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The effect of oral pancreatic extract on jejunal bactericidal activity in protein-deficient vervet monkeys challenged with *Vibrio cholerae*¹

K. GYR, O. FELSENFELD

In memoriam Oscar Felsenfeld

Summary

Eleven vervet monkeys (*Cercopithecus aethiops*) were fed with an “0” protein diet. After the serum albumin level fell below 2.5 g/100 ml the animals and 4 controls, which received regular monkey chow, were orally infected with a monkey-adapted strain of *Vibrio cholerae*. The total bactericidal activity of the jejunal fluid decreased during feeding with “0” protein diet, but increased after challenge with *V. cholerae* in all groups. The non-immunoglobulin-bound bactericidal activity, which also decreased during protein depletion, remained less in those animals receiving placebo instead of pancreatic extract after challenge.

Key words: jejunal vibriocidal activity; protein deficiency; cholera infection; pancreatic extract; vervet monkeys.

Introduction

It has been reported in a previous communication (Gyr et al., 1978) that oral administration of pancreatic enzymes modified the course of cholera during protein deficiency in vervet monkeys and that exocrine pancreatic secretion may be of importance in the local defence against cholera. Whether pancreatic extract directly affected the vibrios (Felsenfeld and Gyr, 1977) or whether its effect was mediated by influencing immunological and other defence mechanisms, is not clear. The present communication presents additional data on jejunal antibacterial activity and its changes after treatment with pancreatic extract.

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Material and methods

Experimental animals

The experimental design has been described previously (Gyr et al., 1978) and is summarized as follows: 15 vervet monkeys (*Cercopithecus aethops*) of either sex, weighing 3.1 to 5.5 kg, and considered healthy after a thorough laboratory and clinical work-up, were divided into 3 groups. One, consisting of 4 animals, received the routine monkey chow (Purina Co.), supplemented with fruits (controls). The other group of 11 vervets was fed a "0" protein diet. When the serum albumin of the monkeys fed the "0" protein diet decreased to levels below 2.5 g/100 ml (in 6–7 weeks), all animals were challenged with 3–5 MID₅₀ of the monkey-adapted *Vibrio cholerae* strain Ogawa 41 by gavage. 6 of the monkeys which were fed the "0" protein diet and challenged with cholera vibrios received 0.5 g pancreatin per day per os (Viokase, a gift of VioBin Co.) after challenge. The others were given placebo consisting of pancreatic extract inactivated by heat. Both groups continued to have the "0" protein diet for another 10 days after infection.

Collection of specimens

The animals were intubated surgically with a sterile double bore intestinal tube (Gyr et al., 1975; Gyr et al., 1978). The tube was advanced into the jejunum and succus entericus was then collected before dieting, when the serum albumin level fell below 2.5 g/100 ml, and 2 weeks after challenge plus pancreatic extract or placebo treatment. The controls underwent the same procedures, but did not receive placebo or pancreatic extract after challenge. The samples were collected in iced tubes and portions separated for assays of pancreatic enzymes, and microbial and immunological studies.

Immunological tests

Bactericidal activity against the challenging organism was determined by the routine opacity method in the presence of 1:20 diluted complement. Strain V41 was used as the inoculum, $0.9\text{--}1.1 \times 10^3$ vibrios in the growth phase per tube. The incubation time was 4 h. The opacity was measured in a Coleman 9 nephelometer. It was expressed in percent of that observed in the control tubes containing only inoculum, broth, and complement but buffered phosphate saline pH 7.3 instead of the jejunal fluid in which the bactericidal potency was determined.

Immunoabsorption was performed by thrice repeated absorption of the filtered jejunal fluid after its pH had been adjusted to 7.0–7.8. Vervet immunoglobulins separated and purified in this laboratory (Gyr et al., 1975a) were used for the preparation of rabbit antisera and for the absorption. As a rule, each absorption was done with 3 times the volume of the antiserum necessary to precipitate the respective immunoglobulin which yielded near-maximum or maximum precipitation, as calculated from the N determinations in antigen, antibody, and precipitate.

Statistical analysis

Student's 2 sample t-test and tests for paired data were performed according to Remington and Shork (1970).

Results

The challenge with *V. cholerae* caused a non-fatal diarrhoea which averaged 1.3 days in the controls and the pancreatin group, and 4.8 days ($p < 0.001$)

Table 1. Jejunal bactericidal activity in percent growth inhibition

Group	Animals	Total activity			Activity after absorption of immunoglobulins		
		B	A	C	B	A	C
I	4 controls* P	48.5 ± 15.5	51.0 ± 11.9 NS	84.8 ± 14.2 ~0.05	25.0 ± 4.2	26.3 ± 5.9 NS	40.8 ± 7.4 NS
II	5 on "0" protein diet, no pancreatin P	44.4 ± 8.3	29.2 ± 6.6 <0.001	78.2 ± 7.6 <0.001	21.2 ± 2.6	12.4 ± 2.6 <0.001	32.2 ± 3.8 <0.001
III	6 on "0" protein diet, pancreatin after challenge P	44.3 ± 5.0	35.7 ± 5.7 <0.01	86.2 ± 8.3 <0.001	24.0 ± 6.6	15.2 ± 2.6 <0.01	44.0 ± 3.7 <0.001
P	I vs II	NS	<0.01	NS	NS	<0.01	<0.01
	I vs III	NS	NS	NS	NS	<0.01	NS
	II vs III	NS	<0.05	NS	NS	NS	<0.001

* receiving regular chow

+ arithmetic mean ± standard deviation

P level of significance of difference

B before dieting

A after dieting; before challenge

C 2 weeks after challenge

in the monkeys receiving placebo. The response of intestinal antibody titers to cholera toxin was also significantly lower in the latter group. These data have been reported previously (Gyr et al., 1978).

The bactericidal activity of the jejunal fluid is shown in Table 1. The total activity was significantly greater ($p < 0.01$ and < 0.001) in animals before feeding with the "0" protein diet. After absorption of IgG, IgA and IgM from the fluid, there still was a considerable amount of activity left which also clearly decreased during protein depletion ($p < 0.01$ and < 0.001). Following challenge with *V. cholerae*, the total bactericidal activity increased significantly without showing a difference between the 3 groups. However, activity after absorption was significantly less in the protein-depleted vervets not receiving pancreatin ($p < 0.001$).

Discussion

It had been reported that protein-depleted animals have lower intestinal trypsin, chymotrypsin, amylase, and lipase levels than monkeys receiving regular chow (Gyr et al., 1975b) and that the course of cholera in these monkeys could be mitigated to a certain extent by the administration of pancreatin (Gyr et al., 1978). The mechanisms involved in the latter observation have still to be elucidated. The present report is dealing with the jejunal bacteriolytic process in this animal group. By separating immunoglobulin (Ig)-absorbable and -not-absorbable bactericidal activity of the jejunal fluid, it could be shown that the role of the non-absorbable antibacterial activity is significant. It appears to be more affected by protein depletion than the Ig-bound activity. The constituents of the non-absorbable activity are as yet unknown in spite of the proof that enzymes participate in the destruction of at least some of the vibrios (Felsenfeld and Gyr, 1977). The fact that administration of pancreatic extract following challenge with *V. cholerae* was connected with an increase of the non-absorbable activity to the level seen in the controls is another point in favour of the exocrine pancreas being involved in the intestinal immunological and unspecific defence against cholera.

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