7377 C

Action of Sodium Salicylate on Fermentation of Salicin and Glucose by Streptococci.

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The action of sodium salicylate on the biological functions of streptococci is of particular interest because of the obviously beneficial effects obtained through the administration of sodium salicylate in rheumatic fever.[1, 2] The etiology of this disease is still considered to be doubtful, yet streptococci seem to be involved, at least in producing complications. Experiments on the direct streptococcidal action of salicylates indicated that this action is not sufficiently strong to explain any curative effect.[3] Curative effect was not found in animal experiments.[4] The opinion that salicylates act in clinical cases mainly by virtue of their antipyretic and analgesic properties has thus come to be almost generally accepted.[5, 6] However, this is in contradiction to the fact that other antipyretic and analgesic drugs cannot as a rule replace salicylates in rheumatic fever.

Studies on the mechanism of the action of different chemotherapeutic agents (neoarsphenamine, Bayer 205) have indicated that in low therapeutic concentrations these do not kill the parasites but merely decrease their virulence.[7] While the activity of these agents can be easily demonstrated in animal experiments, salicylates do not seem to be active in the experimental infections so far studied. This may be due to the fact that there is no experimental infection of streptococci which is strictly comparable with rheumatic fever. The writers have studied skin lesions produced by the intradermal injection of streptococci (green producing and hemolytic) and by the

[1] Meyer, H., Gottlieb, R., and Henderson, V. E., *Experimental Pharmacology*, Lippincott, 1926, 562.

[2] Caussade, Q., and Tardieu, A., *Revue médicale*, 1931, **48**, 287.

[3] Birkhaug, K. E., *J. Infect. Dis.*, 1931, **48**, 212.

[4] Miller, J. L., *J. Am. Med. Assn.*, 1914, **63**, 1107.

[5] Swift, H. F., and Boots, R. H., *J. Exp. Med.*, 1923, **37**, 553.

[6] Davis, D. J., *Arch. Int. Med.*, 1915, **15**, 555.

[7] Reiner, L., and Koveskuti, J., *Deut. Med. Wochenschr.*, 1927, **53**, 1988. Reiner, L., and Leonard, C. S., *Arch. intern. pharmacodynamie*, 1932, **43**, 10, 49.

same organisms treated *in vitro* with sodium salicylate. There was no appreciable difference between the lesions produced by salicylate-treated streptococci and controls. Neither was any effect observed on similar skin lesions in 2 rabbits which received 10 daily injections of one gram each (5 cc. of 20% solution) of sodium salicylate subcutaneously. Seven injections were given prior to and 3 following the intradermal injections of streptococci.

A definite damaging action of sodium salicylate was found, however, when the glucose and salicin fermentation of streptococci was studied in the presence of sodium salicylate. The extent of fermentation was estimated by the total acid produced and by the change of pH of the medium.* Details and results of a typical experiment are given below.

Streptococcus, AB, 13,† was grown for 18 hours in beef heart broth (pH 7.4); centrifuged and the sedimented organisms suspended in one-twentieth of the original volume of peptone solution. Two-tenths cubic centimeter of the heavy culture suspension was added to tubes containing salicin or glucose, both with and without sodium salicylate in a medium of beef extract broth. All tubes were brought to equal volumes (9.0 cc.). After incubation for specified times, a portion of each solution was removed for testing. pH determinations were done by colorimetric method. Non-volatile acid production was determined by titration with N/10 sodium hydroxide with phenolphthalein as indicator, after preliminary addition of a definite amount of hydrochloric acid and boiling to remove CO_2.

The experiment tabulated shows that 0.5% sodium salicylate strongly inhibited the fermentation activity of streptococcus AB, 13, for salicin and glucose. Distinct inhibition was also found with 0.2% salicylate. The pH change and the acid produced in 24 hours was always less in the presence of sodium salicylate than the pH change and acid produced in 4 hours without salicylate. The inhibition of activity by sodium salicylate for salicin was greater than for glucose.

The growth of bacteria after 6 hours incubation in these various

* The inhibiting action of salicylic acid on the fermentation of sugar by yeast was demonstrated by Kolbe.[8] For the action of salicylates on other ferments, see Hanzlik.[9] The activity of yeast lactic dehydrogenase is not inhibited by sodium salicylate (our own experiment).

[8] Kolbe, H., *Chem. Z.*, 1874, 617, 632.

[9] Hanzlik, P. J., *Actions and Uses of the Salicylates and Cincophen in Medicine*, The Williams Wilkins Co., 1927.

† This culture was obtained through the courtesy of Dr. Nicholls and Dr. Stainsby, New York Hospital, Cornell Medical College, New York.

TABLE I.
Fermentation Activity of Hemolytic Streptococcus for Salicin and Glucose with and without the Addition of Sodium Salicylate.

		Observations after Incubation for Various Periods of Time						
		4 hrs.	6 hrs.				24 hrs.	
Carbohydrate .5%	% Sodium Salicylate	pH	pH	Density	Approximate number of colonies per cc. in millions	Approximate number of cocci per chain or clump	pH	Total Acidity for 10 cc.
Salicin	—	5.3	5.0	5	4,400	12-20	4.9	2.36*
"	0.5	6.4	6.4	3½	3,400	8-12	6.3	1.20
"	0.2	6.3	6.3	3½			5.4	1.64
Glucose	—	5.0	4.9	5	3,140	16-20	4.8	2.72
"	0.5	5.6	5.5	3½	2,960	8-12	5.4	1.82
"	0.2	5.4	5.4	4			5.3	2.12
†—	—	6.7	6.7	3½	3,040	8-12	6.4	0.68
—	0.5	6.7	6.7	3	2,780		6.4	0.84

* In terms of tenths milli-equivalents of acid produced per 10 cc. of culture.
† Before incubation, Density = Barium Sulfate Standard No. 3.
Colonies per cc. = 4,000 million.
Approximate number of cocci per chain, 8-12.

media showed interesting differences when measured by the density, smear examination and colony count in blood agar plates. The density in the tubes in which the organisms were grown in salicin or glucose alone had increased from the original value of No. 3 (Barium Sulfate Standard) to a value of 5, while in the tubes which contained sodium salicylate with the sugars, the density had increased only to final values of 3½ to 4. Examination of smears made at this time showed that in the tubes containing carbohydrate, but no salicylate, the number of cocci per chain had increased from the original average number of 8-12 to an average of 12-20 and there was a tendency to form clumps. The number of chains or clumps per field, however, appeared to be unchanged. In the tubes containing salicylate, 0.5%, with the carbohydrates, the number of cocci per chain and the number of chains per field were approximately the same as in the original culture dilution. On the other hand, the number of colonies per cubic centimeter, as evidenced by the growth in blood agar pour plates, incubated for 48 hours was similar in the tubes with or without sodium salicylate. This might be taken as an indication of the fact that the colony count alone is inadequate as a method for studying the growth of streptococci. It is of interest to note that streptococci with short chains sometimes have been considered to be less virulent.

The fermentation of salicin and that of glucose by streptococci was found to be inhibited by sodium salicylate. The chains were

shorter in the presence of salicylate than in the controls without salicylate. The density was also less in the former case than in the latter, largely due to the fact that there were fewer individual cocci, although the number of chains or clumps were approximately the same in both cases as shown by the plate count. It is conceivable that this inhibition of a very important biological function of the streptococci is associated with a decrease of their vitality and their virulence.

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7378 P

Cephalin Content of Prepared Fibrinogen and Prothrombin Solutions.

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Mills[1] prepared fibrinogen and prothrombin solutions from platelet-free plasma; the fibrinogen remained unclotted by both calcium and cephalin for 24 hours at 40°C.; the prothrombin needed cephalin as well as calcium for its activation. Few other investigators have secured preparations which could fulfil these rigorous tests, and Howell,[2] in particular, has held to the idea that calcium alone is sufficient to activate prothrombin, the more especially since he and Cekada[3] were able to obtain active thrombins that were lipoid-free. In view of Mills' demonstration that (a) the prothrombin prepared by Howell's acetone method contained cephalin which could be extracted with cold benzene, leaving a preparation which needed cephalin as well as calcium for its activation, and that (b) an appreciable amount of cephalin-like lipoid could be extracted by boiling ether from platelet-free goose and dog plasma, we wondered whether Howell's pure thrombins had been tested on fibrinogen solutions free from lipoids. The following experiment emphasizes the need for satisfying this inquiry.

A dog, *not fasted,* was anesthetized with sodium amytal. Blood was collected, via a paraffined cannula in the carotid artery, into

1 Mills, C. A., *Chinese J. Physiol.,* 1927 (several papers).

2 Howell, W. H., *Am. J. Physiol.,* 1910, **26,** 453.

3 Cekada, E. B., *Am. J. Physiol.,* 1926, **78,** 512.