

(From the State Bacteriological Laboratory, Stockholm. Head: Prof. G. Olin)

BACTERIAL PRODUCTION AND DESTRUCTION OF HISTAMINE IN VITRO

Continued studies

By

KNUT ALIN

In a preceding paper (Alin, 1950) the results were presented of some preliminary experiments pertaining to the power of certain bacteria to produce and inactivate histamine *in vitro*. The procedure, which had already been used by Werle (1941), was as follows. A suspension of washed bacteria was added to a buffered solution containing histamine 2 hydrochloride or l-histidine hydrochloride. After incubation at 37° C for a certain period, the histamine content of the solution was estimated biologically (with guinea-pig intestine). The continued experiments to be recorded in the following were carried out with the same technique, a more detailed account of which was given in the paper mentioned above. In the present investigation the bacteria were harvested from agar slant cultures incubated at 37° C for about 18 hours. During the growth period the pH of the medium either remained stable or, if the latter contained 2 per cent glucose, its pH was lowered from 7.2 to 5-5.5 by the growth of strains fermenting the carbohydrate. In the buffered bacterial solution, the concentrations of histidine or histamine were 100 µg per ml and 10 µg per ml respectively, and the bacterial concentration was roughly 50 millions of bacteria per ml. The temperature was 37° C.

TABLE I
The incidence of histidine decarboxylase within the separate bacterial groups.

Bacterial group	No. of strains examined	Medium	Histidine decarboxylated into histamine, per cent							
			0	0.1-1.9	2-3.9	4-5.9	6-7.9	8-9.9	10-11.9	12->12
<i>Escherichia</i>	85	glucose agar	14	26	11	10	12	2	4	6
	81	agar pH 5	30	48				2		1
<i>Salmonella</i>	27	glucose agar	1	7	6	6	2	1	2	2
	21	agar pH 5	12	9						
<i>Shigella</i>	2	glucose agar		1	1					
	2	agar pH 5 ¹								
<i>Proteus</i>	17	glucose agar	3	3	2	1			3	5
	8	agar pH 5		2						6
<i>Pseudomonas</i>	5	glucose agar								
	5	agar pH 5								
<i>Micrococcus pyogenes</i> (staphylococci)	6	glucose agar								
		agar pH 5 ¹								
<i>Vibrio comma</i>	1	glucose agar								
		agar pH 5 ¹								

¹ Sparse growth, if any.

The figures within double frames relate to the numbers of strains, the histamine production of which kept within the limits indicated above.

Experimental period, 48 hours, in the tube pH 5.

First, the principal aim of the present investigation was to study the question of to what extent various groups of bacteria, in the first place *Enterobacteriaceae*, were capable of producing or inactivating histamine. About 200 strains, some of them taken from the cultures stored at the State Bacteriological Laboratory and others isolated from material forwarded for examination, were studied in these respects; for the results, see Table 1 and 2.

TABLE 2
The incidence of histaminase within the separate bacterial groups.

Bacterial group	Nr. of strains examined	Nr. of strains inactivating histamine
<i>Escherichia</i>	70	20
<i>Salmonella</i>	68	0
<i>Shigella</i>	5	0
<i>Proteus</i>	26	0
<i>Pseudomonas</i>	12	12
<i>Micrococcus pyogenes</i>	6	0
(staphylococci)		
<i>Vibrio</i> (comma)	1	0
<i>Serratia</i>	3	0
<i>Klebsiella</i>	1	0
<i>Bacillus</i> (subtilis)	5	0
<i>Alcaligenes</i>	1	0
<i>Lactobacillus</i>	2	0
<i>Neisseria</i>	3	0

Growth on agar with pH 7.2.

Experimental period, 24 hours, in the tube pH 7.

To a certain extent the *Escherichia*, *Salmonella*, *Shigella* and *Proteus* groups thus proved capable of decarboxylating histidine into histamine. The decarboxylase activity varied considerably within the same group. The *Salmonella* group comprised strains of the types, *S.typhi*, *S.paratyphi B*, *S.typhi murium*, and *S.odense*. No appreciable difference emerged in respect of the decarboxylase activity between the separate types. Excepting the *Proteus* group, the strains growing on

glucose glucose agar generally were found much more active than those growing on agar with pH 5. As regards certain strains, their growth, if any, was very slight on the latter medium.

Histaminase activity was observed in all the *Pseudomonas* and a proportion of the *Escherichia* strains. Every one of the strains comprised in the former group completely inactivated histamine, which did but few of the latter group. In the remaining *Escherichia* strains capable of inactivating histamine, this effect was only partial.

Second, I endeavoured to study the significance of the hydrogen ion concentration for, on the one hand, the histidine decarboxylase- and histaminase production as well as, on the other, the optimum activity of these enzymes.

Curiously enough, some of the strains growing on agar with pH 7 were found to produce as much, if not more, histamine as the strains growing on more acid media. Even some strains growing at pH exceeding 7 produced histamine. No appreciable difference was noted between the S and R forms of *S.typhi*.

The effect of the medium upon the histaminase activity was also studied with some strains of *P.aeruginosa*. In these experiments, however, no significant difference emerged between bacteria growing on media with pH 5, 6, 7 and 8 respectively. The optimum activity of the histidine decarboxylase was found at a hydrogen ion concentration of pH 5.

Third, my investigations having disclosed a very powerful histaminase activity of *P.aeruginosa*, I tried to find a procedure of extracting histaminase from the bacteria and obtaining sterile histaminase preparations. Having disintegrated the bacteria by means of ultrasound waves, I found that the bacterial suspensions that had been subjected to this agency, and which under the microscope proved to consist principally of bacterial debris, gave rise to a histaminase effect more rapid than that of intact bacteria. On the other hand, repeated attempts at securing histaminase-active, bacteria-free preparations with the aid of filters were unsuccessful. Possibly the

TABLE 3

Medium, agar with pH	Histidine decarboxylated into histamine, per cent									
	pH in the experiment									
	4		5		6		7			
<i>E. coli</i> 5723	5	0	0	11	10	0	0	0	0	0
	6	0	0	14	15	0.5	0.1	0	0	0
	7	0	0	30	32.5	0	0	0	0	0
	8	0	0	17.5	18	0	0	0	0	0
<i>E. coli</i> 3081	5	0			0.6		0		0	
	6	0			0.2		0		0	
	7	0			0.1		0		0	
	8	0			0.1		0		0	
<i>E. coli</i> 11	9	0			0		0		0	
	5	0			6.1		0.6		0	
	7	0			4.0		0.4		0	
<i>Proteus</i> <i>vulgaris</i> 21	9	0			5.1		0.1		0	
	5	0			17.5		0		0	
	7				16.2				0	
<i>Proteus</i> <i>vulgaris</i> 21	5	0			16.0		0.1		0	
	7				35.0				0	
	8									
<i>S. typhi</i> Vi S	5				1.1		1.3		0	
	6				0.8		0.6		0	
	7				0.1		0.1		0	
	8				0		0		0	
<i>S. typhi</i> Vi R	5				1.3		0.6		0	
	6				0.3		0.2		0	
	7				0.2		0.2		0	
	8				0		0		0	
<i>E. coli</i> 9867	5				4					
	6				6.5					
	7				5.5					
	8				5					
<i>E. coli</i> 700	5				4.3					
	6				6.5					
	7				8					
	8				5					
<i>E. coli</i> 9675	5				18					
	6				35					
	7				35					
	8				20					

Experimental period, 48 hours.

enzyme is firmly attached to the bacterial stroma. The more rapid histaminase effect produced by the suspensions treated with ultrasound waves would seem to be due to a larger contact surface between histamine solution and enzyme-laden structure.

DISCUSSION

As regards the incidence, within separate groups of bacteria, of the power to produce or inactivate histamine, the present investigation has yielded results very much like those emerging from previous studies (Eggerth, 1939; Gale, 1940; Werle, 1941; Ehrismann and Werle, 1947/48). As has been seen from Table 1, growth on a medium containing glucose as a rule entailed a considerably more powerful decarboxylase activity of the strains concerned than growth on agar with pH 5. The conversion of amino acids into amines by bacteria growing on acid media is considered due to neutralization mechanism started by the bacterial cell to shift the hydrogen ion concentration towards the neutral zone (Gale, 1948). If bacteria growing on media containing glucose display decarboxylase activity, this would be due to the pH of the medium during bacterial growth through carbohydrate fermentation being displaced towards the acid zone. However, curiously enough the evidence presented in Table 3 shows that histamine production frequently was as marked in the strains grown at neutral or slightly alkaline reactions as in those grown under acid conditions. This observation, already made by Ehrismann and Werle (1947/48), was by these workers considered to suggest the presence of an enzymatic constituent not adaptive to the hydrogen ion concentration. Common to the investigation just quoted and the present one is the use of a biological method of histamine estimation, whereas earlier workers in this field either had used chemical titration (Eggerth, 1939; Gale 1940) or a manometric technique (Gale, 1940), when measuring the power of bacteria to produce carbon dioxide out of histidine. Under the experimental

conditions maintained in the present study, bacterial production of histamine, in a biologically active form, out of histidine would to a certain extent seem to be independent of an acid reaction of the medium on which the bacteria had been allowed to grow. In that case it might be conceived that histaminase produced by the bacteria is capable of destroying the histamine possibly produced by them.

SUMMARY

- (i) Roughly 130 bacterial strains were examined for their capacity of producing histamine, and approximately 200 strains for their power to inactivate histamine. Histamine production was observed in gram-negative rods comprised in the *Escherichia*, *Proteus*, *Salmonella*, and *Shigella* groups, whereas histamine inactivation was none in the *Pseudomonas* and, to a smaller extent, *E. coli* groups.
- (ii) A number of *E. coli* and *Proteus* strains grown on glucose-free media with varying hydrogen ion concentrations were found to produce, if not more, quite as much histamine when grown on a neutral or slightly alkaline medium, as when grown under acid conditions.
- (iii) Attempts at extracting histaminase from the bacterial cells were unsuccessful.

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