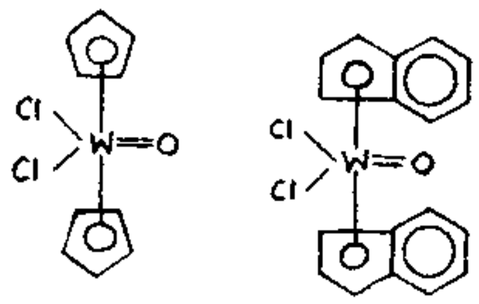
W, 45·88 ; Cl, 17·60%). These crystals do not melt upto 350° C. and cannot be sublimed under vacuum and are stable in air and soluble in petroleum ether, diethyl ether but sparingly soluble in benzene, toluene and carbon tetrachloride.

Preparation of Bisindenyl Tungsten Oxydichloride.—To a cold solution of 3·5 g. tungsten oxytetrachloride (0·1 mole) in 100 ml. of tetrahydrofuran was added 5 ml. of indene (0·2 mole) and the contents were refluxed for 12–14 hr. at 95–100° C. The colour of the solution first changed to light red and then pink and finally brown. The solvent was removed by evaporation under reduced pressure and the solid mass after repeated crystallization from ether gave brown crystals of bisindenyl tungsten oxydichloride [Found : C, 43·05 ; H, 2·71 ; W, 36·39 ; Cl, 14·14%. Calc. for $(C_9H_7)_2WOCl_2$: C, 43·11 ; H, 2·79 ; W, 36·50 ; Cl, 14·1%]. These crystals are stable in air for a short period only, melt at 230° C. and sublime at 140–145°/10 mm. These are insoluble in mineral acids and alkalies but soluble in ether, THF and dimethyl formamide. Tungsten was estimated as oxinate and chloride as silver chloride. Infra-red spectra, taken on Perkin Elmer infra cord model 137, is given in Table I.

Infra-red spectra given in Table I indicate that cyclopentadiene and indene react with tungsten oxytetrachloride forming "Sandwich" compounds analogous to other transition metals.[8] On the basis of their analytical data as well as the infra-red spectra, the following structures are suggested for the compounds (I) and (II).



(I) (II)

TABLE I

Infra-red spectra of biscyclopentadienyl tungsten oxydichloride and bisindenyl tungsten oxydichloride

Name of compound	C–H stretching	$(C_5H_5)_2$-metal stretching
$(C_5H_5)_2WOCl_2$(KBr)	3000 cm.$^{-1}$	960, 1110, 1380, 1470, 1640 cm.$^{-1}$
		(C_9H_7)-metal stretching
$(C_9H_7)_2WOCl_2$(Nujol)	3010 cm.$^{-1}$	1460, 1560, 1600, 1660 cm.$^{-1}$

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1. Cotton, F. A. and Wilkinson, G., *Z. Naturforsch*, 1954, **176**, 417.
2. Wilkinson, W., *J. Amer. Chem. Soc.*, 1954, **76**, 209.
3. Green, M. L., Meclavesty, J. A., Pratt, L. and Wilkinson, G., *J. Chem. Soc.*, 1961, **4**, 4854.
4. Pauson, P. L. and Wilkinson, G., *J. Amer. Chem. Soc.*, 1954, **76**, 2024.
5. Zimmer, H. and Sparmann, H. W., *Chem. Ber.*, 1954, **87**, 645.
6. Ans, J. D., Zimmer, H. and Brachwitz, M. V., *Ibid.*, 1955, **88**, 1507.
7. Lesbre, M., Mazerolles, P. and Manel, G., *Compt. Rend.*, 1962, **255**, 544.
8. Wilkinson, G., Cotton, F. A. and Birmingham, J. M., *J. Inorg. Nucl. Chem.*, 1956, **2**, 95.

ANTIGENIC VARIATION IN *VIBRIO CHOLERAE* RESULTING FROM CHROMOSOMAL TRANSFER BY CONJUGATION*

K. BHASKARAN, V. B. SINHA AND S. S. IYER
Central Drug Research Institute, Lucknow, India

THE identification of a fertility system in *Vibrio cholerae* permitted the isolation of genetic recombinants of marked parental strains.[1] Crosses between strains yielded a larger number of recombinants if parental strains were fixed on membrane filters prior to plating on selective media.[2] This technique obviously facilitated cell pairing and effective contact, likely to be less efficient in fluid media because of the active motility of the organism. By this technique it was shown that P^+ strains (possessing the fertility factor P) functioned as gene-donors while P^- strains (devoid of the fertility factor P) served as gene-recipients.

Earlier studies with mutants of *V. cholerae*, strain 162, suggested a chromosomal sequence of seven genetic factors as given below :

str ... pur ilva ... O ... arg ... leu ... his.

As O (symbolising the genetic determinants of O antigen synthesis) was located between ilva and arg, it was expected that when these are used as selective markers a proportion of the recombinants should inherit the contiguous O antigenic determinants as well. Evidence of this is presented here in which Smooth streptomycin-sensitive P^+ (donor) strains

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were mated with a Rough streptomycin-resistant P^- (recipient) strain on membrane filters for 60 min. and then plated on selective media for the isolation of recombinants. Results are recorded in Table I.

lised by O. Further studies with Rough mutants (derived from Inaba types) will confirm whether genetic determinants of group and type specificity of Smooth strains are separable by recombination.

TABLE 1

Crosses between Smooth (P^+) and Rough (P^-) strains of Vibrio cholerae

Strains *	A.	Inaba	162	P^+	str-s	$ilva^+$	O-In	arg^+	his^+
	B.	Ogawa	V209	P^+	str-s	$ilva^+$	O-Og	arg^+	his^-
	C.	Rough	V58-SR	P^-	str-r	$ilva^-$	O-R	arg^-	his^-

S. No.	Cross	Selective marker P^+	Selective marker P^-	No. of recombinants tested	No. Rough	No. Smooth
1	A×C	$ilva^+$	str-r	50	38	11 (Inaba) 1 (Ogawa)
		arg^+	str-r	75	69	6 (Inaba)
		his^+	str-r	37	37	Nil
2	B×C	$ilva^+$	str-r	60	50	10 (Ogawa)
		arg^+	str-r	65	62	3 (Ogawa)

Key = str-s = streptomycin-sensitive
str-r = streptomycin-resistant
O-In = O antigenic type Inaba
O-Og = O antigenic type Ogawa
O-R = Rough
arg = arginine
ilva = isoleucine + valine
his = histidine
$^+$ = independence
$^-$ = dependence

* Mutants of *V. cholerae*, Ogawa 162/p.

It will be seen that a significant number of Smooth strains could be identified among the recombinants resulting from the two crosses performed. The O antigenic type of these strains, with one exception, corresponded to that of the donor strain. The unselected markers were invariably those of the recipient, confirming that segmental transfer from P^+ to P^- cells was restricted to one or two adjacent loci. If selection was made for his^+ marker of the donor strain (cross 1), all the recombinants tested were Rough indicating absence of linkage between his and O.

As Rough strains of *V. cholerae* are often isolated from Cholera convalescents, and as such strains are known to be avirulent, the present study suggests their possible reversion to Smooth virulent types by conjugation with appropriate fertile strains. This is of interest because of the well-known stability of Rough strains of *V. cholerae* in contrast to type variation (Ogawa to Inaba) observed in Smooth strains.[3,4] The solitary isolation of a recombinant, with an antigenic type different from that of the donor strain, may be due to the persistence of genetic determinants of type-specificity (as distinct from group specificity) in the Rough strain, which is perhaps expressed only when associated with group specific genetic determinants, rendered possible in this case by a cross-over within the region symbolised by O. Further studies with Rough mutants (derived from Inaba types) will confirm whether genetic determinants of group and type specificity of Smooth strains are separable by recombination.

In this study, efforts were made only to *identify* and *type* Smooth strains among the recombinants isolated. This was rendered easy because Smooth strains are not agglutinated by Rough 'O' serum, whereas Rough and partially Rough (Smooth-Rough) strains are invariably agglutinated by this serum. It is possible that some of the recombinants may have been of the intermediate variety, resulting from cross-overs in the region O.

The scope of this study may be profitably enlarged by using non-cholera vibrio strains as donors in such crosses, which, if successful, would result in Rough (as well as Smooth) recipient strains of *V. cholerae* developing O antigens serologically different from O group 1 which characterise *V. cholerae* (and *V. El Tor*). This will be of considerable interest particularly with reference to cholera genicity of these hybrids. Such studies are in progress.

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1. Bhaskaran, K., *Bull. Wrld. Hlth. Org.*, 1964, **30**, 845.
2. —, Sinha, V. B. and Iyer, S. S., *Curr. Sci.*, 1967, **36**, 257.
3. Shrivastava, D. L. and White, P. B., *Indian J. med. Res.*, 1947, **35**, 117.
4. Bhaskaran, K. and Gorrill, R. H., *J. gen. Microbiol.*, 1957, **16**, 721.
5. Gardner, A. D. and Venkataraman, K. V., *J. Hyg, Camb.*, 1935, **35**, 262.