than the incidence of sicklers in two control groups. Wilson (1960) suggested that Garlick's findings of lower than normal parasite densities in sickling children at their first attendance could mean that they developed symptoms earlier and thus presented themselves at hospital sooner than those without haemoglobin S. Miller *et al.* (1956) advocated the mechanical protection theory of sickling consequent on malarial parasitization of erythrocytes containing haemoglobin S, and it is accepted that the pain crises of sickle-cell disease are caused by intravascular sickling in various parts of the body (Zuelzer, 1959). Therefore it is suggested that the lethal effects of intense parasitaemia by *P. falciparum* are prevented by sickling of parasitized cells, and that this protective effect is accompanied by early onset of symptoms. Thus adult policemen with the sickle-cell trait are unlikely to have contracted malaria more often than those without, but have developed symptoms with infections whose intensities were probably below the threshold required to produce symptoms in normal subjects. The incidence of malaria in policemen with the haemoglobin-C trait being lower than the normal suggests that the protective effect of haemoglobin C differs in its nature from that of haemoglobin S, and that it appears to remain demonstrable into adult life, possibly owing to an additive effect with the mechanism of acquired immunity.

Summary

Determination of the rate and intensity of *P. falciparum* infections among 840 Accra police children aged 1 to 6 years, correlated with the results of haemoglobin electrophoresis, confirmed the protective effect of haemoglobin S and demonstrated a similar effect for haemoglobin C in Ghana.

Among 278 adult policemen reporting sick with fever in Accra the incidence of positive blood films was highest in those with sickle-cell trait and lowest in those with the haemoglobin-C trait.

These findings support the theory that haemoglobin C is the result of a favourable mutation at the genetic locus responsible for haemoglobins A and S, and suggest that the protective effect exerted against malaria by haemoglobin S is due to sickling of parasitized erythrocytes and is accompanied by symptoms.

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THE VIBRIOS OF THE RECENT CHOLERA-LIKE OUTBREAK IN HONG KONG

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Outbreaks of cholera were reported in 1961 from widely scattered Far Eastern countries, including Hong Kong, Macao, parts of the Chinese mainland, the Philippines, and Indonesia, after which an extensive campaign of anticholera inoculations was organized in active co-operation with W.H.O. (*J. Indian med. Ass.*, 1961).

We present in this paper the result of our bacteriological investigation of the causal organisms of these outbreaks, which we have identified as El Tor vibrios. This finding has important epidemiological implications because the cholera-like disease caused by El Tor vibrios, as distinct from the classical *Vibrio cholerae*, the causative organism of cholera, has not so far been known to occur outside a limited geographical area in the Indonesian archipelago. And since the El Tor vibrio is taxonomically quite distinct from *V. cholerae*, the protective value of the cholera vaccine (with *V. cholerae* antigens) against El Tor disease is open to doubt.

Attempts were made from this laboratory to collect vibrios isolated in these outbreaks for strain identification by phage-typing and examination of their possible relationship with the *V. cholerae* strains prevalent in the cholera endemic centres of India. A first instalment of six strains labelled as *V. cholerae* was obtained from Dr. D. J. M. MacKenzie, Director of Medical and Health Services, Hong Kong, through Dr. L. Mattsson, of the South East Asia Science Cooperation Office of Unesco. The same six strains were also forwarded by Lieutenant-Colonel O. Felsenfeld, of the S.E.A.T.O. Medical Research Laboratory, Bangkok, for phage-typing. All these strains were agglutinable, and a preliminary test for phage susceptibility indicated that they belonged to the El Tor type, a finding confirmed by the more detailed investigations reported below.

The results were communicated to Dr. MacKenzie with a request for the supply of all the remaining

available strains isolated in the Hong Kong outbreak. Dr. MacKenzie expressed his interest in the finding, and a second lot of 124 strains of vibrios labelled as *V. cholerae* were received by courtesy of Dr. R. E. Alvares, Government Pathologist, Hong Kong. The results obtained in studies of the strains are reported here.

Materials and Methods

All the 130 strains were isolated between August 16 and September 25, 1961, in the Colony of Hong Kong, which comprises the island of Hong Kong, the Kowloon peninsula, and the New Territories extending up to the border with China. One of the 130 strains was obtained from the stool of a Macao patient.

Of the 124 cultures received in the second lot, 76 had been isolated from cases and the remainder came from contacts. The sources of isolation of the first six strains had not been specified.

Standard techniques were followed for biochemical and serological studies of these strains. The Voges-Proskauer reaction was carried out according to Barritt's (1936) modification.

A haemolytic test was done with sheep erythrocytes, using 24-hour culture in alkaline broth (pH 9). The results were read after two hours' incubation at 37° C. and then overnight in the refrigerator.

The phage-susceptibility pattern of the vibrios was determined by the technique described earlier, using the four groups of cholera typing phages maintained in this laboratory (Mukerjee *et al.*, 1957; Mukerjee, 1960).

Results

All the vibrio strains have the typical fermentation reaction of Heiberg (1934) group I and were agglutinable by a cholera O serum. Of the 130 strains, 129 belonged to the Ogawa and one to the Inaba serotype. All strains gave positive cholera-red and negative methyl-red reactions. The Voges-Proskauer test was positive with 126 and negative with three strains. All but three strains were strongly haemolytic, these three strains showing weak but distinct haemolytic properties. All the strains were therefore identified as El Tor vibrios.

The six strains received in the first instalment were also found to give positive soda-serum-agglutination and negative soda-sublimate-precipitation reaction (Tanamal, 1938). The remaining strains were not tested for these two reactions.

The bacteriophage susceptibility of the strains is given in Table I.

TABLE I.—*Susceptibility of the Vibrio Strains to the Four Groups of Cholera Phages*

No. of Strains Tested	No. of Strains Lysable by Phage Group							
	I		II		III		IV	
	R.T.D.	Undil.	R.T.D.	Undil.	R.T.D.	di.	R.T.D.	Undil.
130	—	104	—	—	129	130	—	—

R.T.D. = Routine test dilution for *V. cholerae*. Undil. = Undiluted.

Discussion

Of the 130 strains tested all but one belonged to the Ogawa serotype. The single Inaba strain may have originated from the Ogawa vibrios as a result of loss variation (Shrivastava and White, 1947) or mutation (Bhaskaran and Gorrill, 1957) during the course of the outbreak.

It may be seen from Table I that none of the strains was lysable by group IV phage. This is in conformity

with our previous observations that El Tor vibrios are uniformly insensitive to group IV phage, although the phage of this group is universally lytic for strains of *V. cholerae* and can therefore be used to differentiate cholera from El Tor vibrios on the basis of their susceptibility to it (Mukerjee, 1960, 1961).

It has been reported (Dr. J. C. Azurine, Manila, personal communication) that vibrio strains isolated during the earlier part of the Hong Kong outbreak were non-haemolytic but in later stages of the epidemic haemolytic strains were obtained. However, when tested in the present series all strains were found to be of El Tor type, both by haemolytic test and by their insensitivity to group IV cholera bacteriophage.

Several workers (de Moor, 1949; Tanamal, 1959; Mukerjee and Guha Roy, 1961a) have reported that the haemolytic power of El Tor vibrios may be masked in some newly isolated strains but becomes evident after a few subcultures. It has therefore been concluded (Wilson and Miles, 1955) that too much weight should not be attached to the results of the Greig haemolytic test. The comparative value of five other tests for species identification of the O group I vibrios has been studied (Mukerjee and Guha Roy, 1961b) and the bacteriophage susceptibility test found to give the most consistent results in differentiating the two species of agglutinable vibrios. Precise identification of the strains from the earlier part of the epidemic was missed, probably because the diagnosis was based mainly on the results of haemolytic tests of the freshly isolated vibrios.

It has been stressed by Mukerjee (1961) that prompt and unequivocal identification of the O group I vibrios is of critical importance. This is particularly important in the case of strains isolated in the early part of an epidemic, as the nature and extent of the control measures to be adopted would be determined by the results of identification.

The findings reported here are of considerable epidemiological interest, for outbreaks of paracholera El Tor in epidemic form have till recently been almost exclusively confined to a limited geographical area in the Indonesian archipelago. Phage-typing of strains from 1959 and 1960 cholera epidemics in Thailand showed the occurrence of El Tor strains to the extent of 10% and led to the suggestion (Mukerjee and Guha Roy, 1961a) that the epidemic might be a mixed one arising from both *V. cholerae* and El Tor infections. Felsenfeld and Mukerjee (1961) later found a localized epidemic in Thailand to be of exclusively El Tor origin. It appears now that El Tor infections have spread to a much wider area in the Far East. Besides the Hong Kong strains as reported here, strains of vibrios from the recent epidemics in the Philippines as well as from Indonesia have been confirmed as El Tor in this laboratory (Mukerjee, unpublished work).

Future outbreaks of cholera-like epidemics due to El Tor therefore appear to be likely in areas so far free from them. It is important that particular care is taken in the precise bacteriological characterization of such epidemics and exclusive reliance is not placed on the results of haemolytic tests.

The cholera-typing bacteriophages used in this series are available from us as well as from the American Type Culture Collection, Washington.

In spite of the similarity of O antigens of *V. cholerae* and El Tor vibrios, differences exist not only in the chemical composition of their cells, especially the

proteins, but also in the toxins produced by them (Van Loghem and de Moor, 1938; Felsenfeld, 1960). Further, Tanamal *et al.* (1958) have shown that animals immunized with routine cholera vaccines are not satisfactorily protected against El Tor vibrios. Mass immunization with routine cholera vaccine should therefore be regarded of doubtful value as a measure against outbreaks of paracholera in epidemic form.

Summary

All the 130 strains of vibrios isolated in the 1961 cholera-like epidemic in Hong Kong have been found to belong to the El Tor type by haemolytic and phage-sensitivity tests. The importance of confirmatory tests like phage-susceptibility characteristics for the precise identification of the O group I vibrios has been discussed. The phage-susceptibility test is specially recommended in the case of strains isolated early in an outbreak.

The pattern of change in the geographical distribution of choleraic disease due to El Tor vibrios has been discussed and the consequent importance of precise bacteriological characterization of fresh outbreaks emphasized. In view of the differences in chemical composition and toxigenicity of *V. cholerae* and El Tor vibrios mass immunization with routine cholera inoculations should be regarded as of doubtful value.

Our thanks are due to Dr. J. C. Ray, Director of the Institute, for his help and interest in this work. We are especially indebted to Dr. L. Mattsson, Dr. D. J. M. MacKenzie, Dr. R. E. Alvares, and Lieutenant-Colonel O. Felsenfeld for their ready and willing co-operation in making the strains available. The technical assistance of Shri K. N. Maitra is acknowledged.

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TWENTY-ONE UNCOMMON CASES OF POISONING

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Deaths from poisoning number about 5,000 a year; carbon monoxide, the barbiturates, and aspirin account for most of these cases. The details of other poisonings are rarely of sufficient interest to warrant publication, because they involve such old favourites as arsenic trioxide, strychnine, morphine, nicotine, yellow phosphorus, or the halogenated hydrocarbons. Even when an unusual case involving death from a suicidal or accidental overdose of a new drug is encountered it is often not of enough scientific interest to publish.

Details of uncommon cases are nevertheless of interest to the toxicologist and for the purposes of statistical analysis; the following cases (Tables I and II) have been selected, therefore, from the ones submitted to this laboratory over the past 10 years for the analyses of which I have been responsible. The laboratory serves the whole of Yorkshire, an area of about 3,500,000 acres (1,416,400 hectares). Poisoning, or allergic response (Cases 15 and 16), was in all cases implicated either from the analyses or from circumstantial evidence.

TABLE I.—General Analysis of Cases

Case No.	Poison	Source	Quantity	Sex and Age	Weight		Time between Ingestion and Death
					st. lb.	kg.	
1	Dichloral phenazone	Wellborm	60-100 tablets	M 65	12	76.2	<3 hours
2	Orphenadrine	Elimit tablets	Not known	F 34	8	50.8	5½ "
3	Imipramine	Tofranil	60 tablets, size not known	F 22	9	57.2	<5 "
4	Thioridazine	Melleril	20, 100-mg. tablets	M 61	9 7	60.3	2½ "
5	Ethyl-2: 4-dichloro-phenoxy acetate	Dicotox Extra	3-4 oz. (60-90 g.)	F 49	8	50.8	About 2½ days
6	Insulin	Protamine zinc insulin	Not known. ? 1,200 units	M 53			At least 16 hours
7	"	Soluble insulin 40 units/ml.	Not known	M 22	10	63.5	About 13 hours
8	"	Protamine zinc insulin	? 800 units	M 48			Not known
9	"	Insulin zinc suspension 80 units/ml.	Injections of about 1 ml. on Mon., Tues., Wed., Thurs.	F 19			Admitted to hospital in coma on Fri. 4.30 a.m.
10	Hydrogen fluoride	Movol iron-mould remover	1.5 g. hydrogen fluoride	F 27			About 6½ hours
11	Phalloidin	A. phalloides	Not known	F 72	8 7	54.0	34 hours
12	"	"	"	M 45	11	69.9	2½ days
13	Adrenaline	Accidental injection of nebula adrenaline and atropine co. in hospital	2 ml. = 9 mg. adrenaline	M 37	12	76.2	30 minutes
14	Aminophylline	Corosedine tablets	Not known	F 1½	1 6	9.1	About 17 hours
15	Pollen vaccine	Combined pollen vaccine—strong	1.2 ml.	M 50	—	—	A few minutes
16	" "	Pollen extract of timothy grass and cocksfoot	200 units	F 52	—	—	Immediate collapse
17	Paraldehyde	"	2 dr. (8 g.) in water	M 32			43 hours
18	Glutethimide	Doriden	? 60 tablets	F 60	12	76.2	<12 hours
19	"	"	? 28 "	M 39	10	63.5	Missing several days
20	"	"	? 30 "	F 56	10	63.5	Found dead
21	"	"	? 36 "	F 50	—	—	" "