LYTIC ACTION OF CERTAIN STRAINS OF HEMOLYTIC STREPTOCOCCI ON FRESH STERILE KIDNEY AND OTHER TISSUES

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During a study of the biochemical and immunological characteristics of a group of hemolytic streptococci recovered from throat cultures taken in New Orleans and New York (Teiger and Seegal, 1936), a delicate test for the proteolytic action of these organisms was employed. The kidneys from exsanguinated monkeys[1] were removed aseptically and cut into pieces 0.5 to 1 cm. square by 1 to 2 mm. thick. These were dropped into tubes of meat infusion broth, at pH 7.6, and incubated 24 hours to test for sterility. The tubes then were innoculated with 94 of the strains of hemolytic streptococci obtained in New Orleans and New York and were placed in the incubator at 37.5°C. for 3, 4, or 5 days, together with uninoculated control tubes. At the end of the incubation period the cultures were streaked on blood plates to establish their growth and purity, the pH of the broth was tested, and the pieces of monkey kidney were fixed in Zenker's, sectioned and stained with hematoxylin and eosin.

Histological examination of the sections of kidney grown with the streptococci showed that certain strains of hemolytic streptococci exert an intensely lytic action on monkey kidney tissue. The pieces of kidney taken from cultures of such strains showed destruction of nuclei and cell boundaries with loss of cellular material. Frequently the lysis was so complete that only the

[1] These monkeys were obtained through the courtesy of Dr. C. W. Jungeblut. Most of the animals were paralyzed from experimental poliomyelitis.

connective tissue frame work of the kidney remained to identify the organ. The glomerular and tubular cells were reduced to wraiths of poorly staining amorphous material without cell boundaries or nuclei. The pieces of kidney from tubes inoculated with nonproteolytic hemolytic streptococci and from the tubes of uninoculated broth appeared normal except for minimal autolytic changes. These findings are shown in figures 1 and 2. Figure 1 illustrates a piece of monkey kidney incubated for four days with a nonlytic hemolytic streptococcus and shows only minimal autolytic changes. Figure 2 illustrates a section of monkey kidney incubated for four days with a nephrolytic hemolytic streptococcus which has destroyed cell boundaries and nuclei and most of the cell cytoplasm.

Forty of all the strains tested were lytic for monkey kidney. These strains gave β-hemolysis in rabbit-blood pour plates (Brown, 1919), and belonged to Lancefield's Group A (1933). Fifty-four of the strains failed to lyse monkey kidney tissue. Forty-eight of these were not β-hemolytic streptococci and did not belong to Group A; four other strains were β-streptococci but did not belong to Group A; the remaining 2 strains were β-hemolytic streptococci and did belong to Group A. These latter two hemolytic streptococci were therefore culturally and immunologically similar to the nephrolytic strains but on two testings failed to show nephrolysin. It is possible that prolonged cultivation on artificial media may result in a loss of nephrolytic activity which proceeds faster with some organisms than with others. Among the nephrolytic strains there was apparently a difference in the amount of nephrolysin produced since some organisms produced more complete destruction of the kidney tissue than others. A study of the factors which may influence nephrolysin production is in progress.

CONTROL TESTS

A number of control tests were run in order to rule out a nonspecific cause for the lysis of the kidney tissue. They may be summarized as follows:

1. The final pH of the broth containing monkey kidney did

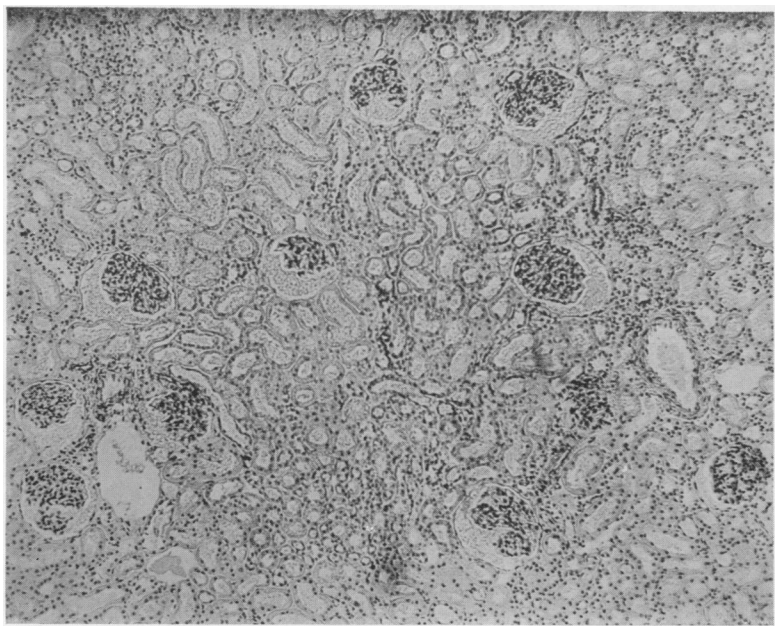

FIG. 1. SECTION OF MONKEY KIDNEY INCUBATED FOUR DAYS WITH A HEMOLYTIC STREPTOCOCCUS WHICH HAD NO NEPHROLYTIC ACTIVITY

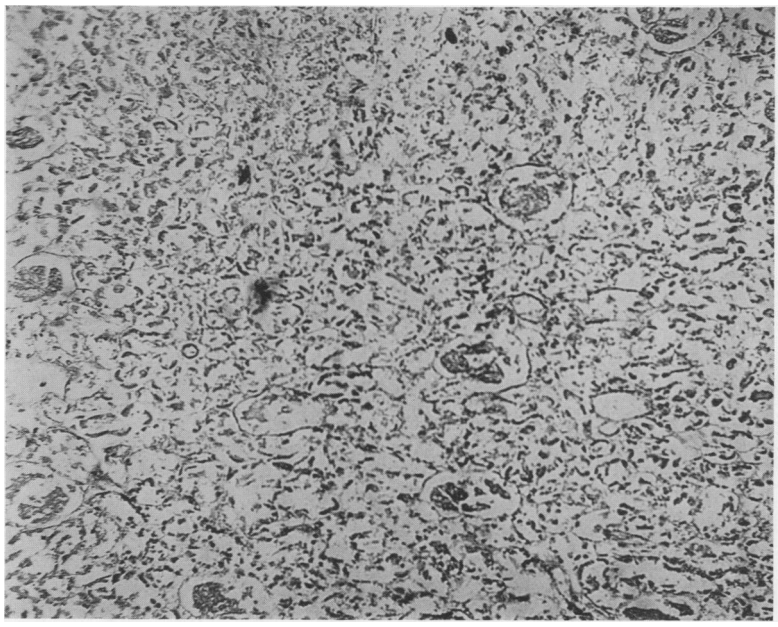

FIG. 2. SECTION OF MONKEY KIDNEY INCUBATED FOUR DAYS WITH A NEPHROLYTIC HEMOLYTIC STREPTOCOCCUS

not vary appreciably irrespective of the strains of streptococci grown. It lay between 6.7 and 7.0. The results of the pH determinations indicate that the lytic effect is not due to the final pH of the culture.

2. The effect exerted by the type of media on the lysis of the kidney tissue was tested by growing 11 selected strains in a variety of media containing the kidney tissue. Nine of the strains were known to lyse monkey kidney, while two did not. The broths employed were beef-heart infusion broth with either 1 per cent bacto- or neo-peptone, beef-muscle infusion broth with either 1 per cent bacto- or 2 per cent proteose peptone, Difco veal broth with 2 per cent proteose peptone, and the supernatant fluid from the chopped beef-heart broth used in Frobisher's histase test (1926) to be described later. No peptone was added to this latter media.

The nephrolytic strains of organisms lysed the monkey kidney in all the media tested. However, the lytic streptococci grown in the peptone free supernantant fluid from Frobisher's chopped beef-heart broth gave the most complete lysis. The nonlytic organisms did not become lytic in this peptone-free medium. This was substantiated by growing twenty-three additional known nonlytic strains with monkey kidney in this medium.

During the course of the experiments on the effects of nephrolysin which have extended over a period of a year and a half, three lots of broth have been encountered in which only slight lysis of kidney tissue occurred. These were 2 lots of beef-muscle infusion broths with proteose peptone added and one supernatant fluid from chopped beef heart without peptone. The reason for these unsatisfactory results has not been determined but probably depends upon a variation in the broth rather than a change in the microörganisms which have proved maximally lytic on subsequent testing in new media.

3. The pieces of monkey kidney varied considerably in size but pieces larger than the average lysed as readily as pieces smaller than the average. The nephrolytic substance was apparently diffusible through the larger pieces of kidney. This was further substantiated by the observation that serial sections

of four pieces of kidney showed practically the same degree of lysis throughout

4. One day's growth was insufficient to demonstrate the lytic effect satisfactorily. At the end of two days, lysis was marked and at the end of three days of growth, practically a maximum of lysis was obtained.

5. Twenty strains of the hemolytic streptococci were grown anaerobically as well as aerobically in the kidney broth. Although all grew anaerobically, the lytic effect was less pronounced anaerobically than aerobically with the exception of the case of one strain which was actively lytic when grown anaerobically, although it was not lytic when grown aerobically. This strain was not a Group A organism nor did it produce β-hemolysis in pour plates.

6. Twelve selected strains of nephrolytic and non-nephrolytic hemolytic streptococci were tested for their lysis of other monkey organs. The nephrolytic strains partially lysed monkey skeletal muscle, heart muscle, spleen, and liver. The muscle cells were more resistant to lysis than the kidney tissue. The control uninoculated sections of spleen and liver showed considerable autolytic disintegration which made the evaluation of the additional lytic effect of the streptococci difficult.

7. The same twelve strains used above were tested for their lysis of kidney tissue from other species of animals. Those strains of hemolytic streptococci which lysed monkey tissue also lysed sections of kidney from the rat, rabbit, guinea pig, and dog. The monkey kidney was the most satisfactory to use, however, since the control sections of this kidney showed only a minor degree of autolysis, even after five days incubation, whereas the kidneys from the other species showed considerable autolysis in the control tubes, rendering the evaluation of the lytic effect of the streptococci more difficult. However, the lytic effect was not species specific.

8. Representative cultures of lytic and nonlytic hemolytic streptococci were planted in broth containing monkey kidney which had been heated at 100°C. for ten minutes. The purpose

of this experiment was to destroy the autolytic enzymes of the kidney tissue in order to rule out the possibility that the lytic effect of the streptococci was merely an enhancement of the action of the proteolytic enzymes already present in the fresh kidney tissue. If lysis by the streptococci were still produced, it might be assumed that this lysis was accomplished by a product of the streptococcus acting directly on the kidney cells and not through an enhancement of the autolytic enzymes of the kidney tissue. The experiments indicated that the lytic effect of the streptococci was independent of autolytic enzymes in the kidney tissue.

9. Filtrates of hemolytic streptococci were tested for the nephrolytic agent with the following results:

a. Filtrates from cultures grown for 24 or 48 hours were slightly lytic when incubated for three or five days with monkey kidney tissue. The addition of kidney tissue to the broth from which the filtrate was obtained did not alter the results.

b. The lytic effect was completely destroyed by heating for two hours at 100°C

A concentration of the lytic activity of the filtrates is essential for further studies with this sterile substance.

A COMPARISON OF THE ABILITY OF STRAINS OF HEMOLYTIC STREPTOCOCCI TO PRODUCE A NEPHROLYSIN, TO LYSE CHOPPED MEAT, TO PRODUCE A SOLUBLE HEMOLYSIN AND TO PRODUCE A FIBRINOLYSIN

The lytic action of hemolytic streptococci on monkey kidney is a relatively delicate reaction since it is impossible to be certain of lysis without histological study. In this respect the reaction apparently differs from the "histase" reaction described by Frobisher (1926) which consists in measuring the amount by which the level of meat in a chopped meat broth tube has been reduced following incubation with a hemolytic streptococcus. The difference may be a quantitative rather than a qualitative one, however. The possible relation of the lytic action on monkey kidney to the fibrinolytic action of hemolytic streptococci described by Tillett and Garner (1933) is also interesting. The nephrolysin appears to be less species specific than the fibrino-

lysin, although Yen (1935) has reported lysis of rabbit as well as human clot with a concentrated preparation of hemolytic streptococcus fibrinolysin.

Fifty-nine strains of hemolytic streptococci from New Orleans and New York were tested simultaneously for their production of a soluble nephrolysin, hemolysin, fibrinolysin, and their ability to lyse chopped meat. The results of the tests are given in table 1. The hemolysin was tested for by the method given in the preceding paper (Teiger and Seegal, 1936). The fibrinolysin was tested in oxalated human plasma by the method of

TABLE 1

A comparison of hemolysis, fibrinolysis, "histase" reaction, and nephrolysis by hemolytic streptococci

NUMBER OF TRAINS	POUR PLATE β-HEMOLYSIS	LANCEFELD'S GROUP A	RABBIT CELL, HEMOLYSIS 2 HOURS	FIBRINOLYSIS, TIME FOR LYSIS	HISTASE REACTION MM. LYSIS	NEPHROLYSIS
29	+	+	+	13 minutes to 1 hour 45 minutes*	5–14	+
1	+	+	+	23 minutes	2	0
1	+	0	+	36 minutes	3	0
3	+	0	+	None to 50 per cent, 24 hours	0–5	0
20	0	0	0†	None, 24 hours	0–2	0
5	M	0	Trace to complete	None, 24 hours	0–4	0

* One organism required 2 hours and 40 minutes.

† One organism showed 50 per cent hemolysis in 2 hours.

M = minute streptococci of Bliss and Long (1934).

Tillett and Garner (1933). The histase reaction was carried out in tubes of chopped beef-heart media incubated aerobically. The degree of lysis as indicated by the reduction in the height of the chopped meat was read after five days incubation and 28 days at room temperature.

It will be seen from the table that a certain correlation of hemolysin, fibrinolysin, histase reaction, and nephrolysin exists in this small group of organisms. Twenty-nine strains of Group A streptococci produced a soluble hemolysin, fibrinolysin, and nephrolysin, and were actively lytic for chopped beef heart.

Another five strains, only one of which was a Group A, failed to show any nephrolytic effect, gave little or no lysis of chopped meat, and were irregular in the production of a soluble fibrinolysin but still produced sufficient soluble hemolysin to lake the test amount of rabbit red blood cells. The remaining 25 strains were relatively inactive in all the lytic tests except for an irregular amount of soluble hemolysin production by the minute streptococci of Long and Bliss.

It would appear from these results that only hemolytic streptococci which produce a soluble hemolysin and fibrinolysin and which give Frobisher's "histase reaction" also produce a nephrolysin. On the other hand, a few strains of hemolytic streptococci which produced a soluble hemolysin and fibrinolysin and had a slight histase reaction, failed to produce a nephrolysin. This may be due to a different mechanism involved in these reactions or to a quantitative difference in the amount of proteolytic substance required for a given reaction. An attempt to answer this question by the use of sterile filtrates is now in progress.

CONCLUSIONS

1. Ninety-four strains of hemolytic streptococci were grown in broth to which pieces of fresh, sterile monkey kidney had been added. Forty of these 94 strains lysed the kidney tissue.

2. The streptococci of this limited series which produced this nephrolysin were β-streptococci of Lancefield's Group A.

3. The nephrolytic effect exerted by these 40 strains was neither organ nor species specific. The same strains of hemolytic streptococci which produced a nephrolysin lysed monkey skeletal and heart muscle, spleen and liver, and the kidney tissue of the rat, rabbit, guinea pig, and dog.

4. However, the monkey kidney tissue proved the most satisfactory test organ of the lytic effect since only slight autolysis occurred in the control sections.

5. Sterile filtrates of nephrolytic hemolytic streptococci produced slight lysis of the tissue when incubated with pieces of monkey kidney.

6. Fifty-nine strains of hemolytic streptococci were tested simultaneously for their production of a nephrolysin, a soluble hemolysin, a fibrinolysin, and a "histase" enzyme (Frobisher). Twenty-nine of these strains produced all four of these lytic effects and 20 of the strains failed to produce any of them. The remaining 10 strains failed to produce any nephrolysin but produced one or more of the other lytic reactions.

REFERENCES

BLISS, E. A., AND LONG, P. H. 1934 Jour. Bact., **37**, 105.
BROWN, J. H. 1919 Mono. Rockefeller Inst. Med. Res. No. 9.
FROBISHER, M., JR. 1926 Jour. Exper. Med., **44**, 777.
LANCEFIELD, R. 1933 Jour. Exper. Med., **57**, 571.
TEIGER, P., AND SEEGAL, B. C.
TILLETT, W. S., AND GARNER, R. L. 1933 Jour. Exper. Med., **58**, 485.
YEN, A. C. H. 1935 Proc. Soc. Exper. Biol. and Med., **32**, 1403.