|
| 1/6 | $7.4 \times 10^4$ | 24/30* | 80.0 | 21/30 | 70.0 | 25/30 | 83.3 |
| 1 | $5.0 \times 10^4$ | 17/25 | 68.0 | 13/25 | 52.0 | 17/25 | 68.0 |
| 3 | $7.1 \times 10^4$ | 11/20 | 55.0 | 8/20 | 40.0 | 6/20 | 30.0 |
| 7 | $1.0 \times 10^1$ | 3/30 | 10.0 | 0/30 | .0 | 1/30 | 3.3 |
| 7 | $1.8 \times 10^1$ | 4/40 | 10.0 | 1/40 | 2.5 | 6/40 | 15.0 |
| 7 | $3.4 \times 10^2$ | 4/20 | 20.0 | 11/20 | 55.0 | 10/20 | 50.0 |
| 7 | $2.8 \times 10^3$ | 6/10 | 60.0 | 2/10 | 20.0 | 5/10 | 50.0 |
| 7 | $1.9 \times 10^4$ | 4/10 | 40.0 | 6/10 | 60.0 | 4/10 | 40.0 |
| 7 | $7.4 \times 10^4$ | 21/50 | 42.0 | 29/50 | 58.0 | 29/50 | 58.0 |
| 7 | $1.2 \times 10^6$ | 6/10 | 60.0 | 8/10 | 80.0 | 6/10 | 60.0 |
| 7 | $1.0 \times 10^7$ | 36/40 | 90.0 | 37/40 | 92.5 | 32/40 | 80.0 |
| 14 | $5.1 \times 10^4$ | 17/30 | 56.6 | 20/30 | 66.6 | 17/30 | 56.6 |
| 21 | $9.8 \times 10^4$ | 29/40 | 72.5 | 27/40 | 67.5 | 34/40 | 85.0 |
| Total | | 182/355 | 51.3 | 183/355 | 51.5 | 192/355 | 54.1 |

* Numerator = No. dead; Denominator = Total No. challenged.

Similarly, splenectomy had no effect on response of mice inoculated with $9.8 \times 10^4$ organisms on the 21st post-splenectomy day; 29 of 40 (72.5%), 27 of 40 (67.5%) and 34 of 40 (85.0%) deaths occurred in the splenectomized, sham-operated and normal groups, respectively. A total of 8 experiments (Table I) were conducted wherein mice were inoculated 7 days following splenectomy with doses ranging from $1.0 \times 10^1$ to $1.0 \times 10^7$ organisms. Again, no significant differences in response could be demonstrated at any dose level among the 3 groups. Summation of observed mortality of 355 mice in each of the 3 challenge groups receiving streptococci, intravenously, showed that 182 (51.3%) splenectomized, 183 (51.5%) sham-operated and 193 (54.1%) normal mice experienced fatal infections (Table I). Table II shows that no significant differences were observed in mortality of splenectomized, sham-operated and normal mice inoculated intranasally with $1.6 \times 10^6$ streptococci 6 or 13 days post-splenectomy or in those receiving $2.0 \times 10^6$ 14 days post-splenectomy. Combined results of these 3 studies demonstrated that 31 of 90 (34.4%), 26 of 90 (28.9%) and 31 of 79 (39.2%) of splenectomized, sham-operated and normal mice died, respectively.

Intravenous inoculation of $3.6 \times 10^8$ staphylococci 7 days post-splenectomy, and intraperitoneal inoculation of $1.0 \times 10^8$ 20 days post-splenectomy resulted in similar mortality rates in all 3 groups (Table II). Similarly, aerosol challenge with $3.0 \times 10^4$ pneumococci 16 days after surgery caused

TABLE II. Response of Splenectomized Mice to Bacterial Agents.

| Organism | No. organisms | Challenge route | Day post-splen. | Mortality Splenectomized No. | % | Sham-operated No. | % | Normal No. | % |
|---|---|---|---|---|---|---|---|---|---|
| *S. hemolyticus* | $1.6 \times 10^6$ | Intranasal | 6 | 7/30* | 23.3 | 8/30 | 26.6 | 11/30 | 36.6 |
| | " | " | 13 | 11/30 | 36.6 | 5/30 | 16.6 | 7/19 | 36.8 |
| | $2.0 \times 10^6$ | " | 14 | 13/30 | 43.3 | 13/30 | 43.3 | 13/30 | 43.3 |
| *S. aureus* | $3.6 \times 10^8$ | Intravenous | 7 | 21/30 | 70.0 | 23/30 | 76.6 | 20/30 | 66.6 |
| | $1.0 \times 10^8$ | Intraperitoneal | 20 | 7/39 | 17.9 | 10/41 | 24.4 | 3/15 | 20.0 |
| *D. pneumoniae* | $3.0 \times 10^4$ | Aerosol | 16 | 3/26 | 11.5 | 3/24 | 12.5 | ND† | ND |
| *P. aeruginosa* | $1.9 \times 10^8$ | Intraperitoneal | 14 | 4/20 | 20.0 | 3/20 | 15.0 | 0/10 | 0.0 |
| | " | " | 21 | 18/40 | 45.0 | 16/40 | 40.0 | 14/40 | 35.0 |
| | " | " | 26 | 2/20 | 10.0 | 1/20 | 5.0 | 0/6 | 0.0 |
| Total—all organisms and routes | | | | 86/265 | 32.5 | 82/265 | 30.9 | 68/180 | 37.8 |

* Numerator = No. dead; denominator = total No. challenged. † Not done.

fatal infections in 3 of 26 (11.5%) and 3 of 24 (12.5%) of splenectomized and sham-operated mice, respectively. When $1.9 \times 10^8$ pseudomonas organisms were given, intraperitoneally, 14, 21 or 26 days, post-surgery, again no differences in mortality rates were observed among the 3 groups (Table II).

*Discussion.* Diverse observations have been made regarding the role of the spleen as related to experimental bacterial infections in animals(4). A recent careful study has demonstrated that the splenectomized mouse has increased susceptibility to pneumococcal infections(6). However, this status could only be induced in pathogen-free mice, and only with small numbers of a particular strain of pneumococcus (type VI). The present studies in white Swiss mice revealed no significant increases in susceptibility to hemolytic streptococci administered intravenously at various dose levels or at times varying from 4 hours to 21 days post-splenectomy. Similarly, splenectomy did not alter resistance to intranasal inoculation of streptococci, intravenous or intraperitoneal inoculation of staphylococci, aerosol challenge with type III pneumococci or intraperitoneal challenge with pseudomonas organisms. Whether one can extrapolate these observations to man or those observed only after intravenous inoculation with a type VI pneumococcus in pathogen-free mice is not clear. With subhuman primates where lethality was not the only measure, but wherein the overall clinical and laboratory observations could be conducted in a manner similar to those in humans, no significant differences in response were observed between normal and splenectomized monkeys following fatal or non-fatal infections with staphylococci, streptococci and pneumococci. It appears that the variability of results of animal studies concerning the role of the spleen in resistance to bacterial infections may better be served by utilization of subhuman primates. Although the interpretation of the present studies with mice parallel those reported from this laboratory in monkeys(1-3), the subhuman primate presents one with an opportunity to make general observations more closely resembling those encountered in patients with severe sepsis. The cumulative studies from this laboratory(1-3,7) have suggested that the underlying disease is more likely to be responsible for increased susceptibility to infection than is the absence of the spleen. A recent critical study(8) in humans supports this hypothesis.

*Summary.* No differences in mortality were observed in splenectomized, sham-operated and normal mice following intravenous or intranasal inoculation of hemolytic streptococci. Similarly, susceptibility to intravenous and intraperitoneal staphylococcal, aerosolized pneumococcal or intraperitoneal pseudomonas infections was not affected by splenectomy.

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