tericidal, infection is readily established in the kidney. While Beeson and Rowley(4) have indicated that kidney inactivates complement and thus destroys the complement-dependent bactericidal activity of serum against *Escherichia coli,* this does not explain the findings in the *Str. faecalis* model. *Str. faecalis* is gram positive and not susceptible to complement since heating sera at 56°C reduces bactericidal activity very little, and guinea pig serum rich in complement has no demonstrable bactericidal effect against this organism.

Once established, renal infection due to *Str. faecalis* persists throughout the normal life span of the rat despite eventual appearance of high titer of circulating antibacterial antibodies. The presence of these antibodies does not enhance bactericidal activity of serum, and thus, would not be expected to contribute to those serum factors involved in elimination of organisms from the kidney. Whether or not these antibodies participate in other host defense mechanisms, such as phagocytosis, is not known.

Studies are in progress to determine whether or not renal metabolic environment has an effect on non-complement-dependent serum bactericidal activity.

Summary. Pyelonephritis produced in rats by intravenous inoculation of *Str. faecalis* becomes established in the presence of normal serum bactericidal activity against the infecting organism. The infection persists throughout the life of the rat despite the eventual appearance of circulating antibacterial antibodies. This finding may be partially explained by the fact that these antibodies do not enhance normal serum bactericidal activity as tested.

1. Kleeman, C. R., Hewitt, W. L., Guze, L. B., *Medicine,* 1960, v39, 3.

2. Needell, M. H., Neter, E., Staubitz, W. J., Bingham, W. A., *J. Urol.,* 1955, v74, 674.

3. Jacobson, D., Braude, A., *Clin. Res.,* 1959, v7, 284.

4. Beesen, P. B., Rowley, D., *J. Exp. Med.,* 1959, v110, 685.

5. Weyrauch, H. M., Rosenberg, M. L., Amar, A. D., Redor, M., *J. Urol.,* 1957, v78, 532.

6. McCabe, W. R., Jackson, G. G., *Biology of Pyelonephritis,* Little, Brown & Co., Boston, 1960, 39.

7. Andersen, B. R., Jackson, G. G., *J. Lab. and Clin. Med.,* 1962, v60, 457.

8. Sanford, J. P., Hunter, B. W., Souda, L. L., *J. Exp. Med.,* 1962, v115, 383.

9. Guze, L. B., Goldner, B. H., Kalmanson, G. M., *Yale J. Biol. and Med.,* 1961, v33, 372.

10. Lancefield, R. C., Perlmann, G. E., *J. Exp. Med.,* 1952, v96, 71.

11. Kaplan, M. H., *ibid.,* 1958, v107, 341.

12. Stavitsky, A. B., Arquilla, E. R., *Int. Arch. Allergy,* 1958, v13, 1.

13. Miles, A. A., Misra, S. S., Irwin, J. O., *J. Hyg.,* 1938, v38, 732.

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Pyelonephritis V. Role of Serum Bactericidal Activity and Antibody in Acute Pyelonephritis in the Rabbit.* (28532)

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Intravenous inoculation of *Str. faecalis* in the rat produces a pyelonephritis which persists for at least 2 years despite the presence of antibacterial antibodies. It has been suggested that at least a partial explanation for the chronicity of the infection may be the lack of increase of bactericidal activity of serum when antibacterial antibodies are present(1). In the rabbit, in contrast to the rat, preliminary studies revealed that the infection "burned out" in relatively few weeks. Comparative studies of antibody production and serum bactericidal activity were performed to determine whether immunological

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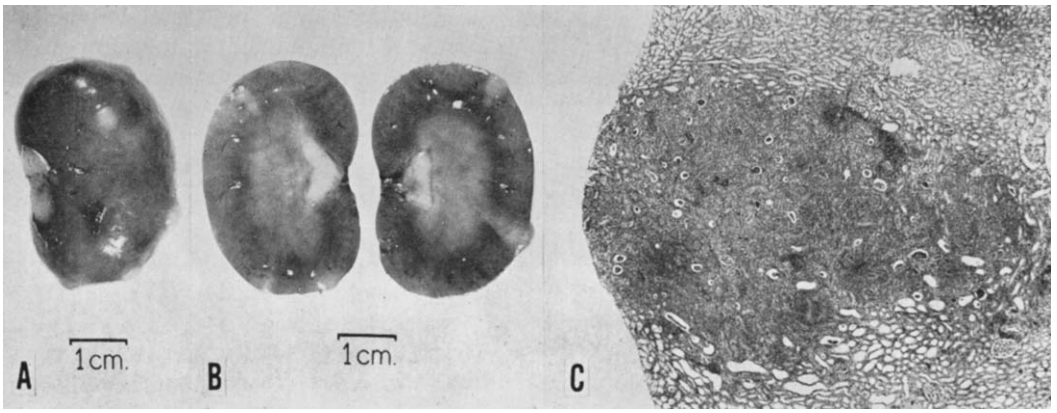

FIG. 1. A. Abscesses on anterior surface of kidney. B. Cut surface of kidney shown in 1A. Note extension of abscesses from medulla to cortex. C. Low-power photomicrograph of abscess.

parameters might provide an explanation for the different behavior of infection in the rat and rabbit.

Materials and methods. Random-bred male albino rabbits obtained from a single commercial source were used. Prior to infection, animals were bled from a marginal ear vein. Bacterial agglutination and antistreptococcal protein hemagglutination titers as well as serum bactericidal activity were determined. Rabbits were individually identified so that the bacteriological and serological course of infection could be accurately related to prior immunological studies. The animals were infected by intravenous inoculation of 3 ml of an 18-hour heart infusion broth culture containing a total of 1.2×10^9 *Str. faecalis*. At intervals thereafter, bacterial populations of kidney, liver and spleen were determined and serological studies made. Techniques used in these experiments have been described(1,2).

Results. Bacteriological studies. Fig. 1A, B, C demonstrate abscess formation of acute pyelonephritis at one week. The bacterial populations of kidney, liver and spleen at intervals after infections are shown in Table I. The listing of rabbits is in order of increasing bactericidal activity of the serum (obtained before infection). The bacterial content per gram of kidney was highest at 1 week, slightly less at 2 weeks, and thereafter only an occasional animal demonstrated small numbers of bacteria in renal tissue. The bacterial content of liver and spleen were lower than that of the kidney at one week, and both of these organs became bacteria-free rapidly. The results of these analyses show no relationship between prior bactericidal activity of serum and extent or outcome of infection.

Serum antibody. The agglutination and antistreptococcal protein hemagglutination titers are shown in Fig. 2. Prior to infection, the rabbits had an agglutination titer of 1:20 to 1:40. A 1:20 hemagglutination titer was present in one rabbit; the remainder were negative at 1:20 (which was the lowest dilution of serum tested). After infection, the agglutination titers rose rapidly at one week, then leveled off for the duration of the study. The hemagglutination titers rose somewhat more slowly and, at about 3 weeks, reached and maintained approximately the same level as did the agglutination titers.

Serum bactericidal activity. The bactericidal activity of sera obtained before infec-

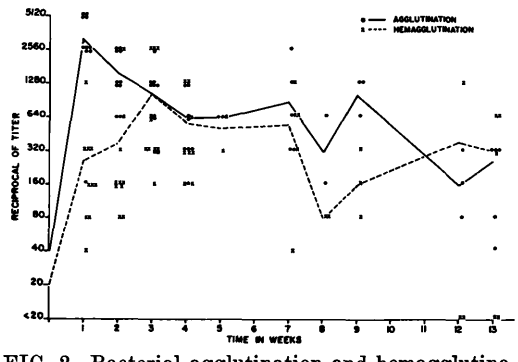

FIG. 2. Bacterial agglutination and hemagglutination titers in rabbits after I.V. injection with *Str. faecalis*.

tion varied greatly. The results (Fig. 3) indicate that the proportion of bacteria remaining viable after exposure to serum for 120 minutes at 37°C ranged from 22 to 118% (average 64%).

TABLE I. Bacterial Populations in the Various Organs of the Rabbit Following Intravenous Injection with *Str. faecalis.*

Duration of infection (wk)	Rabbit No.*	Log No. organisms/g tissue: Left kidney	Right kidney	Liver	Spleen
1	1	4.18	4.76	3.82	3.48
	2	1.95	4.93	0	0
	3	0	4.56	1.65	1.81
	4	6.15	6.18	2.92	5.72
	5	5.49	4.00	0	1.15
	6	5.04	6.43	0	0
	7	7.87	6.38	4.56	4.92
	8	7.61	6.00	0	.88
	9	6.26	4.04	1.04	—†
	10	6.04	7.96	0	1.48
	Mean	5.29		1.39	1.94
2	1	4.58	4.36	0	0
	2	4.61	5.08	0	0
	3	7.08	7.08	3.97	4.06
	4	4.70	0	0	0
	5	2.67	2.23	0	0
	6	1.98	2.20	0	0
	7	4.82	3.39	0	0
	8	3.92	3.23	0	0
	9	5.0	4.91	0	0
	10	0	0	0	0
	Mean	3.39		.39	.40
3	1	4.15	3.93	0	0
	2	0	0	—	—
	3	.78	3.89	2.58	1.30
	4	0	0	0	0
	5	0	0	0	0
	6	2.60	0	0	0
	7	0	0	0	0
	8	0	0	0	0
	9	.84	2.00	0	0
	10	0	0	0	0
	Mean	.91		.25	.13
4	1	0	—	0	0
	2	0	0	0	0
	3	0	1.88	0	0
	4	3.83	3.79	4.74	4.95
	5	1.32	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	2.04	0	0
	Mean	.80		.59	.61
5–8	1	0	.11	0	0
	2	0	.11	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	2.51	0	0
	6	3.23	0	0	0
	7	0	1.28	0	0
	8	.90	0	0	0
	Mean	1.02		0	0
13	1	0	.39	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	Mean	.08		0	0

* The rabbits are listed in order of increasing bactericidal activity of serum (obtained before infection).

† Not done, technical error.

Comparison of the bacterial populations of the kidneys, agglutination and hemagglutination titers and serum bactericidal activity at various intervals after infection are shown in Table II. These findings demonstrate that development of antibacterial antibodies does not appear to increase the bactericidal activity of serum. At each sacrifice time, bactericidal activity of individual serum varied in a random way, but the means did not differ from pre-infection levels.

Discussion. These results indicate that the rabbit with pyelonephritis due to *Str. faecalis,* in contrast to the rat, was able to clear its kidneys of bacteria in a relatively short time. The immunological parameters studied, (*i.e.,* antibacterial antibody production and serum bactericidal activity) demonstrated no differences between these two species. Furthermore, the individual level of bactericidal activity of normal serum (obtained before bacterial inoculation) did not appear to influence the subsequent course of the infection.

It is possible that the normal bactericidal activity of serum, despite the fact that it did not increase, accounted for the ability of the rabbit to eliminate infection from the kidney. However, this is not an entirely satisfactory explanation since similar serum activity was noted in the rat in which infection persists. The different responses may be related to other defense mechanism, *i.e.,* cellular antibody or phagocytosis. On the other hand, the difference may reside in some variation of the metabolic environment of the kidney which is less favorable for *Str. faecalis* in the rabbit than in the rat or which interferes with

TABLE II. Comparison of Bacterial Population of Kidneys, Agglutination, Hemagglutination Titers and Serum Bactericidal Activity of Rabbits After Intravenous Injection with *Str. faecalis.*

Duration of infection (wk)	No. of animals	Mean log No. bacteria/g kidney	Mean agglutination titer (reciprocal)	Mean hemagglutination titer (reciprocal)	% of bacteria surviving at 120 min at 37°C
None (diluent control)					232*
Normal (prior to infection)	51		20–40†	<20	64 (range 22–118)
1	10	5.29	2560	320	59 (range 37–76)
2	10	3.39	1280	320	82 (range 45–150)
3	10	.91	1280	1280	50 (range 26–78)
4	8	.80	640	640	90 (range 31–249)‡
5–8	8	1.02	640	320–640	50 (range 28–88)
13	5	.08	320	320	83 (range 52–116)

* Mean of 8 separate experiments.

† Prior to infection, 17 animals 1:20; 34 animals 1:40. The mean titers obtained after infection are expressed as the nearest 2-fold dilution.

‡ One animal in this group had a bactericidal serum activity of 249; range of remaining animals was 31-98 with a mean of 67.

immune process in the rat, but not in the rabbit.

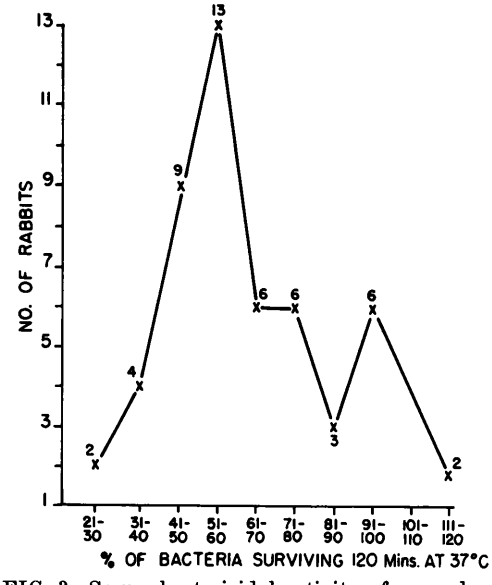

FIG. 3. Serum bactericidal activity of normal rabbits against *Str. faecalis.*

Summary. Intravenous injection of rabbits with *Str. faecalis* produced an acute pyelonephritis which spontaneously resolved in a few weeks. This infection was accompanied by vigorous antibacterial antibody (agglutinating and hemagglutinating) production which was not associated with increase in serum bactericidal activity as tested. There appeared to be no correlation between individual rabbit pre-infection serum bactericidal activity and the quantitative bacteriological findings in the kidney. The difference between the outcome of infection in the rabbit and rat has not been explained by the immunological studies performed.

1. Kalmanson, George M., M.D., Hubert, Earl G., M.A., Guze, Lucien, B., M.D., to be published.

2. Guze, L. B., Goldner, B. H., Kalmonson, G. M., *Yale J. Biol. and Med.,* 1961, v33, 372.

Received February 28, 1963. P.S.E.B.M., 1963, v113.