Central Research Institute,
Kasauli,
28th December 1952.

31 DEC 1952

Dear Sir,

I enclose a reprint from Nature of a preliminary note of our finding of a collagenase in cholera filtrates which supports the hypothesis of Sir Ronald Rogers that damage to the intestinal wall and consequent leakage of water and salts into the intestine are the chief aspects of the pathology of cholera. Sir Franklin M.Burnet, F.R.S. of Australia discovered the first mechanism- a mucinase- capable of directly explaining such intestinal damage. Our finding of a collagenase is the second direct mechanism. Since publishing the note another enzyme- an elastinase too has been observed in some strains of the organism. The three enzymes, if produced in quantity in the bowel of a patient, can do real damage to the intestinal wall as well as the walls of the numerous blood and lymph vessels which course through the intestinal wall. The submucoaa of the intestine is almost pure collagen and is used for making cat-gut and the blood and lymph vessels are composed of collagen and elastin in about equal proportion.

With best regards and best wishes for a Happy New Year!

Yours sincerely,
E. K. Narayanan.
(E.K.Narayanan)

To
Prof. Sir Krishnan, F.R.S.,
National Physical Laboratory,
Delhi.

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Enzymes of *Vibrio choleræ*

BURNET's discovery[1] of an epithelium-desquamating enzyme in *V. choleræ* filtrates has opened up a new line of approach in the investigation of the pathogenesis of cholera.

Using pure collagen prepared by the method of Highberger[2] from buffalo tendo Achillis, we have been able to demonstrate, in culture filtrates of *V. choleræ*, a collagenase with an optimum *p*H of 8·0. This enzyme could not be detected in culture filtrates of *B. coli*. The method employed for this study was as follows. 4-ml. quantities of a Seitz filtrate of an 18–20-hr. culture in 1 per cent nutrient agar of *V. choleræ* (Inaba), after being adjusted to *p*H's ranging from 4 to 9 and made up to the same total volume of 6·4 ml. with distilled water, were treated with 0·2 gm. each of the collagen preparation and incubated for 48 hr. at 37° C. A boiled cholera filtrate – collagen control was also run in parallel. At the end of the incubation period, the undigested collagen was separated off and samples from the clear solution were taken out in duplicate for nitrogen analysis by micro-Kjeldahl method. The average nitrogen content in mgm. per ml. of the solution of closely agreeing duplicates is given in the accompanying table.

Nature of filtrate	*p*H	Nitrogen per ml. of supernatant	Increase in nitrogen/ml. of fresh filtrate incubates over control
V. choleræ, type Inaba	4·0	0·66	0·0
	5·0	0·814	0·154
	6·0	0·941	0·281
	7·0	0·947	0·287
	8·0	0·961	0·301
	9·0	0·918	0·258
Boiled *V. choleræ* filtrate (control)	6·0	0·66	
B. coli filtrate	7·0	0·972	0·013
	8·0	0·995	−0·015
Boiled *B. coli* filtrate (control)	7·0	0·959	
	8·0	1·01	

The increase in the dissolved nitrogen of the collagen–cholera-filtrate incubates over that of the control, with a maximum at *p*H 8·0, shows the presence of a collagenase in *V. choleræ*. This enzyme is capable of acting over a wide range, optimum effect being at *p*H 8·0.

Further work on vibrio enzymes is in progress. The evidence collected so far shows that collagenase

is also present to some extent in culture filtrates of vibrios of non-choleragenic origin. Details of these investigations will be published shortly.

E. K. Narayanan
P. S. Menon

Central Research Institute,
Kasauli. Feb. 26.

[1] Burnet, F. M., and Stone, J. D., *Aust. J. Exp. Biol. and Med. Sci.*, **25**, 219 (1947).
[2] Highberger, J. H., *J. Amer. Leather Chem. Assoc.*, **31**, 93 (1936).

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