

Under anaerobic conditions, high ratios for U^{234}/U^{238} have been reported by Veeh (1967) even in total sediments of the upper continental slope in the eastern Pacific, Gulf of California, when the uranium content is high (4.8 to 39.0 ppm U). In such cases, the decay of excess U^{234} can be followed even in total sediments to determine the sedimentation rates. However, as already pointed out, we are observing U^{234}/U^{238} activity ratios very near 1. This ratio of 1, as well as the consistently small amounts of uranium in the ammonium acetate-acetic acid leach (0.5 dpm/g total sediment) compared to that of uranium from totally destroyed sediment (2.5 dpm/g in the top segment of the core) indicate the selective attack of the authogenic carbonate phase by the buffer. Hence, the method of attacking authogenic carbonate phase as the one reported here above, is of general utility for determining the sedimentation rates using the excess U^{234}/U^{238} activity.

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EVALUATION OF ANTIBACTERIAL AND ANTITOXIC IMMUNITY IN CHOLERA*

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WITH the realization that current cholera vaccines confer only short-term immunity to the disease, research has been stepped up in various laboratories of the world towards identifying more effective immunizing agents. There are two aspects to be considered, both dependent on the pathogenesis of the disease. While the infecting organism, *Vibrio cholerae*, is strictly confined to the gut where it proliferates, the pathological manifestations of the disease such as severe dehydration and electrolyte imbalance are due to an enterotoxin (cholera toxin) released by the organisms during growth.¹ Consequently, immune mechanisms should be operative either against the invader restricting its capacity to multiply in the gut or towards the toxin by neutralizing its activity before any harm results.² Thus, one has to think in terms of either antibacterial or antitoxic immunity or both in cholera immunology.

Sero-epidemiological evidence obtained from cholera endemic areas has provided clues for further research in this field. One of the features observed in East Pakistan was the inverse relationship between age and susceptibility to the disease, children being more susceptible than adults. Correlated with this were higher titres of a complement dependent vibriocidal antibody in the sera of individuals of older age-groups.³ It was also seen that the frequency of clinical cholera in familial contacts of cholera cases was lowest among individuals with high vibriocidal antibody levels and increased progressively among persons with lower titres.⁴ However, the association of this antibody with immunity to the disease is not yet proved as high titres have been observed in healthy individuals never exposed to cholera antigens and in certain other diseases like brucellosis. Passive protection experiments in mice with antibrucella sera showed that there was no correlation between protection and vibriocidal titres.⁵ Further,

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there is no evidence yet that the vibriocidal reaction as observed *in vitro* is the mechanism for suppression of infection in the human gut.

Animals immunized with cholera enterotoxin develop toxin-neutralizing antibodies which can be titrated in rabbit ileal loops.⁶ Such antitoxins appear also in the sera of patients convalescing from cholera and in healthy individuals resident in endemic areas.⁷ Unlike the vibriocidal antibody, antitoxic titres tend to decrease with age and cases of cholera occur in persons with detectable serum antitoxin levels. Woodward *et al.*⁸ attribute this to ineffective immunization occurring in nature and expect, as in tetanus, parenteral immunization with toxoid may induce higher and longer lasting levels of antitoxin. There is a possibility that higher levels achieved thus may be protective, as demonstrated in dogs.⁹ Particularly interesting in this context is the finding that canine ileum, perfused with blood containing antitoxin, did not respond to challenge with cholera enterotoxin.¹⁰

Although in theory it appears feasible to design studies in man and animals to determine independently the relative values of antibacterial and antitoxic immunity in cholera, practical difficulties may be encountered. While parenterally-administered, commercial cholera vaccines generally lead to the production of antibacterial antibodies only, certain preparations may contain sufficient toxin to induce the formation of antitoxins as well.¹¹ In experimental animals, after parenteral immunization with live culture vaccines, which is associated with demonstrable immunity,^{12,13} both antibacterial and antitoxic antibodies may appear in the serum¹¹ rendering interpretation difficult. In contrast, preparations of cholera enterotoxin (choleragen), effectively purified and rendered free from somatic antigenic debris, should induce the production of antitoxin exclusively. Perhaps this is not always easy to achieve as even such purified preparations may occasionally give rise to the formation of agglutinating and vibriocidal antibodies.¹⁴ Another disturbing feature is the high level of reaction observed with such extra pure preparations as manifested by lethal effects in experimental animals.¹⁵

We suggest here simplified methods for assessment of antibacterial and antitoxic immunity in cholera which do not involve expensive and time-consuming procedures of toxin

purification. These methods depend primarily on the use of 'hybrid' strains of *V. cholerae* in which O Group I antigens (Gardner and Venkatraman) have been substituted by the O antigens of a non-cholera vibrio strain (NCV 165) by genetic recombination, as described previously.¹⁶ Further modifications of the techniques, and, the use of streptomycin-resistant mutants, have led to the isolation of such 'hybrids' from three more strains of *V. cholerae* (Inaba, 569 B; Ogawa, Phil 11; and Ogawa, T 50), belonging respectively to Types 1, 3 and 5 of Feeley.¹⁷ These recombinants have been designated as 569B-165, Phil 11-165 and T 50-165 to denote their parentage as well as to indicate O-165 antigenic structure. As chromosomal transfer in *V. cholerae* appears to be confined to short segments,¹⁸ these hybrids should have retained the chromosomal genetic structure of their parent strains including the region concerned with the synthesis of the enterotoxin; the only change being in the gene cluster determining O antigen synthesis (and specificity) derived from O-165 strains by conjugation.

The broad divergence between *V. cholerae* and the 'hybrid' strains is well brought out by agglutination and vibriocidal tests recorded in Table I. Rabbits immunized with *V. cholerae* strains showed good levels of agglutinins and high titres of vibriocidal antibody in their sera, both specific for *V. cholerae* strains only. Likewise, non-cholera vibrio (NCV 165) and 'hybrid' (58SR-165) strains produced serum agglutinins for all strains which contained O-165 antigens. However, in vibriocidal tests with these sera (which showed marked 'pro-zone' effects), only two of the three hybrid strains (Phil 11-165 and T 50-165) were sensitive to this antibody. The other strain, 569 B-165, appeared to be resistant to killing by antibody and complement. Another surprising feature of the tests recorded in Table I was the absence of cross-reaction between O-165 antisera and test cultures of *V. cholerae* in agglutination tests, which could be expected on the basis of common flagellar antigens. This is under investigation. Mouse protection tests, based on the methods of Pittman and Feeley,¹⁹ are shown in Table II. It will be seen that protection is conferred only against 'homologous' challenge.

The finding that strains of *V. cholerae* and O-165 'hybrids' derived from them are antigenically distinct and do not give rise to cross

TABLE I

Agglutinin and vibriocidal titres in rabbit sera after multiple parenteral live culture immunization with cholera, non-cholera and 'hybrid' vibrio strains

Strain used for immunization	Agglutinin titre*						Vibriocidal titre					
	<i>V. cholerae</i>			'Hybrid'			<i>V. cholerae</i>			'Hybrid'		
	1	2	3	4	5	6	1	2	3	4	5	6
<i>V. cholerae</i> , Ogawa V58SR, non-motile	2000	2000	4000	0	0	0	4×10 ⁵	8×10 ⁵	8×10 ⁵	0	0	0
<i>V. cholerae</i> , Inaba V65, non-motile	4000	2000	4000	0	0	0	2×10 ⁵	8×10 ⁴	8×10 ⁴	0	0	0
Non-Cholera Vibrio NCV 165, motile	0	0	0	2000	2000	2000	0	0	0	0	1.6×10 ⁵	1.6×10 ⁵
'Hybrid' Vibrio 58SR-165, motile	0	0	0	2000	1000	2000	0	0	0	0	1.6×10 ⁵	1.6×10 ⁵

* Live cultures employed.

Key:

1 = Inaba 569B
4 = 569 B-165
0 = Negative at 50

2 = Phil 11
5 = Phil 11-165

3 = T 50
6 = T 50-165

TABLE II

Mouse protection tests with cholera, non-cholera and 'hybrid' vibrio strains

Immunized with (circ. 3×10 ⁸ cells*)	Challenge (100 LD 50 doses)		
	<i>V. cholerae</i> Ogawa 162/p	<i>V. cholerae</i> Inaba 162/p	'Hybrid' 58SR-165
<i>V. cholerae</i> Ogawa 162/p	..	1/16	16/16
<i>V. cholerae</i> Inaba 162/p	..	4/16	16/16
Non-cholera vibrio NCV 165	..	16/16	5/16
'Hybrid' 58SR-165	..	16/16	1/16

* Live cultures used.

Numerator : Deaths
Denominator: No. challenged

TABLE III

Immunization and challenge schedules to evaluate antibacterial and antitoxic immunity in cholera

S. No.	Immunization	Challenge	Immune-assay
1	<i>V. cholerae</i> , Inaba 569B killed vaccine (toxin-free)	.. <i>V. cholerae</i> Inaba 569B	Antibacterial
2	'Hybrid' 569B-165 killed vaccine (toxin-free)	.. 'Hybrid' 569B-165	do.
3	*Toxoid from <i>V. cholerae</i> , Inaba 569B	.. do.	Antitoxic
4	*Toxoid from 'Hybrid', 569B-165	.. <i>V. cholerae</i> Inaba 569B	do.
5	1 and 4 or 1 and 3	.. do.	Antibacterial + antitoxic
6	2 and 3 or 2 and 4	.. 'Hybrid' 569B-165	do.
7	Live culture vaccine attenuated <i>V. cholerae</i> ²³	.. <i>V. cholerae</i> Inaba 569B	do.
8	do.	.. 'Hybrid' 569B-165	Antitoxic

* 'Syncase' culture filtrates, formalin treated.

protection permits their use in investigations designed to assess antitoxic and antibacterial immunity in cholera. In particular, *V. cholerae* strain Inaba 569B and the 'hybrid' derived from it, strain 569 B-165, are eminently suitable as they produce detectable levels of cholera toxin and PF toxin during growth in 'Syncase' medium supplemented with sucrose,²⁰ although this has not yet been assayed quantitatively. These strains may, therefore, be used

as source of bacterial cell substance of toxin (toxoid). By virtue of its resistance to the lethal effect of antibody and complement, strain 569 B-165 may be particularly valuable in studies to delineate the role of vibriocidal antibody in cholera immunity.

Table III gives a schedule of immunizing preparations (and combinations), with corresponding challenge strains, to assess antibacterial and antitoxic immunity independently

and collectively. Parenteral routes of immunization may deserve priority because of the current interest in toxoid-induced antitoxic immunity. In experimental animal models and human volunteers, the challenge organism may be administered by mouth to mimic natural infection. Estimates of antibody levels in serum and intestine at different times before and after immunization should prove valuable in the assessment of results. The schedule, though drawn up primarily for active immunization studies, can be adapted for passive immunization as well. Changes could also be made to study type-specific immunity²¹ and the value of purified antigenic preparations in place of whole cell vaccines.²²

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THE S-TECTONITE FABRIC AS A MEANS OF INTERPRETING THE SLIP MOVEMENT AND FOLDED STRUCTURE

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ABSTRACT

A crushed quartzite and two other quartzites taken from a bedded series of Satnur area, Bangalore District, were examined for their microfabric features. All the three samples exhibit an S-tectonite fabric with the maximum at a fabric axis. It is concluded that one single type of movement, either due to faulting or to slipping along bedding planes, would produce an S-tectonite fabric with a single maximum and without a girdle.

INTRODUCTION

ONE of the outstanding contributions of Sander¹ to the petrofabric analyses of rocks is his finding that the mylonites and slickensided rocks conform to a typical S-tectonic fabric. This fabric is characterised by lineation that coincides with the direction of movement, i.e., with the a axis of the fabric. His other findings on the fabric of slickensided rocks are: (i) the strong tendency of quartz grains to lie with the optic axes parallel to a axis, and (ii) the elongation of the quartz grains also parallel to a .

QUARTZITES OF THE SATNUR AREA

Ever since Sander's announcement of the characteristic features of slickensided rocks, reports of such occurrences have been scanty. The present author examined a crushed quartzite selected from along a fault zone about four miles south-east of Satnur, Bangalore District. Slickensides are not found, but the rock has the features of a mylonite, because of its fine-grained nature due to crushing and the presence of closely-spaced slip planes. The slip planes render the rock schistose. An oriented section of this quartzite was taken