A STUDY ON THE ETIOLOGY OF CHOLECYSTITIS AND ITS PRODUCTION BY THE INJECTION OF STREPTOCOCCI *

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In 1914, Rosenow,¹ by making cultures from the emulsified wall of the gallbladder of selected patients, found streptococci in most instances, and reproduced cholecystitis in animals by injecting intravenously the freshly isolated organisms. The work recorded in this report is similar to that of Rosenow, except that all gallbladders removed in operations in the Mayo Clinic, regardless of the degree of pathologic changes, were cultured.

The tissues were cultured as soon as possible after their removal, every effort being made to prevent contamination. Immediately before emulsifying, the tissues were thoroughly washed in large volumes of physiologic sodium chlorid solution. They were then ground in mortars within sterile air chambers or in a hood, the air of which was washed by means of steam from a sterilizer fastened to the end of the hood. The operator wore gloves and sleeves which, with the materials used, were sterilized in the sterilizers opening into the hood.

The emulsions thus made were inoculated in varying concentrations into tall columns of dextrose brain broth, blood broth, litmus milk, ascites dextrose broth, ascites dextrose agar and dextrose agar. Krumwiede plates of dextrose blood agar and plain blood agar plates were poured also. The cultures were studied at the end of twenty-four hours, but those that were negative were examined daily for a week.

Altogether, cultures were made from seventy gallbladders and four ulcers. At first cultures were also made from the contents of the gallbladders, but because of the large number of negative results, regardless of the findings in tissues, this was abandoned.

The duration of the symptoms in the cases studied ranged from three months to thirty years. The pathologic changes ranged from slight to marked thickening of the walls. The results of the cultures are summarized in Table 1.

* From the Division of Experimental Bacteriology, Mayo Foundation.

1. Rosenow, E. C.: *The Newer Bacteriology as Determined by Special Methods*, J. A. M. A. **58**:903, 1914; *ibid.*, *Etiology of Cholecystitis and Gallstones and Their Production by the Intravenous Injection of Bacteria*, J. Infect. Dis. **19**:527, 1916.

In the gallbladders showing slight changes 30 per cent. only yielded streptococci, in contrast to 75 per cent. in those showing marked changes. Moreover, the gallbladders in which marked changes existed showed the larger number of colonies. Some of these contained countless numbers of organisms, while those showing slight changes, with few exceptions, contained a small number. Of the latter, 58 per cent. gave no growth, while only 25 per cent. of those showing marked changes gave no growth. In the cases showing slight changes, colon bacilli were isolated in pure culture from 12 per cent. and in combination with streptococci from 6 per cent. The entire 15 per cent. of those with marked changes contained both colon bacilli and streptococci.

Some of the organisms, when first isolated, produced opaque, indifferent colonies on blood agar, and microscopically were grouped in diplococcus forms with little or no chain formation. Further study, however, proved them to be streptococci. In this connection an interesting observation was made. From one of these cases, showing a pure culture of opaque gray staphylococcus-like colonies, two strains derived from a single colony were studied. The one kept on blood agar alternately aerobically and anaerobically became a green-producing streptococcus. The other, planted alternately in dextrose brain broth and on aerobic and anaerobic blood agar slants, developed hemolytic powers.

TABLE 1.—RESULTS OF CULTURES

Material Cultured	Number	Per Cent. Showing		
		Streptococci	Colon Bacilli	No Growth
Gallbladders showing slight changes.....	50	30	18	58
Gallbladders showing marked changes....	20	75	15	25
Ulcers	4	100	0	0

The different strains varied somewhat in their fermentative powers. Of the eighteen studied, all fermented dextrose, lactose and maltose, three raffinose, four mannite, ten salicin and one inulin. One strain, after a single animal passage, had its fermentative powers changed, but it was still agglutinated, the same as the original strain.

Microscopic examination of the gallbladders failed to reveal bacteria when negative cultures were obtained, but bacteria were found consistently when the cultures were positive. Organisms were found in the lesions produced in rabbits, but were not found in normal tissue. At the suggestion of Dr. E. S. Judd, microscopic examination of liver sections which he removed were made in ten cases. Interlobular cirrhosis was found in six, no change in two, and a bile-duct involvement

in two. The livers which were normal and those showing fibrotic changes were found in cases in which the gallbladders showed marked and slight changes, while in those showing cholangitis there was little or no change.

ILLUSTRATIVE CASES AND ANIMAL EXPERIMENTS

CASE 55.—A man aged 34 years, for the past two years had had gastric symptoms. Pains were aggravated by food, and soda gave no relief. He was operated on Aug. 20, 1918. The stomach was normal, the gallbladder showed slight changes and contained one large stone, the appendix showed slight changes; these two organs were removed. About 3 c.cm. of the fundus of the gallbladder was cultured.

August 21, all the cultures showed indifferent streptococci in pure form. Five c.c. of the dextrose brain broth culture was injected intravenously into Rabbit 1662.

August 22, the rabbit appeared to be well.

August 23, the rabbit appeared to be well; it was chloroformed, and the gallbladder was found greatly distended with watery bile; the walls were edematous. The stomach, spleen, kidneys, appendix, lungs and heart were normal. No other lesions could be found.

August 24, cultures made from the blood of the rabbit were negative, while those from the bile and gallbladder showed countless numbers of the injected streptococcus.

August 26, cultures were made from the pus expressed from the patient's tonsils.

August 27, the blood agar plate cultures showed indifferent and green-producing streptococci and colon bacilli. The dextrose brain broth cultures from the tonsil, containing both streptococci and colon bacilli, were injected intravenously into Rabbits 1682 and 1683.

August 28, both animals were found dead. Necropsy showed marked post-mortem changes but no evidence of specific localization. Five c.c. of the dextrose brain broth culture made from one of the indifferent colonies of streptococci was injected intravenously into Rabbit 1686.

August 29, the rabbit seemed well.

August 30, the rabbit seemed well; it was chloroformed, and the gallbladder was found edematous and distended. There were several small hemorrhages and white necrotic areas in the fundus. No other lesions were found.

August 31, cultures from the blood were negative. Cultures from the gallbladder showed streptococci in pure form. Microscopic examination of the gallbladder revealed streptococci in the tissues.

The primary cultures from three other cases of cholecystitis were injected intravenously in rabbits. The gallbladders in two of these showed marked changes and cholecystitis developed in each of the two rabbits injected. The streptococcus was recovered from the gallbladders in each, while the blood was sterile. The third strain isolated from a gallbladder having chronic changes showed no definite localization.

CASE 58.—A man, 68 years of age, had had intermittent attacks of pain in the region of his stomach for the past thirty years. The pain was worse in the afternoon and was not affected by food. He was operated on Aug. 21, 1918. An ulcer, 1.5 cm. in diameter and 4 mm. deep, with markedly indurated walls, was found on the lesser curvature of the stomach about 5 inches from the pylorus. The ulcer was excised.

August 22, cultures of the emulsified ulcer showed streptococci in pure form. Five and 8 c.c. of the dextrose brain broth culture from the ulcer were injected intravenously into Rabbits 1675 and 1676, respectively.

August 23, Rabbit 1675 seemed well. Rabbit 1676 was found dead. The cardiac end of the stomach showed fourteen punctate hemorrhages with sur-

face erosion. There were no other lesions except coccidial abscesses in the liver and in the inguinal region.

August 24, cultures from the blood and bile were negative. Cultures from the lesions in the stomach gave streptococci in pure form. Rabbit 1675 seemed well. It was chloroformed, and the cardiac end of the stomach showed several punctate hemorrhages with beginning ulceration. Other lesions were absent.

August 25, cultures from the blood and bile gave no growth. Cultures from the affected areas of the stomach gave streptococci in pure form.

August 26, cultures were made from the pus expressed from the patient's tonsils.

August 27, the blood agar plate cultures contained colon bacilli and green-producing streptococci. Three c.c. of the dextrose brain broth culture containing colon bacilli and streptococci from the tonsils were injected intravenously into Rabbit 1681.

TABLE 2.—RESULTS OF AGGLUTINATION EXPERIMENT

Serums	Dilutions of Serums	Tonsil	Gallbladder	Ulcer	Ulcer	Ulcer
		Case 55	Case 55 After One Animal Passage	Case 58	Case 58 After One Animal Passage	Case 75
Case 55 (Cholecystitis)	1-2	0 cloudy	++	+	0	0
	1-4	++	++	+	0	0
	1-8	++	++	+	0	0
	1-20	0	++	0	0	0
	1-100	0	—	0	0	0
	1-500	0	0	0	0	0
Case 58 (Ulcer of Stomach)	1-2	0	++	+	++	0
	1-4	0	0	++	+++	++
	1-8	0	0	++	++	++
	1-20	0	0	++	+	0
	1-100	0	0	+	0	0
	1-500	0	0	+	0	0
Normal Control	1-2	0	0	+	++	0
	1-4	0	0	+	+	0
	1-8	0	0	+	0	0
	1-20	0	0	0	0	0
	1-100	0	0	0	0	0
	1-500	0	0	0	0	0
NaCl Control	1-1	0	0	0	0	0

August 28, the animal was found dead. There were marked postmortem changes, but no evidence of localization.

August 29, cultures from the bile were negative. Cultures from the blood showed countless numbers of green-producing streptococci.

One other rabbit was injected with streptococci from an ulcer. It showed definite lesions in the stomach as did the others. Cultures made from the lesions showed streptococci, while those from a normal portion of the stomach were negative.

To determine further the specificity of the organisms isolated, serums were obtained from the cases cited for agglutination purposes.

As shown in Table 2, the serum of the patient with cholecystitis agglutinated both the strains from the tonsil as isolated, and the strain from the gallbladder after one animal passage, but failed to agglutinate the ulcer strains. The serum of the patient with ulcer, on the other hand, agglutinated the homologous ulcer strain as isolated, and after one animal passage, and the strain from another case of ulcer, but not the cholecystitis strains. The normal human serum had little or no agglutinating power over any of the strains.

SUMMARY

By making cultures of the emulsified tissues of gallbladders or adjacent lymph glands, streptococci are found to be the chief microorganisms associated with cholecystitis. The direct etiologic relationship of the streptococcus is established by their presence, often in numbers proportionate to the degree of gross and microscopic changes, by their having elective affinity for the gallbladder of animals and by the specific agglutinating power of the serum of the patient from whom isolated. The elective affinity for the gallbladder of animals of the strains from the tonsils indicates strongly that cholecystitis is commonly a blood borne infection from a focal source.