THE PATHOGENICITY FOR MICE OF GROUP B STREPTOCOCCI OF BOVINE ORIGIN

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(With Plates 3 and 4 and 1 Figure in the Text)

It is the purpose of this paper to record results of experiments designed to examine the validity of the opinion generally held (Minett & Stableforth, 1931; Lancefield, 1934; Brown, 1939; Simmons & Keogh, 1940; Kaplan, Raiziss & Moetsch, 1942; Little & Plastridge, 1946, p. 168; Pomales-Lebrón, Morales-Otero & Baralt, 1947; Francis & Peters, 1947) that the majority of bovine strains of group B streptococci are of low lethal power for mice.

MATERIAL AND METHODS

Details of the twenty bovine strains of group B streptococci used are given in Table 1. It will be noted that eight of the strains have been classified serologically as type 1 ? subtype, the reason being that all were closely related to, but not identical with, the subtype 1*b* described by Stableforth (1937). All strains were dried over phosphorus pentoxide and sealed *in vacuo* until required; when in use the strains were maintained on bacto-tryptose ox-blood agar.

Table 1. *Year of isolation, source, and serological type of twenty bovine strains of group B streptococci*

Strain	Isolated	Source	Serological type
S 1	1946	Teat sore	1 ? subtype
S 2	1943	Mastitis	3*a*
S 3	1946	Teat sore	1 ? subtype
S 4	1946	,,	1 ? subtype
S 5	1946	,,	3*b*
S 13	1943	Mastitis	1 ? subtype
S 20	1943	,,	Failed to type
S 37	1944	,,	1 ? subtype
S 46	?	,,	Failed to type
S 90	1945	,,	1 ? subtype
S 113	1945	,,	Failed to type
S 115	1945	,,	3*a*
S 117	1945	,,	1 ? subtype
S 133	1946	,,	3*b*
S 148	1946	,,	1 ? subtype
S 172	?	,,	3*b*
S 175	?	,,	3*a*
S 186	?	,,	Failed to type
S 196	?	,,	3*b*
S 216	?	,,	3*a*

The five liquid media used were:

(*a*) Glucose broth, prepared as for Hartley's broth (Mackie & McCartney, 1948, p. 146) with 0·5% glucose added and the final pH adjusted to 7·2.

(*b*) Todd & Hewitt's broth (Todd & Hewitt, 1932).

(*c*) Todd & Hewitt's broth with 20% sheep serum added.

(*d*) McKenzie's broth (McKenzie, 1941), without the addition of thallium acetate or crystal violet.

(*e*) Bacto-tryptose broth, prepared by adding 10 g. Bacto-tryptose and 5 g. sodium chloride to 1000 ml. Hartley's broth and adjusting the final pH to 7·0.

The number of living organisms in a culture was determined by a surface plate count on bacto-tryptose ox-blood agar. Dilutions in normal saline were prepared at 10^{-2}, 10^{-4}, and 10^{-6}, with dropping pipettes standardized to deliver 0·02 ml. drops. Three separate series of dilutions were always made, so that the resulting count was the mean of three counts, each made from a dilution independent of the other two.

White Swiss mice from a closed population, each weighing 14–25 g., were used; in any one experiment test and control animals were of the same sex and of comparable weight. All mice that died were examined bacteriologically, or, if the number was so great as to make this impracticable, a random sample of the dead animals was examined. Animals killed for examination were destroyed with chloroform vapour.

All tissues for histological examination were fixed in 10% formol-saline and embedded in paraffin. Two sections of every tissue were examined, one stained with haematoxylin and eosin and the other by Gram's method. Tissues stained by the same methods and obtained from healthy animals in the same colonies served as controls.

EXPERIMENTAL OBSERVATIONS

Pathogenicity of the same strain in different media

Strain S 13 was grown simultaneously for 18 hr. in four different media, and mice were inoculated intraperitoneally in groups of 30 with 0·5 ml. of the four

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cultures respectively. The experiment was repeated with groups of twenty mice, and the results (Table 2) showed marked variation in pathogenicity according to the medium in which the strain was grown.

Table 2. *Results of intraperitoneal inoculation of mice with 0·5 ml. of strain S 13 grown simultaneously for 18 hr. in different media*

Exp. no.	Medium	No. of mice inoculated	Died within 5 days
1	Todd & Hewitt's broth	30	25
	Bacto-tryptose broth	30	16
	Glucose broth	30	5
	McKenzie's broth	30	1
2	Todd & Hewitt's broth	20	14
	Bacto-tryptose broth	20	9
	Glucose broth	20	1
	McKenzie's broth	20	0

To test whether the lethal effect of a culture might depend on the number of living organisms it contained, groups of ten mice were inoculated intraperitoneally with 0·5 ml. of strain S 13 grown for varying periods in (*a*) glucose broth, (*b*) Todd & Hewitt's broth, the number of living organisms inoculated being determined at the time of each inoculation. Results (Tables 3 and 4) with both media showed a positive correlation between lethal effect and viable count that was confirmed by repetition of both experiments. This correlation was emphasized by the fact that growth curves in the two media were quite different.

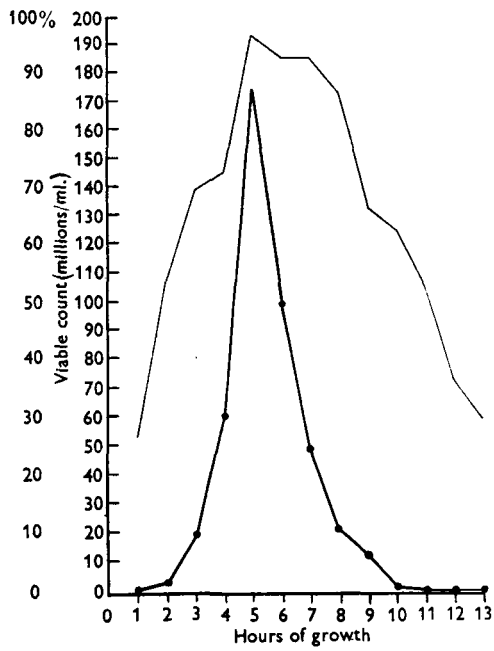
Table 3. *Results of intraperitoneal inoculation with 0·5 ml. of strain S 13 grown for varying periods in glucose broth*

Age of culture (hr.)	Viable count in millions per ml.	No. of mice inoculated	Died within 5 days
2	50	10	2
4	350	10	8
6	400	10	9
8	150	10	8
10	50	10	1
12	3	10	0
14	7·5	10	0
16	1·5	10	0
18	0·5	10	0
20	0·025	10	1
22	0·005	10	0
24	0·005	10	0

A further experiment was carried out to examine the possibility that a given number of organisms in a rapidly growing culture might be more virulent than the same number of organisms in a rapidly dying culture. 0·02 ml. of an overnight growth in Todd & Hewitt's broth of strain S 13 was seeded into each of thirteen flasks containing 20 ml. of glucose broth warmed to 37° C. A viable count was made every hour from the first to the thirteenth hour of growth, and at each hour ten mice were inoculated intraperitoneally with 0·1 ml., ten with 0·5 ml., and

Table 4. *Results of intraperitoneal inoculation with 0·5 ml. of strain S 13 grown for varying periods in Todd & Hewitt's broth*

Age of culture (hr.)	Viable count in millions per ml.	No. of mice inoculated	Died within 5 days
2	2	10	1
4	12	10	3
6	4·5	10	0
8	4	10	6
10	17·5	10	9
12	45	10	7
14	450	10	10
16	450	10	8
18	500	10	6
20	350	10	10
22	600	10	8
24	550	10	8
26	450	10	10
28	350	10	8
30	350	10	9



Text-fig. 1. Growth curve of strain S 13 in glucose broth over 13 hr. and percentage mortality of mice over the same period (thirty mice inoculated at each hour). •—•, viable count; —, mortality.

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Table 5. *Results of intraperitoneal inoculation of mice with (a) 0·1 ml., (b) 0·5 ml., (c) 1·0 ml. of strain S 13 grown for varying periods in glucose broth*

Age of culture (hr.)	Viable count in millions per ml.	Dose (ml.)	No. of mice inoculated	Died within 5 days
1	0·32	0·1	10	1
		0·5	10	2
		1·0	10	5
2	4·0	0·1	10	1
		0·5	10	5
		1·0	10	10
3	20·0	0·1	10	1
		0·5	10	10
		1·0	10	10
4	61·0	0·1	10	4
		0·5	10	8
		1·0	10	10
5	175·0	0·1	10	10
		0·5	10	9
		1·0	10	10
6	100·0	0·1	10	9
		0·5	10	9
		1·0	10	10
7	50·0	0·1	10	8
		0·5	10	10
		1·0	10	10
8	22·5	0·1	10	6
		0·5	10	10
		1·0	10	10
9	13·5	0·1	10	1
		0·5	10	9
		1·0	10	10
10	2·0	0·1	10	1
		0·5	10	8
		1·0	10	10
11	0·43	0·1	10	0
		0·5	10	7
		1·0	10	9
12	0·27	0·1	10	0
		0·5	10	2
		1·0	10	9
13	Less than 0·27	0·1	10	0
		0·5	10	0
		1·0	10	9

ten with 1·0 ml. Results (Table 5) failed to show a significant difference in pathogenicity between inoculations of the same number of living organisms in the rapidly multiplying phase and in the rapidly dying phase. In Text-fig. 1 these results are shown graphically, mortality being expressed as a percentage of all mice inoculated, i.e. thirty mice at each hour.

Foster (1921) established by detailed experiments the relationship of pH of medium to growth and viability of a strain of '*Streptococcus haemolyticus*', and the close similarity of his findings to those recorded here suggests an intimate relationship between growth (i.e. viable count), acid production, and virulence for mice.

Table 6. *Results of intraperitoneal inoculation of mice with varying numbers of viable strain S 13 streptococci contained in 0·5 ml. doses of cultures in a variety of liquid media*

Viable count in millions per ml.	No. of mice inoculated	Died within 5 days	Mortality (%)
Less than 1	50	1	2
1–50	150	58	39
51–100	110	74	63
101–200	127	107	84
201–300	141	115	82
301–400	114	103	90
401–500	100	90	90
501–600	139	102	73
Over 600	221	156	71

Table 6 records the results of 130 experiments with strain S 13 grown for varying periods in a variety of media, and emphasizes the correlation between the lethal effect of a culture and the number of living organisms it contains. It will be noted, however, that there is a minimum viable count (in the case of these experiments about 100 million per ml.) above which percentage mortality does not vary greatly, and that the resistance of individual mice to doses of group B streptococci lethal to the majority of mice is an important reason for assessing the lethal effect of a strain by inoculation of several animals.

Pathogenicity of different strains in the same medium

To examine the lethal effect of other strains of bovine group B streptococci, ten mice were inoculated intraperitoneally, and ten mice intravenously with 0·5 ml. of cultures of eighteen strains grown in the same batch of Todd & Hewitt's broth containing 20 % sheep serum. All cultures inoculated contained a minimum of 250 million living organisms per ml.

Results (Table 7) have been arranged to illustrate (*a*) mortality following intraperitoneal and intravenous inoculation, (*b*) time of death following intraperitoneal and intravenous inoculation, (*c*) comparative lethal power of strains of different serological type. In the case of fifteen of the eighteen strains more mice died following intravenous than intraperitoneal inoculation; there was a general tendency for deaths after intravenous inoculation to be delayed longer than after intraperitoneal inoculation; there was a significant difference in total mortality between groups of strains of different serological type, although strains of similar lethal power occurred in different serological groups (e.g. strains

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Table 7. *Results of inoculating (a) ten mice intraperitoneally, (b) ten mice intravenously, with 0·5 ml. of cultures containing not less than 250 million viable organisms per ml. of eighteen bovine strains of group B streptococci*

		Died after intraperitoneal inoculation					Died after intravenous inoculation				
Strain	Serological type	1 day	2–5 days	6–10 days	11–21 days	Total	1 day	2–5 days	6–10 days	11–21 days	Total
S1	1 ? subtype	3	1	2	—	6	2	6	1	—	9
S3	,,	6	1	—	1	8	—	7	2	—	9
S4	,,	7	2	—	—	9	1	4	4	1	10
S37	,,	1	—	—	—	1	1	—	4	—	5
S90	,,	7	1	—	—	8	1	2	5	2	10
S117	,,	7	2	—	—	9	—	6	4	—	10
S148	,,	6	1	2	—	9	—	4	6	—	10
	Total	37	8	4	1	50	5	29	26	3	63
S2	3*a*	—	—	—	—	0	—	—	3	—	3
S115	,,	—	—	—	—	0	—	2	—	—	2
S175	,,	—	—	—	—	0	—	—	—	2	2
S216	,,	—	2	1	—	3	—	—	3	—	3
	Total	0	2	1	0	3	0	2	6	2	10
S5	3*b*	—	—	—	—	0	—	4	1	3	8
S133	,,	1	2	4	—	7	—	2	6	1	9
S172	,,	—	—	1	2	3	—	—	2	2	4
S196	,,	—	1	2	2	5	—	4	4	1	9
	Total	1	3	7	4	15	0	10	13	7	30
S20	Failed to type	—	1	—	1	2	—	—	—	1	1
S113	,,	3	1	—	—	4	—	—	—	—	0
S186	,,	1	1	—	—	2	—	—	3	—	3
	Total	4	3	0	1	8	0	0	3	1	4

Total mortality for groups of known serological type: (*a*) 1 ? subtype, 81 %; (*b*) 3*a*, 16 %; (*c*) 3*b*, 56 %.

S1 and S133). Subsequent experiments, with these and other strains of group B streptococci, have confirmed the general principles that more mice are likely to die after intravenous than after intraperitoneal inoculation of equal doses of the same culture, and that death following intravenous inoculation is generally delayed longer than after intraperitoneal inoculation.

Several authors (e.g. Minett, 1935; Lancefield, 1940–1; Little & Plastridge, 1946, p. 173) have suggested that virulence and serological type of bovine group B streptococci may be related; the results in Table 7 support this view but require confirmation on a considerable scale before the true relationship between lethal power and serological type can be established.

Examination of four strains for the presence of toxin

In an effort to demonstrate toxin in bacteria-free filtrates of cultures that were themselves pathogenic, strains S3, S13, S90 and S117 were grown overnight in Todd & Hewitt's broth and groups of fifteen mice were inoculated with 0·5 ml. of whole culture or with twice that quantity of bacteria-free filtrate of the same culture. All whole-culture inocula were lethal to the majority of mice, but no mouse was killed by any of the filtered inocula (Table 8).

Table 8. *Results of intraperitoneal inoculation of mice with (a) 0·5 ml. of overnight whole culture in Todd & Hewitt's broth, (b) 1·0 ml. Seitz EK filtrate of the same culture, of four bovine strains of group B streptococci*

Strain	Inoculum	Viable count in millions per ml.	Dose (ml.)	No. of mice inoculated	Died within 5 days
S3	Whole culture	100	0·5	15	15
	Seitz filtrate		1·0	15	0
S13	Whole culture	100	0·5	15	15
	Seitz filtrate		1·0	15	0
S90	Whole culture	100	0·5	15	13
	Seitz filtrate		1·0	15	0
S117	Whole culture	500	0·5	15	14
	Seitz filtrate		1·0	15	0

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The virulence for guinea-pigs of four strains inoculated intraperitoneally

Strains S 3, S 13, S 90 and S 117 were grown overnight in Todd & Hewitt's broth, and 3·0 ml. doses of the resulting cultures were inoculated intraperitoneally into groups of fifteen guinea-pigs weighing 200–250 g. Results are given in Table 9.

Table 9. *Results of intraperitoneal inoculation of guinea-pigs weighing* 200–250 *g., with* 3·0 *ml. of four bovine strains of group B streptococci grown overnight in Todd & Hewitt's broth*

Strain	Viable count in millions per ml.	No. of guinea-pigs	Died (days after inoculation)
S 3	200	15	8 (1)
S 13	400	15	13 (1) 1 (2)
S 90	150	15	14 (1) 1 (2)
S 117	200	15	14 (1)

The progress of the disease in mice following intraperitoneal inoculation of a lethal dose of strain S 13

Thirty-five mice were inoculated intraperitoneally with 0·5 ml. of an overnight growth in Todd & Hewitt's broth of strain S 13 containing about 350 million viable organisms per ml., and were then killed in groups of three for bacteriological and histological examination at ½, 1, 2, 4, 6, 8, 10, 12, and 14 hr. after inoculation. Mice killed at 6 hr. and later were clinically ill, and those killed at 14 hr. were moribund. In addition to these groups, one mouse died of the disease at 11 hr., one at 13 hr., one at 13½ hr., one at 14½ hr., and three mice were moribund at 15 hr. and were killed at that time. One mouse showed no clinical evidence of disease when destroyed at 15 hr.

(*a*) *Bacteriological findings.* Glucose broth cultures of heart blood, pleural fluid, liver, spleen, kidney, and peritoneal fluid were made from all mice; sufficient urine for culture was obtained from thirteen mice killed from 2 to 15 hr. after inoculation. All cultures, except pleural fluid of one mouse killed at 6 hr. and spleen and kidney of the healthy mouse killed at 15 hr., were positive. Seven of the positive cultures, selected at random, were typed serologically and belonged to group B.

To confirm the rapid appearance of streptococci in the heart blood (i.e. 30 min. after inoculation), twelve other mice were inoculated intraperitoneally with a similar dose of the same strain, and two of these were killed for culture of heart blood every 5 min. from 5 to 30 min. after inoculation. All cultures were positive. This rapid penetration of intraperitoneal inocula into the blood stream has been noted by several workers, whose findings have been reviewed by Wilson & Miles (1947, p. 1039).

(*b*) *Histological findings.* Histological examination was made of lung, liver, spleen, and kidney of all mice, and the outstanding finding was an intense peritonitis detectable from the fourth hour onwards; peritoneal adhesions to liver (Pl. 3, fig. 1) and spleen were noted in many sections. From the sixth hour cloudy swelling of liver cells and karyolysis of some liver-cell nuclei were noted, and from the tenth hour there was a slight subcapsular mononuclear infiltration (Pl. 3, fig. 2). Occasional homogeneous, eosinophilic casts were found in kidney tubules as early as 4 hr. after inoculation and there was some tubular dilatation. Some congestion of pulmonary vessels was occasionally noted. Spleen sections were not obviously abnormal. Cocci were only numerous in the greatly thickened peritoneum or on the liver capsule (Pl. 3, fig. 3), but were also found in pulmonary macrophages, on the capsules of spleen and kidney, and, rarely, in spleen pulp, Kupffer cells, and liver sinusoids.

Francis & Peters (1947) made a histological examination of mice killed by intraperitoneal inoculation of *Strep. agalactiae*, but did not state the time that elapsed between inoculation and death. Their failure to record peritonitis and their finding of considerable numbers of cocci in the liver substance suggest that their mice survived longer than those examined in this experiment. Their conclusion that the lungs contained fewer cocci than the liver or spleen was founded, apparently, on histological examination only.

Barnard & Todd (1940) described as a bacteraemia the disease caused by intraperitoneal inoculation of mice with group A streptococci; there was cloudy swelling of parenchymatous organs.

Attention has been drawn to the fact that lethal inocula of bovine group B streptococci were usually more rapidly fatal intraperitoneally than intravenously. The explanation may be referable to the profound toxaemia of acute intestinal obstruction so frequently related to severe peritonitis (Boyd, 1938, p. 621), rather than to the coexisting bacteraemia.

The progress of the disease in mice following intravenous inoculation of a lethal dose of strain S 13

Thirty-three mice were inoculated intravenously with 0·5 ml. of an overnight growth in Todd & Hewitt's broth of strain S 13 containing about 100 million viable organisms per ml. Groups of three mice were killed for bacteriological and histological examination at ½, 1, 2, 4, 6, 8 and 10 hr. After 10 hr. all mice were clinically ill and were killed at intervals thereafter—one at 12 hr., two at 14 hr., one at 24 hr., one at 27 hr., one at 29½ hr., and two at 30 hr.

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In addition, one mouse died of the disease at 11 hr., one at 24 hr., and two between 36 and 48 hr.

(*a*) *Bacteriological findings.* The same organs were cultured as in the intraperitoneal inoculation experiment, and in this case sufficient urine for culture was obtained from nineteen mice that were killed or died from ½ to 30 hr. after inoculation. All cultures were positive, except seven peritoneal fluid cultures between ½ and 6 hr., one urine culture at 1 hr., and one urine culture at 2 hr. Five positive cultures, selected at random, were typed serologically and belonged to group B.

To confirm the rapid appearance (½ hr. after inoculation) of streptococci in the urine, nineteen other mice were inoculated intravenously with 0·5 ml. of the same strain containing 600 million viable organisms per ml., and all were killed within 1 hr. of inoculation. Sufficient urine for culture was obtained from ten of these nineteen mice, and six of the ten cultures were positive—one at 30 min., one at 40 min., three at 50 min., and 1 at 60 min.

Sherrington (1893) expressed the opinion that bacteria only appeared in the urine after damage to kidney tissue; this view has been generally upheld by subsequent workers, whose findings have been reviewed by Book (1933). It is recorded here that *Strep. agalactiae* strain S 13 was isolated from the urine 2 hr. after intraperitoneal inoculation and 30 min. after intravenous inoculation, these being the earliest cultures made in either case, and it is therefore concluded that this strain inoculated as described caused early and significant renal damage.

(*b*) *Histological findings.* Degenerative changes were noted in livers of all mice that died or were killed beyond the fourth hour after inoculation. The only abnormalities noted in the lungs were slight patchy congestion and some macrophage infiltration into the alveolar walls. Sections of spleen were not obviously abnormal. There was slight to marked abnormality in all kidney sections, the abnormalities being cloudy swelling, tubular dilatation, and homogeneous, eosinophilic cast formation (Pl. 3, fig. 4); in some sections there was shrinkage of glomeruli but in others the glomeruli were swollen and congested.

In contrast to mice inoculated intraperitoneally, streptococci were found easily in all mice—in pulmonary macrophages, in Kupffer cells and liver sinusoids, in kidney glomeruli (Pl. 3, fig. 5), inside blood vessels—and in mice that survived beyond the tenth hour dense clumps of streptococci were found in various tissues but notably in the kidney. The usual lack of cellular reaction round these clumps of cocci was a notable feature, and when a cellular reaction did occur it was almost exclusively of mononuclear type. In some kidney sections there was necrosis of tubular epithelium round the clumps of streptococci (Pl. 3, fig. 6).

Small yellowish spots were noted macroscopically on the hearts of eight mice that survived beyond 24 hr., and histological examination of these lesions showed dense clumps of streptococci and, frequently, myocardial necrosis (Pl. 4, fig. 7). Examination of the brains of two mice that died of the disease revealed foci of cocci in the meninges and brain substance (Pl. 4, fig. 8).

Histological examination of the kidneys of mice inoculated intraperitoneally or intravenously with group B streptococci

The histological appearance of kidneys of mice that died within 15 hr. of intraperitoneal inoculation or within 48 hr. of intravenous inoculation of strain S 13 has been described. It was noted in other experiments, however, that when mice died several days after intraperitoneal or intravenous inoculation of bovine group B streptococci, post-mortem examination usually showed the kidneys to be pale, enlarged, and studded with yellowish white spots. Histological examination of kidneys showing these lesions revealed typical pyaemic nephritis (Pl. 4, fig. 9). The same condition was noted in two mice that died 5 days after subcutaneous inoculation of about 150 million living strain S 13 organisms.

DISCUSSION

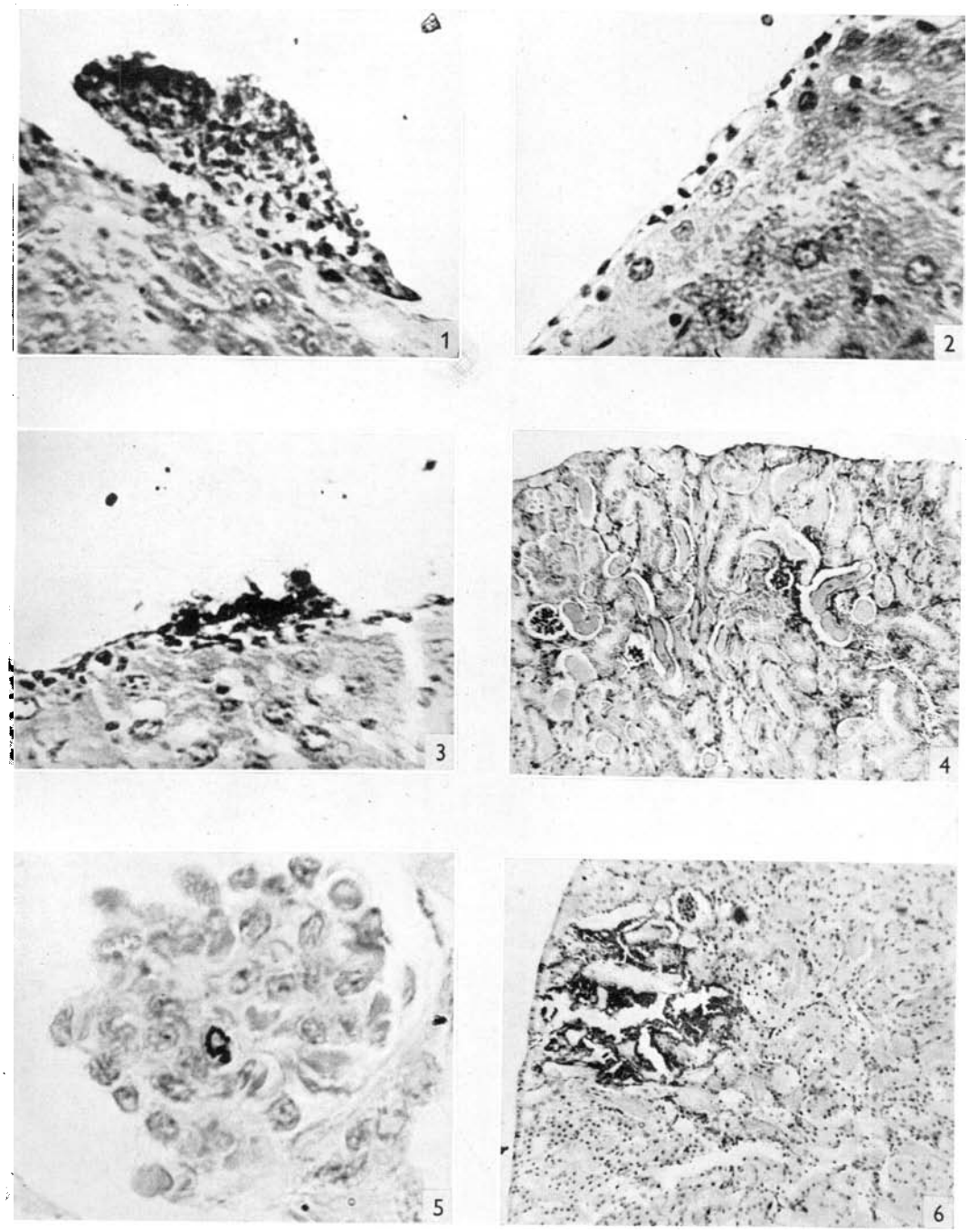
The results of experiments detailed in this paper indicate that some modification is necessary in the opinion generally held that bovine group B streptococci are of low lethal power for mice. There is now no doubt that many strains are capable of killing mice regularly when inoculated in suitable numbers by a suitable route, and although death depends on inoculation of relatively enormous numbers of living organisms, my experience has been that the mouse is a most useful animal for immunological and chemotherapeutic studies with bovine group B streptococci. However, in view of methods used by several previous authors, it seems necessary to emphasize the importance of determining the number of *living* organisms in a culture of group B streptococci used for virulence tests; failure to make such a count, or estimation of numbers by an opacity test, leaves doubt as to the viability of the culture injected.

SUMMARY

The following conclusions were drawn from these experiments with bovine group B streptococci:

1. A relationship existed between lethal power and the number of living organisms inoculated, and, therefore, the number of living organisms inoculated must be determined when the killing power of a strain is under investigation.

2. Equal doses of the same strain were likely to



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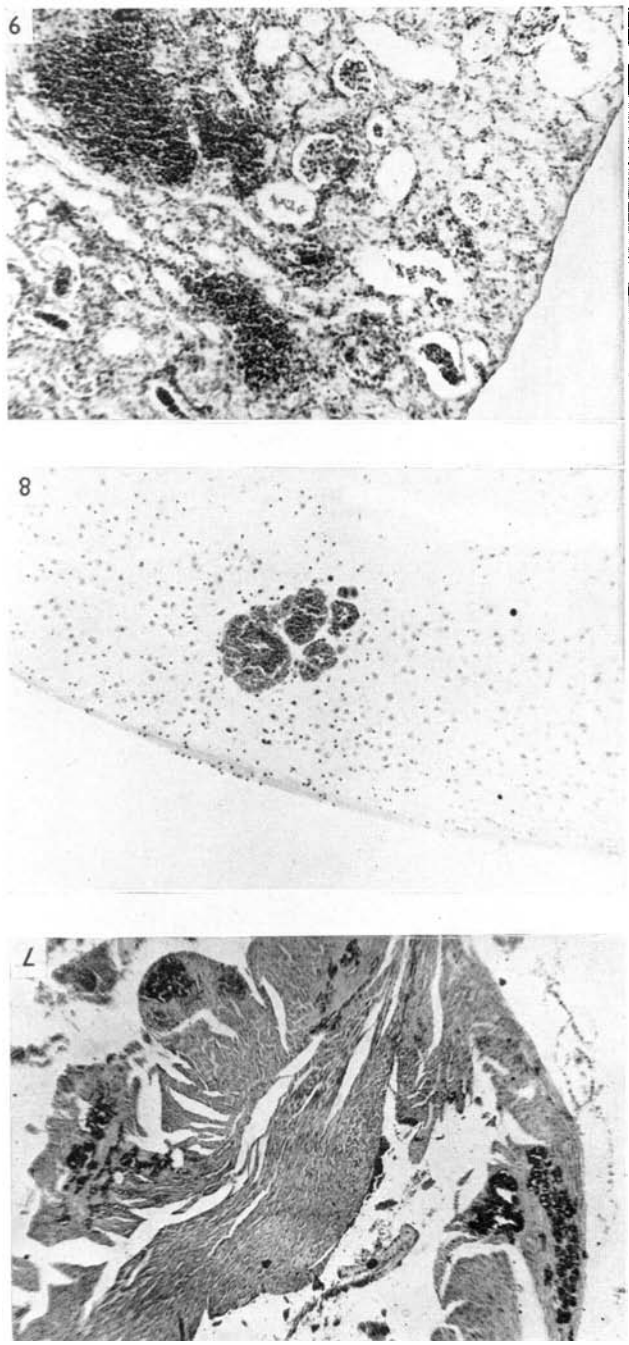

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kill more mice, but less rapidly, when inoculated intravenously than when inoculated intraperitoneally.

3. Virulence for mice might be related to serological type.

4. The disease caused by intraperitoneal inoculation of strain S 13 was a rapidly fatal peritonitis with concomitant bacteraemia.

5. The disease caused by intravenous inoculation of strain S 13 was a fatal bacteraemia with a tendency for the streptococci to form dense clumps in widely separated tissues.

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EXPLANATION OF PLATES 3 AND 4

PLATE 3

Fig. 1. Liver of mouse killed 6 hr. after intraperitoneal inoculation of *Strep. agalactiae*. Fragment of greatly thickened peritoneum swarming with cocci attached to liver capsule (H. and E., × about 400).

Fig. 2. Liver of mouse killed 12 hr. after intraperitoneal inoculation of *Strep. agalactiae*. Subcapsular infiltration with cells of mononuclear type (H. and E., × about 400).

Fig. 3. Liver of mouse killed 8 hr. after intraperitoneal inoculation of *Strep. agalactiae*. Cocci heaped on the capsular surface (Gram, × about 400).

Fig. 4. Kidney of mouse killed 1 hr. after intravenous inoculation of *Strep. agalactiae*. Tubular dilatation, homogeneous, eosinophilic casts, and some shrinkage of glomeruli (H. and E., × about 75).

Fig. 5. Kidney of mouse killed half an hour after intravenous inoculation of *Strep. agalactiae*. Cocci in a glomerulus (Gram, × about 900).

Fig. 6. Kidney of mouse that died 36–48 hr. after intravenous inoculation of *Strep. agalactiae*. Necrosis of tubular tissue in close relation to a dense focus of cocci. (H. and E., × about 75).

PLATE 4

Fig. 7. Heart of mouse that died 36–48 hr. after intravenous inoculation of *Strep. agalactiae*. Multiple dense foci of cocci (H. and E., × about 30).

Fig. 8. Brain of mouse that died 29½ hr. after intravenous inoculation of *Strep. agalactiae*. Dense focus of cocci deeply situated in the cerebral cortex (H. and E., × about 75).

Fig. 9. Kidney of mouse that was clinically ill when killed for examination 5 days after intraperitoneal inoculation of *Strep. agalactiae*. Pyaemic nephritis (H. and E., × about 75).

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