CHARACTERIZATION OF THE ANTIDIURETIC FACTOR IN THE LYSATE OF *V. CHOLERAE*

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One of the important symptoms of clinical cholera is marked anuria. Fraenkal (6) observed it as early as in 1892 and found 4.7% mortality amongst his patients with diminished urinary secretion and 57.2% with the group having anuria.

The anuria has been attributed to several reasons. Lebert (12) thought that anuria could be due to dehydration or due to renal nephritis which is frequently found in choleraic kidney. Consequently, it might be the result of loss of fluid, diminished arterial pressure, distention of the veins, or antomical changes in the cortical substance. Several workers (2,19,11) attributed this symptom primarily to low blood pressure and circulatory failures. Rogers (17) observed that antidiuresis is established as soon as the diarrhoeal evacuations begin.

Klebs (10) Aufrecht (1), Fraenkel and Simmonds (8) and Fraenkel (7) advocated that renal manifestations in cholera patients were due to the action of the "toxin" of *V. cholerae* on the kidney. Leyden (13), however, emphasized that the alterations observed in the kidney were altogether different from the nephritic process casued by bacterial toxins in other infectious diseases.

It has been shown by Dutta and Habbu (4) that live *V. cholerae* causes a disease in infant rabbit which closely resembles clinical cholera, so also, when cell free vibrio lysates are orally administered to these animals (5,16). The most prominent features of cholera in rabbits are profuse diarrhoea, dehydration and anuria. In the present work attempts have been made to locate the factor which is responsible in inducing anuria.

MATERIALS AND METHODS

The extract of *V. cholerae* was prepared as described by Gallut (9), using rabbit passaged strain of Inaba 569 B. The sonicate vibrio lysate was prepared with the same strain according to the method described by Oza and Dutta (16). It was dialyzed separately against distilled water to obtain dialysate and against running water to have the dialysand. The dialysis was carried out for 36-48 hours. The separated constituents were lyophilized and then reconstituted as required. All the preparations were sterile before use.

The antidiuretic activity was determined by using rat hydration method described by Burn *et al* (3). The procedure adopted was as follows.

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Groups of 4 male albino rats within the weight range of 140-240 *gms* were chosen. Food was taken out on the previous evening of the day the test was to be carried out. Five ml of sterile distilled water per 100 *gm* body weight was given by stomach tube to each rat.

Soon after the animal was hydrated, the *V. cholerae* lysates, or the dialysate, or the dialysand of the sonicate lysate was injected subcutaneoulsy in suitable doses.

A group of hydrated rats was injected with equivalent volume of saline and kept as control to note the normal urinary output.

Collection of urine : Four rats were kept in each metabolic cage and the urine collected through a funnel in a measuring cylinder under toluene layer. The amount of urine excreted was measured every 30 min. from the mid-time of the period of hydration and the injection of test samples. The animals were observed for a period of 3 hours or more.

The degree of antidiuresis was ascertained by comparing the total urinary output in the control group with that of the experimental group (Tables I, II, III, IV).

TABLE I

Antidiuretic effect of acid lysate of V. cholerae (Gallut) on rats when injected subcutaneously (40 mg/100 gm body wt.)

Time (min.)	Volume (ml) of urine excreted at the end of each 30 min.	
	Control Group	Experimental Group
0	..	..
30	3.9	0.25
60	4.1	..
90	4.0	3.55
120	3.0	2.60
150	2.5	1.50
180	1.0	..
210	1.0	..
	Total 19.5	Total 8.00

Reduction in urinary output in the experimental group=60%

TABLE II

Antidiuretic effect of sonicate! ysate of V. cholera on rats when injected subcutaneously (52 *mg*/100 *gm body wt.*)

Time (min.)	Volume (ml) of urine exerted at the end of each 30 min. Control Group	Experimental Group
0	..	..
30	..	..
60	..	..
90	6.6	2.2
120	3.4	0.9
150	6.0	0.9
180	2.0	..
210	..	..
	Total 18.0	Total 4.9

Reduction in the urinary output in experimental group=77%

TABLE III

Effect of dialysate of V. cholerae on albino rats (*sonicate lysate*) *when injected subcutaneously in grade doses*

Time min.	Volume (ml) of urine excreted at the end of each 30 min. Control group;	Experimental group (30 *mg*/100 *gm*)	(52 *mg*/100 *gm*)	(105 *mg*/100 *gm*)
0	..	..	..	..
30	0.1	..	0.1	..
60	0.1	..	0.1	0.1
90	0.8	0.5	0.3	0.1
120	0.4	0.8	0.9	1.1
150	..	..	..	..
180	3.6	3.6	3.6	3.6
210	3.6	3.6	3.6	3.6
	Total 8.6	Total 8.5	Total 8.6	Total 8.5
		% Reducation—Nil.	% Reducation—Nil	% Reduction—Nil.

TABLE IV

Antidiuretic effect of dialysand of V. cholerae (sonicate lysate) on rats when injected subcutaneoysly (12 mg/100 bodywt

Time (min)	Volume (ml) of urine excreted at the end of each 30 m Control groups	Experimental group
0	..	..
30	7.2	4.0
60	0.5	0.2
90	2.6	0.3
120	4.6	0.5
150	1.0	..
180	4.0	1.0
	Total 19.9	Total 6.0

Reduction in the urinary output of the experimental group=70%.

RESULTS

In Table I is presented the urinary output of the control group and the group of rats which had extract of vibrio lysate. The total excretion of urine in 210 min. with control group was 19.5 ml. while that in experimental group it was 8 ml. The percentage reduction was about 60.

In a similar condition the control group excreted 18 ml of urine as compared to 4 ml excreted by experimental group receiving sonicate vibrio lysate. The percentage reduction was found to be 77 *i.e.* 17% higher than Gallut's vibrio lysate. The findings are given in Table II.

Table III gives data on the urinary output of the control group as compared with the three different doses of the dialysate derived from the sonicate vibrio lysate, injected according to dry weight basis. Graded doses of 30 mg, 52 mg and 105 mg per 100 gm of body weight were given. Irrespective of dose levels, the dialysate produced no differences on the urinary output from the control animals.

DISCUSSION

Cessation of urine is one of the classical symptoms evidenced by majority of the cholera patients although its onset and duration vary considerably. Rumpf and Fraenkel (18) observed that urine secretion took place hand in hand with the general improvement of the cholera

patient and it could also persist even after the complete recovery. There has been great deal of agreement amongst workers in that the anuria is caused due to the lowering of blood pressure well below the rate at which it could be filtered through the glomerules (2,19,17,14). Thus, the evidences have been largely based on symptomatology and clinical experience. Anuria has been observed in the infant rabbits infected with *V. cholerae* (4) or when they were fed with lysed vibrio (5,16). It has been demonstrated here that vibrio lysates could cause anuria in healthy normal rats. Evidence is thus provided that *V. cholerae* produces some substance(s) which is responsible for antidiuresis in infant rabbits and probably in clinical cases too. In this respect the extract of *V. cholerae* (Gallut's toxin) has shown a marked antidiuresis and still greater effect was observed with the sonicate lysate.

The dialysate of the sonicate *V. cholerae* lysate was free from antidiuretic activity, while dialysand was highly active. On quantitative basis 12 mg of dialysand per 100 *gm* weight induced even pronounced antidiuresis than that produced by a dose of 52 *mg* per 100 *gm* weight of the whole lysate. Fifty-two *mg* dry weight corresponds to 1 ml of sonicate whole lysate in which about 65% was the dialyzable matter and 25% nondialyzable moiety. It was the non-dialyzable constituent (the dialysand) which was the chief source of nitrogenous matter of the vibriolysate, the dialysate being free from protein nitrogen (15). The findings here are suggestive that the antidiuretic activity is associated with the dialysand moiety of the lysate of *V. cholerae* which is chiefly constituted of protein portion of the lysate.

SUMMARY

The antidiuretic activity of lysates derived from classical *V. cholerae* strain has been studied on hydrated rats. The lysates of *V. cholerae* have been found to cause anuria in the experimental animals. The sonicate lysate has been separated into two parts by dialysis. The dialysate, comprising of major physical constituents of the whole lysate, was found to be free from antidiuretic activity. The dialysand which is mainly proteinous in nature accounted for the antidiuretic activity of the lysate.

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