never exposed to dengue.[z] The SLE epidemics in Florida and Texas occurred 28–40 years after the last dengue fever outbreaks and primarily affected the most recent immigrants into the areas. Our findings suggest significant cross-protection between dengue fever virus (particularly dengue-2) and SLE. We would encourage similar studies with dengue and MVE, JE, or WN viruses. If our hypothesis on cross-protection is correct, it may be necessary to administer attenuated dengue virus vaccines to large groups in the tropics and subtropics to restore the ecological balance after the eradication of *Aedes aegypti* mosquitos.

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[z] Hammon, W. M., Tigertt, W. D., Sather, G. E., Berge, T. O. & Meiklejohn, G. (1958) *Amer. J. trop. Med. Hyg.*, 7, 441–467.

Non-Agglutinable Vibrios Isolated in the 1966 Epidemic of Cholera in Iraq

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During the 1966 epidemic of cholera in Iraq, many organisms biochemically identical to *Vibrio cholerae* were isolated but they did not agglutinate with cholera O group 1 antiserum. Some of these organisms were isolated from patients with clinically diagnosed cholera or from individuals suspected of being carriers. Others were isolated from water or from sewage during routine examinations for the presence of cholera organisms. The question of the pathogenicity and origin of these non-agglutinable (NAG) organisms has been the subject of many reports in the literature. Many consider that they are non-pathogenic and are to be found free-living in water or sewage. Others are of the opinion that these organisms were originally agglutinable forms which have lost their agglutinability owing to loss of antigen. Bhattacharji & Bose [a] claimed that they could achieve transformation of *Vibrio cholerae* into NAG vibrios and *vice versa*.

In this communication we report a study of those organisms isolated during the 1966 epidemic of Iraq which were described as NAG vibrios, and an attempt to repeat Bhattacharji & Bose's experiments.

[a] Bhattacharji, L. M. & Bose, B. (1964) *J. med. Res.*, 1771.

Materials and methods

Isolation of the organisms. Stool or sewage specimens were inoculated on alkaline peptone water for 6 hours. Water specimens were filtered through a Millipore filter and the filter was put in alkaline peptone water for the same length of time. Subcultures were made on TCBS plates and subcultures from suspicious colonies were inoculated on Kligler iron-agar (both stab and streak inoculations). Tubes which showed an acid butt and an alkaline slant, with no gas, were regarded as suspicious and subcultures were made from these, on peptone water to test for the production of indole, on semi-solid mannitol to test for acid production and motility and on agar slants for agglutination tests. Cultures which were indole-positive, motile and mannitol-positive but which gave a negative agglutination reaction with polyvalent cholera antisera, were provisionally described as NAG vibrios; further tests were carried out before the organism was definitely classified.

Maintenance of cultures. Strains of NAG vibrios, as well as El Tor vibrios, were maintained by stab cultures on semi-solid nutrient agar (0.5% agar). The cultures were kept at room temperature (20°C–28°C). Cultures were transferred on to semi-solid

TABLE 1
CHARACTERIZATION OF ISOLATES

Species	No. of isolates	Gelatin liquefaction	Nitrate reduction	Indole production	Ulrich milk peptonization	Glucose fermentation
V. agarliquefaciens	16	–	+	+	AR	+
V. cyclosites	4	–	–	+	–	+
V. percolans	5	–	–	+	–	–
V. tyrogenus or *X. xenopus*	1	+	+	–	–	–
Flavobacterium proteus	3	–	+	+	–	–
Flavobacterium devorans	1	+	–	+	–	+
Flavobacterium sewenens	1	+	–	+	–	+
NAG vibrios	22	+	+	+	–	+

nutrient agar every 2 weeks, and every 3 months subcultures were made on to nutrient broth and TCBS plates (Eiken) for the study of colonial morphology and other reactions of the organism. Subcultures on to semi-solid nutrient agar were repeated from 3 colonies from the surface of solid media.

Further biochemical tests. Strains provisionally described as NAG vibrios were tested, according to *Bergey's Manual*,[b] for gelatin liquefaction, nitrate reduction, indole production, Ulrich milk peptonization (Difco) and glucose fermentation.

As a result of these tests many of the cultures could be classified as species belonging to the genus *Vibrio* and some as another genus. Some of the isolates were true NAG vibrios. These were oxidase-positive and they fermented glucose; these true NAG vibrios were classified into Heiberg groups according to the fermentation of sucrose, arabinose and mannose.

The NAG strains were tested for utilization of amino acids by the method of Falkow.[c] The medium consisted of 0.5% peptone, 0.3% yeast extract, 0.1% glucose and 0.5% of the amino acid. Each of the following amino acids was used: arginine, tyrosine, alanine, tryptophane. Phenol-red was added as indicator and the pH was adjusted to 7.2. The media were sterilized by filtration and distributed into screw-capped bottles and inoculated. As acid is produced from glucose fermentation, the colour of of the indicator becomes yellow during the first 12 hours after inoculation. Later as the amino acid is decomposed the pH becomes alkaline again and the colour returns to red. The results were read after 48 hours.

The NAG strains were also subjected to tests which differentiate El Tor vibrios from classical *Vibrio cholerae*, i.e., agglutination of chicken erythrocytes, haemolysis of sheep erythrocytes, sensitivity to 50 units of polymyxin B and the Voges-Proskauer reaction.

Phage-susceptibility of the strains. To test for phage-susceptibility, a nutrient-agar plate was uniformly inoculated from a broth culture of the strain. Single drops of each type of Mukerjee's 4 phages (1:100 dilution of the original phage suspension) and of a polyvalent phage suspension were put on separate sectors of the plate and the plates were incubated at 37°C for 24 hours. Afterwards, they were examined for the presence of zones of lysis or inhibition of growth.

Experiments to test for the transformation of El Tor vibrios. Water samples of various degrees of salinity were collected from marshes and rivers of Iraq and were sterilized by filtration through a Millipore filter and then inoculated with a suspension of El Tor vibrios (1 000 000/ml). From each source 10 water samples were taken and they were inoculated with 10 different strains. They were kept at room temperature (20°C–28°C) and aliquots were taken daily and tested for the presence of vibrios. This procedure was continued until no vibrios could be isolated from the samples.

Results

Table 1 shows that the strains which were provisionally described as NAG vibrios are actually a

[b] Breed, R. S., Murray, E. G. D. & Smith, N.R., ed., (1957) *Bergey's manual of determinative bacteriology*, 7th ed., Baltimore, Williams & Wilkins Company.

[c] Falkow, S. (1958) *Amer. J. clin. Path.*, **29**, 598.

heterogeneous group of organisms, many of which are non-cholera vibrios: among the 49 cultures isolated, there were 22 NAG vibrios.

The Heiberg classification of the NAG strains isolated was rather difficult because some of the organisms changed from one group to another after being left at room temperature for 3 months (Table 2).

One of the organisms which changed from group I to group II reverted to group I again after 4 weeks. Similarly, one of the organisms which changed from group IV to group I, later changed to group II.

TABLE 2
CLASSIFICATION OF NAG STRAINS

Heiberg group at original testing	Heiberg group after 3 months				Total
	I	II	IV	V	
I	5	5	—	—	10
II	2	1	—	—	3
IV	2	3	—	—	5
V	3	1	—	—	4

The NAG organisms behaved differently in the tests which differentiate between the classical and El Tor vibrios, i.e., the Voges-Proskauer reaction, sensitivity to polymyxin B, the agglutination of chicken erythrocytes, the haemolysis of sheep erythrocytes and the susceptibility to Mukerjee's 4 phage types. Among the 22 NAG strains all were resistant to polymyxin B, 10 were Voges-Proskauer-positive, 10 agglutinated chicken erythrocytes and 9 caused haemolysis of sheep erythrocytes: 6 of the isolates were susceptible to Mukerjee's phage type III, 3 were susceptible to type IV and 1 isolate that belonged to Heiberg group I was susceptible to phage type II. This is extremely interesting since NAG vibrios are normally not susceptible to phage type II.[d] The rest of the isolates were resistant to all 4 phage types. There was no evidence of any correlation between the results of the different tests.

All the NAG strains decomposed tryptophane, glycine, isoline, valine, D-cystine, methionine and thionine. Only 1 strain did not decompose alanine and 3 did not decompose tyrosine. Table 3 shows that 7 strains of this collection did decompose arginine, and this is contrary to the usual finding: of these 7 strains, 3 were ornithine-negative and 4 were ornithine-positive.

TABLE 3
UTILIZATION OF AMINO ACIDS BY NAG VIBRIOS

Decomposition of amino acids (+ or −)			No. of isolates	Heiberg group
Lysine	Arginine	Ornithine		
+	−	+	9	I
			6	II
+	+	+	1	I
			3	II
+	+	−	2	I
			1	II

El Tor vibrios of the Ogawa serotype survived for different periods of time, up to 39 days, in different water samples sterilized by filtration through a Millipore filter. However, during this time none of the 10 strains used for inoculation changed into a non-agglutinable form.

Discussion

Bhattacharji & Bose [a] artificially inoculated water with classical *Vibrio cholerae*, of the Inaba serotype, and claimed that within 24 hours the organisms had changed into NAG organisms of Heiberg group 1. In the course of another 48 hours the NAG organisms of group 1 changed again to NAG organisms of group II. In our experiments we were not able to isolate NAG organisms from the water samples that were artificially inoculated with the agglutinable organisms. One difference between our experiments and those of Bhattacharji & Bose [a] was that they used classical *Vibrio cholerae*, of Inaba serotype, while we used Ogawa El Tor vibrios, which were isolated in the 1966 epidemic in Iraq.[e] Another difference between the experiments was that they added sterile soil to the water samples; we did not do this as it is difficult to believe that sterile soil can have mutagenic properties.

It is true that the NAG vibrios were rather unstable and changed their Heiberg groups, but none of our NAG vibrios changed into an agglutinable form during maintenance in the laboratory for 18 months.

[d] Mukerjee, S. (1961) *J. Hyg. (Lond.)*, **59**, 109.

[e] El-Shawi, N. & Thewaini, A. J. (1968) *Bull. Wld. Hlth Org.*, **38**, 319.

Three of our strains of El Tor vibrios lost their agglutinability after being maintained in the laboratory for longer than a year, and one changed from the serological type Ogawa to Hikojima. This strain was sent to Dr S. Mukerjee for testing and phage typing and was found to be a Hikojima El Tor of phage type VI. The Ogawa El Tor vibrios isolated in the epidemic in Iraq in 1966 were also of phage type VI.[e] Neither the strains which lost their agglutinability, nor the converted strains, were changed in any of their other characteristics.

The results of typing our NAG vibrios with Mukerjee's 4-phage types showed that only 3 of the 22 were susceptible to phage type IV. The rest were like the Ogawa El Tor in their resistance to this phage: 6 were susceptible to phage type III and 1 was susceptible to phage type II. NAG vibrios are usually not susceptible to phage type II.[f]

Some of these NAG vibrios were very similar to the agglutinable *Vibrio cholerae*. More attention should be paid to these organisms since some of them were isolated from patients with clinically diagnosed cholera. Bhattacharji & Bose [a] administered a suspension of NAG vibrios to suckling rabbits and observed conversion into agglutinable organisms *in vivo*.

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[f] Felsenfeld, O. (1966) *Bull. Wld Hlth Org.*, **34**, 161.

A Comparison of the Nutritional Indices in Healthy African, Asian and European Children

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The purpose of this study was to estimate normal values of different biochemical tests that have been recommended as indicators of protein metabolism and that were used in our surveys on Africans living in rural conditions. For this purpose, it was necessary to choose a sample of children in which we could expect normal growth and a good nutrition status.

Methods

Altogether, 45 African, 14 Asian and 28 European children in an elite kindergarten in Nairobi were considered for the study. The children ranged from 4 to 5 years of age. The study was started 4 hours after the children had eaten breakfast. It is therefore supposed that the blood and urine samples were obtained from children in the " fasting state ".

For the urine collection, each male child was provided with a specimen tube 8.0 cm by 2.5 cm and the female children were given a small plastic basin. After the collection, each urine sample was acidified with 0.1 N H_2SO_4 to a pH 3.0. The urine samples were then transported to the laboratory and frozen.

Each child was measured for weight, height, triceps skinfold and circumference of the mid-upper arm. All the measurements were made by the same observer. Weight was measured by means of a platform scale and height by a vertical measuring rod. The arm circumference was measured with a narrow flexible steel tape. Harpenden calipers were used for the triceps skinfold.

Harvard standards [a] were used for the evaluation of both weight and height. The standard given by Tanner & Whitehouse [b] was used for the triceps skinfold and the values of Wolanski (personal communication, cited in Jelliffe) [c] for the arm circumference.

[a] Stuart, H. C. & Stevenson, S. S. (1959) In: Nelson, W. E., ed., *Textbook of pediatrics*, 2nd ed., Philadelphia, Saunders.

[b] Tanner, J. M. & Whitehouse, R. H. (1962) *Brit. med. J.*, **1**, 446.

[c] Jelliffe, D. B. (1966) *The assessment of the nutritional status of the community*, Geneva (*World Health Organization: Monograph Series*, No. 53).