TABLE II. Comparison of Plaque Formation of 2 JBE Virus Strains in Primary HLTC by Double Agar Overlay.

Virus strains	Temp of incubation, °C	2nd Agar overlay system, day applied	Titer/0.1 ml PFU	Size, cm	Description
Nakayama*	37	6	18.5×10^5	.4–.7	Clear, sharp boundaries, round and ellipsoid
OCT-wild† 1	37	6	11×10^5	.4–.7	*Idem*
2	37	6	17.5×10^6	.3–.6	

* Nakayama mouse brain virus strain passed 34 times in HKTC.
† Oct-wild strains 1 = 3 passages in HKTC; 2 = 3 passages in HKTC, and 10 passages in HLTC.

Summary. 1. Monolayer hamster liver tissue cultures proved to be highly susceptible to JBE virus on the basis of CPE titers. Primary HKTC, HLTC and mixed cultures were found to be equally sensitive for several virus strains. Secondary HLTC was less sensitive than the primary. 2. Nakayama and OCT-wild strains of JBE produced similar plaques on HLTC using the double agar overlay technique at 37°C, but the method was not as sensitive as CPE for virus detection. Plaques were 0.4-0.7 cm in diameter and clear with sharp boundaries. 3. Plaques were produced on secondary HLTC using the double agar overlay technique at 37°C. These were smaller, and hazy but in increased numbers. Mixed kidney and liver cultures failed to produce plaques.

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Streptococcal Infection in Normal and Splenectomized Monkeys.* (29598)

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The question of post-splenectomy hypersusceptibility to infection has been undergoing a period of critical appraisal in various clinics. There is not general agreement as demonstrated by recent reports(1,2). Most experimental studies have been conducted in non-primates with varying results dependent on animal species and agent employed(3); relatively little work has been done with primates. Increased susceptibility of splenectomized monkeys to poliomyelitis(4) and plasmodium infection(5,6) have been suggested. Studies in this laboratory have been concerned with the comparative response of normal and splenectomized monkeys to a variety of agents. In this report are described the results following aerosol and intravenous challenge with streptococci.

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Materials and methods. Young pre-puber-

tal monkeys (*Macaca mulatta* and *M. irus*) were splenectomized under pentothal-ether anesthesia.† *Streptococcus hemolyticus*, Group A, Strain S23(7) (mouse intraperitoneal LD 50 of 200-400 organisms) was used. Bacto-Brain Heart Infusion (BHI) blood agar was inoculated with 0.1 ml of a 24-hr Todd-Hewitt (Oxoid) broth culture and incubated 18 hours. Suspensions in 0.1% Bacto-gelatin in physiologic saline were used in the Henderson apparatus aerosol generator while inocula to be given intravenously were prepared in physiologic saline.

Aerosol challenge was conducted in a Model 3 Henderson apparatus(8). Preliminary studies demonstrated that the streptococcus could be aerosolized efficiently by accepted procedures(8). Intravenous challenge was made into the saphenous vein.

Base-line studies included physical examinations, hematologic and serologic studies and nasopharyngeal cultures for 2 weeks prior to challenge. After challenge, monkeys were given a complete physical examination twice daily. Femoral vein blood was used for blood counts, cultures and C-reactive protein (CRP) tests daily or every other day, and weekly for serologic studies.

For blood cultures, 1.5 ml of blood was added to vials containing 20 ml of slanted BHI agar and 15 ml of BHI broth. Nasopharyngeal cultures were made from swabs moistened with BHI broth and streaked on BHI-blood agar plates. Tests for CRP were done as recommended by Schieffelin and Co., New York, using their anti-human CRP serum. Antistreptolysin O (ASO) titrations were performed by the method of Rantz and Randall(9).

Results. Aerosol challenge. Four monkeys, splenectomized 10 months previously, and 2 normal controls were exposed for 2 minutes to an aerosol containing 4.3×10^6 streptococci per liter. No fever was observed in any. Mild dyspnea appeared in 2 of 4 splenectomized animals the first day, and in both controls 2 to 3 days after challenge. Leukocytosis was observed in only 1 of 4 splenectomized monkeys on the 2nd day, and in both controls on the 1st and 2nd days, respectively. None of the animals showed positive blood cultures or CRP. Nasopharyngeal cultures were positive in 3 of 4 splenectomized monkeys for 6 days, and in both controls for 1 and 7 days, respectively. ASO titers showed significant rise by the 19th day in all 4 splenectomized monkeys from a baseline of 12 units to 100, 100, 50 and 160 units, respectively, as compared to 166 and 333 units, respectively, in the 2 controls.

Intravenous challenge. Three separate experiments (Table I) were conducted comparing response of splenectomized and normal rhesus monkeys following intravenous challenge. In Experiment 1 (Table I), 2 monkeys (Nos. 96, 102), splenectomized 24 days previously, and 2 controls (Nos. 3, 4) were challenged intravenously with 9.2×10^9 organisms. Within 24 hours both splenectomized animals showed fever, lethargy, weakness and anorexia; râles were detected bilaterally in No. 96. Both animals exhibited leukocytosis, positive CRP and blood culture by the 1st day, and expired on the 2nd day. At autopsy, streptococci were isolated from lung, liver, kidney, pericardial and spinal fluid, and bone marrow of No. 96 while no positive cultures were obtained from 102. One control (No. 3) became ataxic and weak by the 1st day, and exhibited leukocytosis, positive CRP and blood culture, and died on the 2nd day. All cultures were negative at autopsy. The other monkey (No. 4) exhibited significant fever (104-105°F) for the first 4 days associated with minor illness symptoms consisting mostly of anorexia; râles were detected bilaterally for 15 days. Significant leukocytosis was observed for 7 days, but CRP and blood cultures were negative throughout. The ASO titer rose from negative to a peak of 1280 units by the 14th day.

In Experiment 2 (Table I), 2 monkeys splenectomized 45 days previously, and 2 controls were challenged with 1.2×10^{10} organisms. One splenectomized (No. 85) and 1 normal monkey (No. 73) became acutely ill and died 36-40 hours after challenge. The splenectomized monkey exhibited tempera-

† Kindly performed by Dr. R. D. Williams.

TABLE I. Effect of Intravenous Streptococcal Challenge in Normal and Splenectomized Rhesus Monkeys.

	Exp 1—24 days P.S.* Dose: 9.2×10^9			Exp 2—45 days P.S. Dose: 1.2×10^{10}				Exp 3—3 mo P.S. Dose: 2.3×10^{10}						Total—3 experiments	
	Splen	Controls		Splen		Controls		Splen			Controls			Splen	Controls
Monkey No.	96	102	3	4	85	90	69	73	80	89	37	95	1	2	13	10		
Fever	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	8/8†	8/8
Illness	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	7/8	8/8
Deaths	+	+	+	|	+	|	+	|	|	+	|	|	+	|	+	4/8	5/8
Leukocytosis	+	+	+	+	|	+	|	+	|	+	|	+	|	|	|	|	7/8	4/8
CRP	+	+	+	|	+	+	+	+	+	+	+	+	+	+	+	+	8/8	7/8
Blood culture	+	+	+	|	+	+	+	+	+	+	+	+	+	+	+	+	8/8	7/8
ASO titer rise	ND‡	ND	ND	8§	ND	6	8	ND	6	ND	8	8	10	ND	ND	ND	4/4	3/3
Autopsy culture	+	—	—		+		+			+				+	+	+	3/4	4/5

* P.S. = Post-splenectomy. † Numerator = No. positive; denominator = total No. ‡ N.D. = Not done. § Tubes increase in titer.

ture of 105°F 12 hours after challenge, and then showed subnormal temperatures prior to death. Blood culture and CRP were positive 24 hours after challenge. At autopsy, the streptococcus was isolated from heart blood, lung, liver, spleen, kidney, brain and bronchial lymph nodes. The nonsplenectomized monkey (No. 73) showed fever, weakness, lethargy and ataxia, and died on the 2nd day. He also had positive blood culture and CRP 24 hours after challenge, but no significant leukocytosis. At autopsy, the streptococcus was isolated from lung, spleen, liver, kidney, and ileocecal node. The surviving splenectomized monkey (No. 90) exhibited fever for 5 days, lethargy, weakness, and ataxia for 12 days, but recovered spontaneously. Leukocytosis and positive CRP were observed for 7 days while blood cultures remained positive for 14 days. ASO titer rose from 80 to a peak of 5120 units by the 14th day. The surviving non-splenectomized monkey (No. 69) exhibited significant fever with temperatures as high as 107°F, lethargy and weakness for 29 days, but recovered. Blood cultures were positive for 22 days and CRP for 29 days. ASO titer rose from negative to a peak of 1280 units by the 29th day.

In a 3rd experiment (Table I) 4 monkeys, splenectomized 3 months previously, and 4 controls were challenged with 2.3×10^{10} organisms. Only 1 of 4 splenectomized and 3 of 4 controls died after this challenge. This splenectomized monkey (No. 89) died on the 2nd day after showing fever, lethargy and weakness beginning the day after challenge. There was a modest increase in leukocytes from 5,500 per cu mm base-line to 14,000 per cu mm, and then a drop to 2,100 per cu mm prior to death. Blood cultures and CRP were positive 24 and 48 hours after challenge. At autopsy, the streptococcus was isolated from heart blood, liver, spleen, kidney and bone marrow. The following observations were made in the 3 surviving splenectomized monkeys: No. 80 exhibited fever 24 hours after challenge up to 105.4°F, and continued to show fever for 22 days. He was lethargic and weak for 10 days, and on the 15th day developed cellulitis of the left hand, arm and

axilla from which the streptococcus was isolated. This cleared spontaneously by the 31st day. Blood cultures and CRP were positive through the 28th and 25th days, respectively. ASO titer rose from 40 to a peak of 2560 units on the 15th day. Significant leukocytosis varying from 20 to 46 thousand white cells per cu mm was observed for 35 days. When sacrificed on the 60th day, no positive cultures were obtained. Monkey 95 showed lethargy and weakness for 3 days post-challenge. Fever from 104-105°F was observed for 24 days; blood cultures were positive through 15 days and CRP through 25 days. ASO titer rose from negative to a peak of 1280 units at 28 days. This monkey also developed a streptococcal cellulitis of the left hand on the 21st day which cleared spontaneously by the 31st day. All cultures were negative when the monkey was sacrificed on the 60th day. Monkey 37 did not become ill after challenge, but exhibited fever for 3 days, and significant leukocytosis of 25 to 50 thousand white cells per cu mm from the 1st to 6th days. Blood cultures were positive the 1st 4 days, and again between the 25th to 31st days, but with no associated symptoms. CRP was positive from the 1st to 25th days, and ASO titer rose from 0 to a peak of 1280 units on the 15th day. Sacrifice at 60 days yielded no positive cultures.

Of the 4 controls, 3 died (Table I). Monkey 10 expired 36 hours after challenge after exhibiting fever, marked lethargy and weakness followed by a moribund state. The day after challenge, CRP was positive, but there was no leukocytosis (11,500 leukocytes as compared to base-line of 11,700 per cu mm). On the day of death his count was 7,900 per cu mm with 88% neutrophiles as compared to 49% the day before. The streptococcus was isolated from blood on the 1st day, and from blood, spleen, liver and kidney at autopsy. Monkey 2 exhibited a similar picture clinically and died within 2 hours of No. 10. He also showed positive blood cultures and CRP the day after challenge with no leukocytosis. At autopsy, positive cultures were obtained from heart blood, liver, spleen, bone marrow and kidney. The 3rd monkey (No. 13) which showed lethargy and weakness for 3 days, but fever the 1st day only, became moribund and died on the 3rd day. Blood cultures and CRP were positive on the 1st and 2nd days, and again no significant leukocytosis was observed. At autopsy, positive cultures were obtained from blood, spleen, liver, bone marrow, kidney and lung. The surviving monkey (No. 1) showed fever, lethargy, weakness and anorexia for 3 days, and then recovered spontaneously. Blood cultures were positive for the first 15 days and CRP from the 1st through 10th days. ASO titer rose from base-line of 40 units to a peak of 10,240 on the 15th day. Leukocytosis was observed on the 7th day. At sacrifice 60 days later, all cultures were negative.

For comparative purposes, 3 cynomolgus monkeys, splenectomized 13 days previously, and 2 controls were challenged intravenously with 1.0×10^9 organisms. Two of the splenectomized monkeys, Nos. 17 and 41, died on the 5th and 6th days, respectively. Monkey 17 exhibited positive blood cultures and CRP for the 1st 3 days, but at autopsy all cultures were negative. Monkey 41 showed positive cultures and CRP from the 1st day to death, and at autopsy streptococci were isolated from heart blood, liver, kidney, lung and spinal fluid. Both monkeys had base-line ASO titers of 160 units. The 3rd monkey (No. 45) showed no signs of illness except for fever on the evening of the 1st day. However, blood cultures were positive from the 1st to 12th days and CRP for the 1st 3 days. Leukocytosis up to 56,000 per cu mm was noted up to the 22nd day. ASO titer rose from 160 units to a peak of 10,240 on the 15th day. The monkey when sacrificed 2 months later yielded no positive culture. Both controls became ill, but recovered spontaneously. Monkey 9 showed mild illness with fever and anorexia for 2 days. Leukocytosis (21,000 per cu mm) was observed on the 3rd day, and then again between the 7th and 22nd days when counts of 20 to 30,000 per cu mm were observed. Blood cultures were positive from the 1st through 29th days and CRP from the 1st through 9th days. ASO titer rose from base-line of 320 to a peak

of 2560 units on the 15th day. At sacrifice after 2 months, all cultures were negative. The other monkey, No. 16, showed a similar illness and fever for 2 days, and recovered spontaneously. Blood cultures were positive through the 19th day and CRP between the 1st and 5th days. ASO titer rose from 40 to a peak of 10,240 units on the 15th day. Sacrifice at 60 days yielded no positive cultures.

Effect of intravenous inoculation after previous aerosol challenge. Four splenectomized rhesus monkeys (Nos. 30, 31, 32, 33) and 2 controls (Nos. 40, 41) challenged via aerosol 2 weeks previously (*vide supra*) were challenged intravenously with 1.5×10^7 organisms. In addition, 2 normal monkeys (Nos. 72, 73) not previously exposed to aerosol were included. All 4 splenectomized monkeys exhibited fever. Monkey 30 showed fever for 2 days, but had no other symptoms. Blood cultures were positive for the 1st 2 days and CRP for 4 days. Base-line ASO titer rose from 100 units to a peak of 625 on the 16th day. Monkey 33 also showed no symptoms, but had fever for 24 hours. He also exhibited a modest leukocytosis for 3 days. Blood cultures were positive for 2 days and CRP for 4. ASO titer rose from 166 units to a peak of 625 after 1 week. Monkey 31 showed fever with chills the 1st day, and fever continued until the 6th day. A leukocytosis was observed for the first 7 days. Blood cultures were positive on the 1st day only and CRP for 4 days. ASO titer rose from 100 to a peak of 250 units by the 15th day. Monkey 32 also exhibited chills and fever on the 1st day, and the temperature returned to normal by the 6th day. No change in white cell count was observed. Blood culture and CRP were positive on the 1st day only. ASO titer rose from 50 units to a peak of 333 on the 15th day. Of the 2 previously aerosolized controls, one (No. 41) showed fever only for 3 days. There was a modest leukocytosis for 7 days. Blood cultures were positive for 2 days and CRP for 7 days. ASO titer rose from 333 to a peak of 2500 units on the 15th day. Monkey 40 showed significant fever for 3 days, and appeared lethargic and anorectic on the 3rd and 4th days. Significant leukocytosis was observed on the 4th day. Blood cultures were positive on the 1st day and CRP on the 4th day. ASO titer rose from 166 to a peak of 833 units by the 7th day. Of the 2 monkeys not previously areosolized, 1 (No. 72) showed fever for 1 day only, with no other symptoms. There was a modest leukocytosis on the 4th day. Blood cultures and CRP were positive for the first 4 days. ASO titer rose from 12 to a peak of 166 units on the 15th day. The other animal, monkey 73, showed no fever or symptoms, but did have a leukocytosis on the 2nd and 10th days only. Blood cultures and CRP were positive for the 1st 2 days. ASO titer rose from 0 to a peak of 50 units on the 15th day.

Discussion. These studies demonstrated that normal monkeys exposed to hemolytic streptococcal aerosols showed no evidence or mild clinical evidence of infection. This agrees with previous studies from this laboratory where challenge was performed by intranasal inoculation(10). Splenectomized and normal monkeys showed no significant differences after challenge. Comparable antibody response was observed.

Intravenous inoculation with streptococci induced more obvious signs of infection. Normal and splenectomized monkeys showed a constant similarity in reference to clinical and laboratory findings. As for mortality, 4 of 8 splenectomized and 5 of 8 normal rhesus monkeys died 2 to 3 days after challenge. It would be tempting to relate relative mortality to days elapsed since splenectomy, but the groups were too small to do so. For example, 24 days after splenectomy both monkeys died after challenge as compared to 1 of 2 controls while 1 of 2 in each group died 45 days post-splenectomy. When challenge was performed 3 months after splenectomy, only 1 of 4 died as compared to 3 of 4 controls. This suggestion is augmented by the fact that 2 of 3 cynomolgus monkeys challenged 13 days, post-splenectomy, died while both controls survived. However, studies with pneumococci and staphylococci, to be reported separately, do not suggest any dif-

ference in relation to species or time of splenectomy.

Another aspect of this study was to determine if past experience would be reflected differently in splenectomized monkeys. Thus, 2 weeks after aerosol challenge, intravenous challenge was performed. Again the response was similar in 4 splenectomized and 2 normal controls clinically and laboratory-wise. Thus, these studies do not demonstrate increased susceptibility to infection with hemolytic streptococci in splenectomized monkeys. Since most of the reports supporting the concept of increased susceptibility have been concerned with infants, young pre-pubertal monkeys were employed in these studies. Studies in younger monkeys, recently weaned, are in progress at present.

The similar antibody response in normal and splenectomized monkeys following aerosol or intravenous challenge with viable streptococci is in agreement with our previous studies using killed bacterial antigens in monkeys(11) and humans(12).

Summary. No significant differences in susceptibility were noted between normal and splenectomized monkeys following aerosol or intravenous challenge with a Group A hemolytic streptococcus. No mortalities were observed after aerosol challenge. Intravenous challenge induced obvious disease with mortality in 6 of 11 splenectomized and 5 of 10 normal monkeys. Laboratory findings were essentially similar including C-reactive protein and antistreptolysin response.

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Haptenic Properties of Paralytic Shellfish Poison Conjugated to Proteins by Formaldehyde Treatment. (29599)

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Paralytic shellfish poison (PSP) is a potent toxin produced by the dinoflagellate, *Gonyaulax catenella,* and may be isolated from toxic mussels and clams obtained from waters inhabited by the *Gonyaulax*(1-3). The poison has no adverse effect on the shellfish, which use the *Gonyaulax* as a food source. Human ingestion of PSP-contaminated shellfish results, however, in paralytic poisoning and occasionally death. The mouse bioassay test of Sommer(1), and modifications of this test (4,5) are the only practical methods for detecting and measuring the poison in contaminated clams. A chemical technique, a modification of the Jaffe reaction, has also been employed for determination of poison(6,7). This is, however, an involved procedure and its limit of sensitivity is less than that of

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