CHAPTER - 7

Studies on the antigenic make-up of choleragenic vibrios and antigenic variation in *V. cholerae* by action of mutagens
INTRODUCTION

Several observations made in the past to reveal the antigenic structure of *V. cholerae* have been reviewed by Pollitzer and Burrows (1955) and Pollitzer (1959). In recent years some workers have also attempted to elucidate the antigenic mosaic of *V. cholerae*, NAG vibrios and their antigenic relationship with the aid of gel-diffusion and intra-gel absorption techniques and immunoelectrophoresis (Misra and Shrivastava, 1959; Gullut, 1960; 1965; Ghosh and Mukerjee, 1960; 1961; Misra and Shrivastava, 1961; Kaur and Shrivastava, 1965; Ghosh *et al.*, 1970; Sil *et al.*, 1971; Farbakyova *et al.* 1974).

Antigenic variations in *V. cholerae* both in *vivo* and in *vitro* have also been recorded (Pollitzer, 1959; Bhaskaran and Gorrill, 1957; Sheehy *et al.*, 1966; Ganguly *et al.*, 1966b; Lin and Hwang, 1966; Gangarosa *et al.*, 1967; Sack and Miller, 1969).

In the light of above findings it appears that the antigenic structure of vibrios is complex and it still needs further investigations. Moreover, up to now no extensive work has been done to show that the antigenic variation could be displayed by auxotrophic mutants isolated after exposure to chemical mutagens.
The present chapter deals with our observations regarding the antigenic make-up of choleragenic vibrios and some interesting antigenic variations displayed by the auxotrophic mutants isolated after exposure to NTG and mitomycin C.

MATERIALS AND METHODS

Strains: *V. Cholerae* Inaba strain 569B (described earlier) and *V. cholerae* Ogawa strain CRC 375/70 (isolated at All India Institute of Hygiene and Public Health, Calcutta). Two *V. cholerae* biotype *eltor* Ogawa strain EW6 and GS9/65 (described earlier) and one *V. cholerae* biotype *eltor* Inaba strain CRC 90/69 (isolated in 1969 at Rangoon). Four non-agglutinable vibrio (NAG) strains, N91, CRC 2082/70, CRC 1539/70, CRC 1540/70 (all isolated from human sources) were taken from stock cultures maintained at the Cholera Research Centre, for this present study.

Auxotrophs: Auxotrophic mutants isolated after exposure to NTG and mitomycin C, used in this present investigation are shown in Tables (7.1 - 7.7). Purity of each strain and auxotrophs was confirmed before use accordingly as described earlier.
Serotyping of auxotrophs: Serotyping of the auxotrophic mutants was done by slide agglutination technique with polyvalent 0-1 cholera anti-serum and with monospecific (Ogawa and Inaba) anti-serum. Agglutinability with \( V. cholerae \) R (rough) serum was tested with anti R-serum prepared against Finkelstein's strain C 385A (rough). All the sera were supplied by WHO International Vibrio Reference Centre.

Preparation of soluble antigens: Pure and smooth colonies were taken for the purpose. Eighteen hour culture of each of the cholera vibrio strain in Roux bottles containing nutrient agar media, was washed twice and resuspended in 10 ml. of normal saline. The saline cell-suspension of each strain was then heated in boiling water in thermostatic water bath at 100°C for two hours. The heat killed suspension was centrifuged at 4,000 r.p.m. in MSE centrifuge for 30 minutes, and the supernatant was discarded. Bacterial pellet was suspended in 10 ml. of cold saline to obtain a concentration of \( 10^{11} \) vibrio cells / ml. (measured by Klett-Summerson Photoelectric colorimeter). Saline cell suspension was disintegrated by exposure to ultrasonic vibrations for 20 minutes in a ultrasonic MSE disintegrater at a frequency of
20 Kc/s. Lysed cell suspension was centrifuged in cold at 10,000 r.p.m. for 30 minutes in a Sorvall superspeed RC2-B automatic refrigerated centrifuge. The supernatant was used as the crude soluble antigen source.

Preparation of antisera: For preparation of precipitin sera smooth and pure colonies of Inaba and Ogawa strains of both biotypes of *V. cholerae*, and non-agglutinable vibrio strains described earlier in the chapter, were taken. Precipitin sera were raised by biweekly injections of adult healthy rabbits (weighing 2 to 2.5 Kgs. in weight), first intramuscularly and then intravenously, with 2 hour heat killed saline culture suspensions (3,000 million organisms per ml. approximately) in graded doses from 0.25 ml. to 3.0 ml. Bleeding was done from ear vein one week after the last injection. Agglutination titre of the serum samples was determined according to the procedure described by Cruickshank (1965). After obtaining the desired agglutination titre, final bleeding was done and sera were separated aseptically, and stored in ampoules with merthiolate (1 in 10,000).

Gel-diffusion: Diffusion of antibodies and antigens was carried out according to the gel-diffusion technique as
described by Ofichterlony (1953) with certain modifications to fit with the requirements of the present work. 0.9 gm of Ion-agar No.2 (Oxoid) was dissolved in 100ml. of sterile normal saline with pH. adjusted to 7.4. The properly dissolved agar was melted in steam and cooled to 45°C. Few drops of marthiolated solution were added to give a final concentration of 1 in 10,000. The molten agar was aseptically poured over sterile plastic petri dishes and depth of agar was maintained at 4 mm. Agar-gel was allowed to set at 25°C overnight. Wells were punched out with borer (8 mm. bore) equal to and equidistant from each other. The wells were properly sealed with few drops of molten agar. Equal volume of respective antigens and antisera were placed in different wells (illustrated in respective figures) with the help of sterile capillary pipettes under aseptic precautions until there was disappearance of surface reflection. Lines of precipitation which formed between reacting antigens and anti-bodies on standing at room temperature in a moist chamber were recorded after seven days. Plates were kept under observation for two weeks. Final results were recorded after examining the antigen and antibody reactions against artificial light. Photographic records were taken wherever possible.
Intra-gel absorption: Intra-gel absorption test was done by incorporating soluble antigen in equal proportion (v/v) in the gel-media according to the method of Feinberg (1958).

RESULTS

Fig. 7.1 shows that the antigens of the different strains in the peripheral wells (illustrated in the legends) developed a common thermostable precipitation band against the antiserum of *V. cholerae* Inaba strain 569B in the central well. Ogawa and Inaba antigens of both the biotypes of *V. cholerae* developed an additional dense band analogous to \( \alpha \) against 569B Inaba antiserum. Antigen of the NAG strain N91 did not develop a band analogous to \( \alpha \) against the 569B antiserum in the central well.

Fig. 7.2 depicts that the antigens of different strains in the peripheral wells (illustrated in the legends) formed a common heatstable precipitation band against antiserum of *V. cholerae* Ogawa strain CRC 375/70 in the central well. Ogawa antigens of both the biotypes developed a band analogous to \( \beta \). Antigens of Inaba strains of both the
biotype did not develop any band analogous to \( \alpha \) against antiserum of \textit{V. cholerae} Ogawa strain. Antigen of the NAG strain N91 also did not develop any band analogous to \( \alpha \) against the antiserum of \textit{V. cholerae} Ogawa strain 375/70.

Fig. 7.3 Shows that the antigens of different strains in the peripheral wells (Illustrated in the legends) formed a common heatstable precipitation band against antiserum of \textit{V. cholerae} biotype eltor Ogawa strain W6 in the central well. Ogawa antigens of both the biotypes developed a band analogous to \( \alpha \). Antigens of Inaba strains of both the biotype did not develop any band analogous to \( \alpha \) against antiserum of \textit{V. cholerae} biotype eltor Ogawa strain W6. Antigen of the NAG strain N91 developed a band analogous to \( \alpha \) against the antiserum of \textit{V. cholerae} biotype eltor Ogawa strain W6.

Fig. 7.4 Shows the reactions given by the antigens of different NAG strains and antigen of \textit{V. cholerae} biotype eltor Ogawa strain W6 and antigen of \textit{V. cholerae} Inaba strain 569B against the antiserum of Inaba strain 569B. The antigens of different strains in the peripheral wells, formed a common thermostable band against the antiserum of Inaba strain 569B (illustrated in the legends). Antigens of Inaba and Ogawa strains developed a band analogous to \( \alpha \). However,
none of the NAG strains developed a band analogous to against the antiserum of *V. cholerae* Inaba strain 569B.

Fig. 7.5. Shows the reactions given by the antigens of different NAG strains and antigen of *V. cholerae* biotype *eltor* Ogawa strain W6 and antigen of *V. cholerae* Inaba strain 569B against the antiserum of Ogawa strain W6 in the central well. The antigens of different strains in the peripheral wells (illustrated in the legends) formed a common thermostable band against the antiserum of *V. cholerae* biotype *eltor* Ogawa strain W6. Antigens of the Ogawa strain W6, and NAG strain N91 developed a band analogous to against the Ogawa strain W6. Other NAG strains did not develop any band analogous to against antiserum of Ogawa strain W6.

Fig. 7.6. Shows that the antigens of different NAG strains, Ogawa strain W6 and Inaba strain 569B in the peripheral wells (illustrated in the legends) developed a common heatstable band against the antiserum of NAG strain N91 in the central well. Antigens of Ogawa strain W6 and NAG strain N91 developed a band analogous to against the antiserum of NAG strain N91. Antigens of other NAG strains and *V. cholerae* Inaba strain 569B did not develop a band analogous to against antiserum of NAG strain N91.
Fig. 7.7. Shows that the antigens of different NAG strains, antigen of Ogawa strain W6 and antigen of Inaba strain 569B in the peripheral wells (illustrated in the legends) developed a common thermostable band against NAG strain CRC 1539/70 antiserum in the central well. Antigen of NAG strain CRC 1539/70 developed an additional band analogous to a common band developed between the antigens of different strains against the antiserum in the central well.

Fig. 7.8. Shows the reactions given by the antigens of different NAG strains, antigen of Ogawa strain W6 and antigen of Inaba strain 569B (illustrated in the legends) against the antiserum of NAG strain CRC 1540/70 in the central well. Antigen of NAG strain CRC 1540/70 formed a band analogous to a common band developed between the antigens of different strains against the antiserum in the central well.

Fig. 7.9. Shows intra-gel absorption with NAG antigen of CRC 1539/70 in the gel-media. Central well containing the antiserum against the NAG strain CRC 1540/70. All the common
band were absorbed. The band analogous to \( \alpha \) formed by the NAG strain CRC 1540/70 against its homologous antiserum remained (illustrated in the legends).

Fig. 7.10. Shows intra-gel absorption with the antigen of the NAG strain CRC 1540/70. Central well containing the homologous antiserum. All the bands were absorbed (illustrated in the legends).

Fig. 7.11. Shows intra-gel absorption with the antigen of NAG strain N91 in the gel-media. Central well containing homologous antiserum. All the common bands developed by the NAG strains, Ogawa strain W6 and Inaba strain 569B including the band analogous to \( \alpha \) formed by the antigen of NAG strain N91 were absorbed. The band analogous to \( \alpha \) formed by the antigen of Ogawa strain W6 against the antiserum of NAG strain N91 remained (illustrated in the legends).

Fig. 7.12. Shows when the gel-media was absorbed with the antigen of Ogawa strain W6, the central well containing the antiserum of NAG strain N91. All the common band and the band analogous to \( \alpha \) developed by the antigen of Ogawa strain W6 were absorbed. The band analogous to \( \alpha \) formed by the antigen of NAG strain N91 against homologous antiserum remained (illustrated in the legends).
Fig. 7.13. Shows intra-gel absorption with the antigen of NAG strain W91. Central well containing antiserum of Ogawa strain W6. The common bands and the band analogous to \( \alpha \) developed by the antigen of NAG strain W91 against the antiserum of Ogawa strain W6 were absorbed. The band analogous to \( \lambda \) formed by the antigen of Ogawa strain W6 against the homologous antiserum remained (illustrated in the legends).

Fig. 7.14. Shows intra-gel absorption with the antigen of Ogawa strain W6 in the gel-media. Central well containing antiserum of W6. The common bands and the band analogous to \( \lambda \) developed by antigen of Ogawa strain W6 against the homologous antiserum were absorbed. The band analogous to \( \alpha \) developed by the antigen of NAG strain W91 against the antiserum of Ogawa strain W6 remained (illustrated in the legends).
Table 7.1 shows that the auxotrophic mutants (BR/EN1-10) isolated from a *V. cholerae* biotype *eltor* Ogawa strain W6 after exposure to NTG, agglutinated with both Inaba and Ogawa antiserum but not with rough antiserum.

Table 7.2 shows that out of 27 auxotrophic mutants isolated from a *V. cholerae* biotype *eltor* Ogawa strain W6 after exposure to mitomycinC, 17 auxotrophs (BR/EN1-17) agglutinated with only Inaba antiserum but not with Ogawa and rough antiserum. Ten auxotrophs (BR/EN18-27) agglutinated with both Inaba and Ogawa antiserum but not with rough antiserum.

Table 7.3 shows that three auxotrophs (BR/GN9-11) isolated from a *V. cholerae* biotype *eltor* Ogawa strain GS9/65 after exposure to NTG agglutinated with Inaba antiserum but not with Ogawa or rough antiserum.

Table 7.4 shows that seven auxotrophs (BR/GM10-16) isolated from a *V. cholerae* biotype *eltor* Ogawa strain GS9/65 after treatment with mitomycin C agglutinated with Inaba antiserum but not with Ogawa or rough antiserum.
DISCUSSION

The present chapter reports about the findings on the antigenic make-up of choleragenic vibrio strains obtained by the gel-diffusion and intra-gel absorption techniques carried out with immune sera prepared against heat killed vibrio cells at 100°C for two hours and crude antigens prepared from ultra-sonic disintegration of two hour heat killed vibrio cultures at 100°C. Some interesting observations regarding the antigenic variation displayed by the auxotrophs, isolated after exposure to NTG and mitomycin C have also been discussed.

The present study has indicated the presence of a common thermostable band between Inaba and Ogawa serotypes of *Vibrio cholerae* and *V. cholerae* biotype *el tor* strains (Figs. 7.1 - 7.2). Inaba and Ogawa strains irrespective of their biotypes developed a concave precipitation zone nearest to their respective antigenic reservoirs, when allowed to react with homologous Inaba and Ogawa antisera. Such band has been named as $\alpha$ by Misra and Shrivastava (1959). This band has been considered to be the thermostable antigenic fraction of the vibrio cell, possibly
lipopolysaccharide, in nature and located in the cell wall (Shrivastava, 1965a). In this present study Inaba antigens did not develop any band analogous to \( \alpha \) when allowed to react with heterologous Ogawa antiserum (Figs. 7.2, 7.3). On the other hand, Ogawa antigens reacted with both homologous and as well as heterologous Inaba antiserum and resulted in the development of a band analogous to (Figs. 7.2, 7.1). Such observations have relevance with Misra and Shrivastava (1961), who have found that \( \alpha \) antigen of Ogawa reacted with both the Ogawa and Inaba antisera. Whereas, \( \alpha \) antigen of Inaba reacted only with its own antiserum. According to these workers \( \alpha \) antigen might be structurally different in Ogawa and corresponding Inaba and rough strains. Shrivastava (1965a) had expressed the view that antibodies of Inaba strains are capable of combining with cell wall antigens of both the Ogawa and Inaba strains whereas, antibodies elicited by Ogawa subtype are incapable of doing so. The present observation receives further support from Watanabe and Verwey (1965) and Yoshioka et al (1969). However, this observations do not corroborate with findings of Kaur and Shrivastava (1965), Gallut (1965) and Sil et al (1971) who observed cross reactions between
Inaba polysaccharide or antigens with Ogawa antiserum. Possible explanation for such contradictory observations may be that as has been pointed by Watanabe (1974).

NAG strains studied in this present investigation possessed a specific heat stable band of their own in addition to a common thermostable band between NAG strains and *V. cholerae*. Gallut (1960) pointed out the existence of a non-specific heat stable somatic protein antigen of NAG vibrios common with *V. cholerae*. Ghosh and Mukerjee (1961) observed that NAG strains, beside heat labile antigens also possessed one non-specific heat stable somatic antigen identical with that of *V. cholerae*. NAG vibrio strains studied by these workers had their own heat stable band which differed from strain to strain as has been found with Ogawa and Inaba subtype of *V. cholerae*. Kaur and Shrivastava (1965), reported that NAG polysaccharide produced precipitin band against homologous antiserum. Gallut (1965), studying the NAG vibrio cell walls reported 'At least two lines of
precipitation are obtained: one corresponding to the thermostable 'O' antigen, the other (or others) to one or several thermolabile antigens. Sil et al. (1971) reported that the NAG strains tested by them had their own specific antigenic fraction analogous to \( \alpha \). These workers also observed more than one antigenic fraction common between all or some of the 'O' subgroup I vibrios and some of the NAG vibrios. Recent immunodiffusion and immunoelectrophoretic studies (Ghosh et al., 1970) also lent strong support to the existence of antigenic sharing between 'O' subgroup I vibrios and some of the NAG strains.

It was further observed in this present study that the Ogawa antigen of a \( V. cholerae \) biotype eltor strain W6 developed a band analogous to \( \alpha \) when allowed to react with the antiserum prepared against a NAG strain N91. It appears from the Figure (7.11) that when the gel-media was absorbed with N91 antigen with N91 antiserum in the central well, the band analogous to \( \alpha \) developed by N91 antigen was absorbed but the band analogous to \( \alpha \) developed by Ogawa antigen.
against N91 antiserum remained. This observations may permit us to assume that the NAG strain N91 contained minor antigenic fraction apparently identical with the antigen of the Ogawa strain W6 which on repeated injections might had produced sufficient quantity of antibodies to react and precipitate corresponding Ogawa antigen but the Ogawa antigen content of the NAG strain was insufficient enough to absorb the antibodies present in high titre. It can be seen from the Figure (7.12) that when the gel-media was absorbed with Ogawa antigen with N91 antiserum in the Central well, the band analogous to developed by the N91 antigen remained, while the band analogous to developed by the W6 Ogawa antigen against the N91 antiserum was absorbed. These observations permit us to assume that the band analogous to developed by the NAG strain N91 against N91 antiserum was specific for the particular NAG strain N91, and this antigen may differentiate this NAG strain from the V. cholerae strains agglutinable with O-1 cholera antiserum. The Figure 7.5) reveals that the NAG strain N91 developed a band analogous to against the antiserum of an Ogawa strain W6. This band was absorbed, when the gel-media was absorbed with N91 antigen.
with Ogawa W6 antiserum in the central well but the band developed by Ogawa strain W6 analogous to \( \lambda \) against the Ogawa antiserum was not absorbed by N91 antigen (Fig. 7.13). This observation may suggest that the antigen of the NAG strain N91 was incapable to absorb the specific band of agglutinable vibrios. Figure (7.14) shows that when the gel-media was absorbed with Ogawa antigen W6, the central well containing W6 Ogawa antiserum, the band developed by N91 antigen analogous to \( \lambda \) against the antiserum of the Ogawa strain W6 in the central well was not absorbed but the band analogous to \( \lambda \) developed by Ogawa antigen against Ogawa antiserum was absorbed. Possible explanation for this observation may be that the Ogawa strain W6 contained a small antigenic component apparently identical with the \( \lambda \) antigen of NAG strain N91, which on repeated injections produced sufficient quantity of antibodies to precipitate corresponding NAG antigen but the NAG antigen content of the Ogawa strain W6 was not sufficient enough for absorption of antibodies present in high titre.

Sil et al. (1971) observed that both Inaba and Ogawa strains developed a band analogous to \( \lambda \) against NAG antiserum. However, in this present study Inaba antigen did
not react with NAG antisera to develop a band analogous to \( \mathcal{L} \) (Figs. 7.6 - 7.8). There was also no cross reaction between NAG antigens with Inaba antiserum to develop a band analogous to \( \mathcal{L} \) (Fig. 7.4). Kaur and Shrivastava (1965) also reported that NAG polysaccharide produced no zone against Inaba antiserum. Formation of additional dense precipitation zones against the Ogawa antiserum by boiled saline extracts of some NAG strains was observed by Ghosh and Mukerjee (1961). These workers mentioned that NAG strains may contain variable quantity of heat denatured antigens which are precipitable by Ogawa antiserum and absorbable in boiled extracts of Ogawa antigen.

This present study in general is supported by the earlier observations of Misra and Shrivastava (1961) and Ghosh and Mukerjee (1961). Some of the findings also are in agreement with Sil et al (1971) with some minor variations with regard to number of antigenic bands. However, it appears that the precipitating antigenic bands may vary from strain to strain depending upon the preparation of vibrio extracts and as well as the techniques adopted. This present study in the light of the past observations indicates a close immunological resemblance among the strains of \( V. cholerae \).
studied herein. It also permit us to assume that one, if not more, thermostable common antigen is shared by *V. cholerae* and NAG vibrios.

In the present study it was interesting enough to note that the auxotrophic mutants isolated from a *V. cholerae* biotype *elter* Ogawa strain W6 after exposure to NTG and mitomycin C displayed the antigenic characteristics of Hikojima and Inaba strains. Auxotrophic mutants isolated from another *V. cholerae* biotype *elter* Ogawa strain GS9/65 after treatment with NTG and mitomycin C also displayed the antigenic specificity of Inaba strains. However, none of the auxotrophs isolated from a *V. cholerae* Inaba strain after exposure to NTG or mitomycin C showed an antigenic variation towards Ogawa type. None of the auxotrophs isolated from NAG vibrio strain N91 after exposure to NTG agglutinated with 0-1 cholera antiserum. Auxotrophs isolated from 0 subgroup I vibrios after exposure to mutagens also could not display the antigenic specificity of NAG vibrio.

Change in *V. cholerae* serotypes by cultivating in homologous antiserum was observed by some workers in the past (Pollitzer, 1959). The change from Ogawa to Inaba has been considered as a case of antigen loss by a group of workers
both in the past and present (Shrivastava and White, 1947; Sakazaki and Tamura, 1971). However, Bhaskaran and Gorrill (1957) suggested a possibility that Inaba mutants were preexistent in Ogawa cultures and were subsequently isolated when the predominant organism was agglutinated by type specific serum. Misra and Shrivastava (1960) considered that change from Ogawa to Inaba would bring about some alteration in the polysaccharide moiety in the antigenic mosaic. Transformation of Inaba and Hikojima type from Ogawa type *V. cholerae* biotype *el Tor* strain by the DNase activity has been reported by Lin and Hwang (1966). Change from Ogawa to Inaba and *vice versa* after exposing typical *V. cholerae* strains to the action of chlorine was observed by Ganguly et al (1966b). According to these investigators, conversion of Inaba to Ogawa might be due to a change in the biosynthetic activity of the strains when exposed to chlorine. However, not much is known about such change from Inaba to Ogawa in *vitro*. Mukherji and Mukherji (1966) observed that changes in vibrio subtypes could be brought about by changes in nutrients. Bhaskaran (1974) pointed out that variations between Inaba and Ogawa antigenic subtypes can be achieved by genetic recombination.
Recently, Bhattacharya and Ray (1975) observed that an auxotrophic mutant of *V. cholerae* biotype *el tor* Ogawa strain isolated after exposure to mitomycin C showed antigenic variation towards Inaba type.

Sen Gupta (1951) reported that during a cholera epidemic at Calcutta, vibrios changed from Ogawa to Inaba in 10 cases, from Inaba to Ogawa in 8 cases. In several countries during the last few years the prevalent serotype has been found to change from Ogawa to Inaba (Sakazaki and Tamura, 1971; Barua, 1972). In vivo changes in serotypes in individual patients have also been recorded in few instances. Sheehy *et al* (1966) isolated Inaba serotype on two successive days from a laboratory technician but on the third day the strain isolated belonged to the Ogawa type. Isolation of various serotypes from a single patient at different times has been reported by Gangarosa *et al* (1967). Pierce *et al* (1970) observed a change from Ogawa to Inaba in a patient in their convalescent carrier study. Change in serotypes in experimental germ-free mice has been observed by Sack and Miller (1969). Recently, conversion of a rough strain of *V. cholerae* biotype *el tor* to the smooth state after passages in germ-free mice has been demonstrated by Miller *et al* (1972).
All these observations point to the possibility of in *vitro* and in *vivo* mutation in *V. cholerae* serological subtypes.

Change from 'O' subgroup I vibrios to NAG strains and *vice versa* still appears to be highly controversial. Several claims made in the past about transmutability of 'O' subgroup I vibrios to NAG and *vice versa* by a group workers has been critically reviewed by Pollitzer (1959). In recent years, a possibility of evolution of non-cholera vibrios from *V. cholerae* has been pointed out by Bhaskaran and Sinha (1971b) on the basis of their hybridization experiment.

This present study along with the references cited provided further support to the possibility of a change from Ogawa subtype to Inaba subtype in *vitro*. This change in serotypes may be considered as a matter of significance from epidemiological point of view.
**TABLE - 7.1**

Antigenic behaviour of the 10 auxotrophic mutants isolated after exposing *V. cholerae* biotype *eltor* strain W6 to the action of NTG

<table>
<thead>
<tr>
<th>Strain</th>
<th>Nutritional requirements</th>
<th>Agg. with N. Saline</th>
<th>Agg. with R. antiserum</th>
<th>Agg. with Poly. O-1 serum</th>
<th>Agg. with Ogawa O-1 serum</th>
<th>Agg. with Inaba O-1 serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>W6</td>
<td>Prototroph</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>BR/EN 1</td>
<td>Ade-Ser</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>BR/EN 10</td>
<td>Ade-Ser</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>569B</td>
<td>Prototroph</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: 
- Agg= Agglutinability, 
- R antiserum = Rough antiserum, 
- Agg +++ = Positive agglutination in slide, 
- Agg- = Negative agglutination in slide, 
- Ade-Ser = Adenine and serine requiring auxotrophs, 
- BR/EN 1 -10 = Auxotrophs, 569B = *V. cholerae* Inaba strain, W6 = *V. cholerae* biotype *elctor* Ogawa strain.
TABLE 7.2

Antigenic behaviour of the 27 auxotrophic mutant strains isolated after exposing the Y. cholerae biotype alter strain WS to the action of Mitomycin C

<table>
<thead>
<tr>
<th>Strain</th>
<th>Nutritional requirements</th>
<th>Agg. with N. Saline</th>
<th>Agg. with R. antiserum</th>
<th>Agg. with Poly. O-1 serum</th>
<th>Agg. with Ogawa O-1 serum</th>
<th>Agg. with Inaba O-1 serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>WS</td>
<td>Prototroph</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>BR/EM 1 to BR/EM 17</td>
<td>Ade-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>BR/EM 18 to BR/EM 27</td>
<td>Ade-Ser</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>569B</td>
<td>Prototroph</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
</tr>
</tbody>
</table>

Key: Agg= Agglutinability, R antiserum = Rough antiserum, Agg +++ = Positive agglutination in slide, Agg- = Negative agglutination in slide, Ade-Ser = Adenine and serine requiring auxotrophs, Ade- = Adenine requiring auxotrophs, BR/EM 1-27 = Auxotrophs, 569B = Y. cholerae Inaba strain, WS = Y. cholerae biotype alter Ogawa strain.
## TABLE 7.3

Antigenic behaviour of the 5 auxotrophic mutants isolated after exposing V. cholerae biotype eltor strain GS9/65 to the action of NTG.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Nutritional requirements</th>
<th>Agg. with W. Salmon</th>
<th>Agg. with R. antisem</th>
<th>Agg. with Poly. 0-1 serum</th>
<th>Agg. with Ogawa 0-1 serum</th>
<th>Agg. with Inaba 0-1 serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS9/65</td>
<td>Prototroph</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>BR/CN 9-11</td>
<td>Ade-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>539B</td>
<td>Prototroph</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Key:
- Agg = Agglutinability, R antiserum = Rough antiserum, Agg +++ = Positive agglutination in slide,
- Agg- = Negative agglutination in slide, Ade- = Adenine requiring auxotrophs, BR/HBD-11 = Auxotrophs,
- 539B = V. cholerae Inaba strain, GS9/65 = V. cholerae biotype eltor Ogawa strain.
TABLE 7.4

Antigenic behavior of the 7 mutrophic mutant strains isolated after exposing the V. cholerae biotype 01 strain 039/65 to the action of mitoxacin C.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Nutritional requirements</th>
<th>Agg. with H. Salina</th>
<th>Agg. with R. antiseraum</th>
<th>Agg. with Poly. 0-1 serum</th>
<th>Agg. with Ogawa 0-1 serum</th>
<th>Agg. with Dkta 0-1 serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS9/65</td>
<td>Prototroph</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>BR/01 30-36</td>
<td>Ade-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>GS9B</td>
<td>Prototroph</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>

Key: Agg = Agglutinability, R antiseraum = Rough antiseraum, Agg +++ = Positive agglutination in slide,
Agg- = Negative agglutination in slide, Ade- = Adonine requiring ausrotrophs, BR/01 30-36 = Ausrotrophs,
GS9B = V. cholerae India strain, GS9/65 = V. cholerae biotype 01, Ogawa strain.
Legends of Figures

Fig. 7.1. Central well contains antiserum against heat killed *V. cholerae* Inaba strain 569B. Peripheral wells contain (1) Crude antigen of 569B (Inaba), (2) crude antigen of W6 (Ogawa), (3) Crude antigen of N91 (NAG), (4) Crude antigen of GS 9/65 (Ogawa), (5) Crude antigen of CRC 90/69 (Inaba), (6) Crude antigen of CRC 375/70 (Ogawa).

Fig. 7.2. Central well contains antiserum against heat killed *V. cholerae* Ogawa strain CRC 375/70. Peripheral wells contain the same antigens as in Fig. 7.1.

Fig. 7.3. Central well contains antiserum against heat killed *V. cholerae* biotype *el tor* Ogawa strain W6. Peripheral wells contain (1) Crude antigen of 569B, (2) Crude antigen of W6 (Ogawa), (3) Crude antigen of N91 (NAG), (4) Crude antigen of CRC 2082/70 (NAG), (5) Crude antigen of CRC 90/69 (Inaba), (6) Crude antigen of CRC 375/70 (Ogawa).

Fig. 7.4. Central well contains antiserum against heat killed *V. cholerae* Inaba strain 569B. Peripheral wells contain (1) Crude antigen of 569B (Inaba), (2) Crude antigen of N91 (NAG), (3) Crude
Central well contains antiserum against heat killed \textit{V. cholerae} biotype \textit{eltor} Ogawa strain W6. Peripheral wells contain (1) Crude antigen of 569B (Inaba), (2) Crude antigen of W6 (Ogawa), (3) Crude antigen of N91 (NAG), (4) Crude antigen of CRC 1540/70 (NAG), (5) Crude antigen of CRC 1539/70 (NAG), (6) Crude antigen of CRC 2082/70 (NAG).

Central well contains antiserum against heat killed NAG strain N91. Peripheral wells contain the same antigens as in Fig. 7.5.

Central well contains antiserum against heat killed NAG strain CRC 1539/70. Peripheral wells contain the same antigens as in Fig. 7.5.

Central well contains antiserum against
heat killed NAG strain CRC 1540/70. Peripheral wells contain the same antigens as in Fig. 7.5.

**Fig. 7.9.** Intra-gel absorption with NAG antigen of CRC 1539/70 in the gel-media. Central well containing antiserum against heat killed NAG strain CRC 1540/70. Peripheral wells contain the same antigens as in Fig. 7.5.

**Fig. 7.10** Intra-gel absorption with NAG antigen of CRC 1540/70. Central well containing the same antiserum as in Fig. 7.9. Peripheral wells contain the same antigens as in Fig. 7.5.

**Fig. 7.11** Intra-gel absorption with NAG antigen of N91. Central well containing antiserum against heat killed NAG strain N91. Peripheral wells contain the same antigens as in Fig. 7.5.

**Fig. 7.12** Intra-gel absorption with Ogawa antigen of W6. Central well containing the same antiserum as in Fig. 7.11. Peripheral wells contain the same antigens as in Fig. 7.5.

**Fig. 7.13** Intra-gel absorption with NAG antigen of N91. Central well containing antiserum against
heat-killed *V. cholerae* biotype *altor*

Ogawa strain W6. Peripheral wells contain the same antigens as in Fig. 7.5.

**Fig. 7.14.**

Intra-gel absorption with Ogawa antigen of W6. Central well containing the same antiserum as in Fig. 7.13. Peripheral wells contain the same antigens as in Fig. 7.5.