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Failure to Confirm Rosenow's Work on Encephalitis in its Relation to Green Streptococcus.*

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The writer visited the laboratory of Dr. E. C. Rosenow in Rochester, Minnesota, in June, 1929, to observe the work of this investigator on epidemic encephalitis and poliomyelitis. Rosenow very kindly demonstrated to me his methods and technique with material taken from cases of epidemic encephalitis which had come to the Mayo clinic. The writer frankly expressed his skepticism of the rôle of the green streptococcus in epidemic encephalitis and the rather large group of diseases which Rosenow regards as allied conditions. (It should be recalled that Rosenow regards a green streptococcus as etiologically related to a group of 12 or more disease conditions among which are epidemic encephalitis, poliomyelitis, spasmodic torticollis, epidemic hiccup, ulcerative colitis, gastric ulcer, arthritis deformans, rheumatic fever, epidemic influenza, infectious arrhythmia, chorea, pulmonary embolism, etc.).

Rosenow was able to demonstrate to me that one could take swabs from the tonsils and nasopharynx of encephalitis cases, wash these swabs in a small quantity of gelatin-Locke's solution, inject healthy rabbits with 0.1 cc. to 0.2 cc. of the unfiltered suspension intracerebrally, produce a meningo-encephalitis in most of the animals so injected and recover *only* a green producing diplostreptococcus from the brains of the dead rabbits. Likewise cultures from the swabs in glucose-brain-broth media revealed green producing streptococci when plated out on blood agar. Similar green producing streptococci were found upon direct inoculation of blood plates with the infected swabs. Gram-positive diplostreptococci were demonstrated in smears from the surface of the brains of animals dying (usually in 24 to 48 hours) following injection, as well as from smears prepared with pipettings from the brain substance.

The writer felt that Rosenow's demonstration was valid, his technique unimpeachable, that each detail of the demonstration should be accepted without question. The interpretation of this

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work, however, is another matter, and one is not readily convinced that such a demonstration proves anything regarding the relation of the green streptococcus to encephalitis, poliomyelitis, etc. Since most investigators have failed to confirm Rosenow's results and since few, if any, have up to the present used the precise methods of Rosenow we thought, in fairness to Rosenow, that his methods, as employed at the present time, should be applied to the problem in an effort to confirm his findings.

In Porto Rico the bacterial flora of the upper respiratory tract is somewhat different than in the North. Nearly all healthy individuals harbor the green streptococcus in the tonsils and nasopharynx. We thought it would be of interest to study a series of *normal healthy individuals* here employing the precise technique of Rosenow as he uses with encephalitis and poliomyelitis cases in Rochester. Twenty-five of the personnel of this institution have been so studied. The results are briefly as follows:

1. The precise technique of Rosenow has been used throughout.
2. Swabs were taken from the tonsil and from the nasopharynx. These swabs were washed off in gelatin-Locke's solution (2 cc.) and 0.2 cc. of this mixture (containing everything from the swab) was injected intracerebrally into rabbits.
3. Fifty rabbits were injected in the above manner. Two rabbits for each normal subject. Forty rabbits died with typical symptoms as produced by Rosenow with material from encephalitis cases. In other words, 80% of the animals died. Rosenow obtained 74% deaths in his series with material taken from the tonsils and nasopharynx of encephalitis cases; 40% with material taken from contacts with these cases and only 23% with material taken from normal people. Our animals died as follows:

15 rabbits died in.....	1 day
13 " " "	2 days
7 " " "	3 "
2 " " "	4 "
2 " " "	5 "
1 animal died in.....	10 "

4. Green producing streptococci were cultured from the tonsils in all of the 25 subjects. Green streptococci were cultured from the nasopharynx in all of the 25 normal subjects. In both tonsil and nasopharynx cultures the glucose-brain-broth in long tubes, as advocated by Rosenow, were employed. In fact every step throughout these experiments was exactly the same as demonstrated to us by Rosenow.

5. Green streptococci were recovered from the brains of 35 out of the 40 rabbits which died. In two instances a hemolytic staphylococcus was cultivated along with the green streptococci. In one case a non-hemolytic staphylococcus alone was cultured. Two rabbits died in which no organisms could be demonstrated. Hemorrhages in the brain were found, probably due to mechanical injury of the injection.

6. Two animals out of a total of 50 injected developed chronic lesions.

7. In 31 instances direct smears from the surface of the base of the brain revealed Gram-positive diplococci or definite streptococci. (It should be mentioned that Rosenow also finds for the most part diplococci and not chains.) Nine animals were negative.

8. Positive blood cultures from the heart blood were obtained in 18 animals. Direct smear from the heart blood was positive in only 2 animals.

9. Streptococci or diplococci were found in smears from the various organs at autopsy as follows:

<i>Organ</i>	<i>No. instances positive</i>
Kidney	5
Liver	5
Adrenal	4
Spleen	4
Lung	5

10. Fermentation reactions:

<i>Rosenow Strains (47)</i>		<i>McKinley Strains (42)</i>
47	Glucose	40
22	Lactose	36
38	Maltose	39
31	Saccharose	38
21	Raffinose	34
10	Salicin	15
13	Inulin	13
0	Mannite	1 (?) (Definitely negative third test)

11. Agglutination reactions: Rosenow's anti-encephalitis serum and anti-poliomyelitis serum were used against our strains. The results were as follows: (These tests were run on 26 strains isolated directly from the tonsil or naso-pharynx of the healthy subjects and 25 additional strains isolated from the brains of rabbits which died

following injection.) 51 strains tested in all. All agglutination tests were carefully controlled.

No. of strains giving positive agglutination:	Anti-Encephalitis Serum (Rosenow)				Anti-Poliomyelitis Serum (Rosenow)			
	1/40	1/100	1/200	1/400	1/40	1/100	1/200	1/400
	48	39	30	25	43	38	28	22

12. Virulence tests with 24 strains chosen at random show these streptococci to be practically non-virulent for mice (intraperitoneal injections—1 cc.) and for guinea pigs in the same dosage. Only three out of 24 mice died and only 7 guinea pigs. This is in accordance with virulence tests run with green streptococci by other workers.

13. Three monkeys injected intracerebrally with 1 cc. of 3 different strains chosen at random remained perfectly well. No doubt strains might have been found which would infect monkeys if sufficient numbers were tested.

14. The picture in histologic sections is one of acute meningoencephalitis the same as demonstrated to me by Rosenow in his laboratory. The meningi are hyperaemic and slightly infiltrated with lymphocytes, leucocytes (polymorphonuclears) and plasma cells. There is perivascular infiltration consisting of leucocytes, lymphocytes and plasma cells, as well as diffuse leucocytic infiltration of the subependymal tissue. Gram-positive diplococci, sometimes in short chains and often phagocytized by leucocytes, are found in the subependymal tissue. We have experienced the same difficulty as Rosenow in always finding the microbe in the brain sections, sometimes searching for a long time, but usually they are found to be present though in most instances they are very few.

15. Rosenow's "soaked-pledget-in-the-nose" method of infection has been found very unsatisfactory. Seven rabbits were treated in this manner with pledgets soaked in streptococci but only 3 succumbed to infection.

16. One case of epidemic encephalitis has been studied thoroughly as a control in this series and from this case a green producing streptococcus has been isolated from the tonsils and nasopharynx. With the same technique as employed in all of the other cases precisely the same results have been obtained. In other words, it is impossible to discern any difference between the results obtained by this method with material from encephalitis from those obtained with material from healthy individuals.

17. We have found it perfectly easy and simple to produce a fulminating meningo-encephalitis in the brains of rabbits by injecting a very small quantity of *B. subtilis* (a known saprophyte) and we attach no more importance to this, in relation to the etiology of epidemic encephalitis, than we do to the green streptococcus. In the light of this control work on healthy subjects we believe we would be as much justified in incriminating the Hay bacillus as we would the green streptococcus as the etiological agent in epidemic encephalitis.

We believe that Rosenow's work proves *only* that one can inject into the brains of rabbits material taken from the tonsils or the naso-pharynx of diseased or *healthy* individuals and most of the animals will die of meningo-encephalitis (74% his highest figure with material from encephalitis cases—80% our figure with material from healthy individuals.) One recovers in most instances *only* a green streptococcus—the other microbes having no effect. (In one case we recovered a non-hemolytic staphylococcus alone and in 2 instances we cultured both a hemolytic staphylococcus and green producing streptococcus from the brains of our rabbits.) This phenomenon may be explained on the basis of greater virulence of the green streptococcus or by the fact that they predominate in the flora at the time the swab is taken. In any case it apparently has no bearing upon the etiology of epidemic encephalitis or poliomyelitis and is entirely a problem of more or less academic nature.