Similarities of two hepatitis A virus strains *

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In outbreaks of type A hepatitis in Los Angeles, USA, and Rosario, Argentina, virus particles were isolated from faeces. The geographically diverse strains were identical in appearance and serological reactivity. They differed only in buoyant density, but other workers have also obtained inconsistent results in estimating this. We conclude that the virus strains in the two epidemics were identical.

Epidemiological observations have suggested that hepatitis A virus (HAV) occurs throughout the world. Documentation of this inference and exploration of any strain differences were not possible, however, without laboratory methods. Only the fact that immunoglobulin preparations from one area were effective against HAV infections in other areas (*1*) provided evidence for immunological similarity.

These questions concerning distribution and variations in HAV can now be approached. Feinstone et al. (*2*), by immune electron microscopy (IEM), showed the attachment of specific immunoglobulins (anti-HA) in convalescent serum to virus-like particles in the faeces of infected subjects. Conversely, using faecally derived HAV, IEM and immune adherence haemagglutination (IAHA) can be used to quantify anti-HA. This report summarizes the physical properties and immunological specificities of HAV strains responsible for outbreaks of viral hepatitis in Los Angeles, USA and Rosario, Argentina.

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MATERIALS AND METHODS

An IEM technique (*2*) was used to identify HAV particles in stool specimens. A 2% suspension of faeces was incubated with an aliquot of a standard serum (LA 1), centrifuged, stained with 2% phosphotungstic acid, and examined under the electron microscope. After coding, all specimens were examined in groups, with appropriate positive and negative controls in each. Specificity of identification was kindly confirmed for selected specimens by Dr Jules L. Dienstag, National Institutes of Health, Bethesda, MD, USA.

We determined buoyant density (δ) in a preformed continuous gradient of cesium chloride (δ= 1.10–1.58 g/ml). Centrifugation was at 189 000 *g* and 4°C for 24 h. The density of each fraction was determined by weighing a 100-μl sample. HAV particles were negatively stained with 2% phosphotungstic acid and their distribution was determined by counting antibody-coated particles in 10 squares of good quality on a 400-mesh Formvar-carbon grid.

The antigenic specificities of HAV strains were evaluated by IAHA. The procedure for anti-HA was based on that described by Mayumi et al. (*3*) for the surface antigen of hepatitis B virus. The reagents included: IAHA units of HAV antigen purified by isopycnic banding with cesium chloride as described, and concentrated seven-fold by means of an Amicon 52 cell with a PM 30 filter; bovine serum albumin–barbital buffer; fresh-frozen guinea-pig complement; and type O human red blood cells, which were found to be suitably reactive in the system. The serum to be evaluated was added to an equal volume of HAV in a U-bottom Microtiter plate, and incubated for 1 hour at 37°C. Freshly diluted (1 : 200) guinea-pig

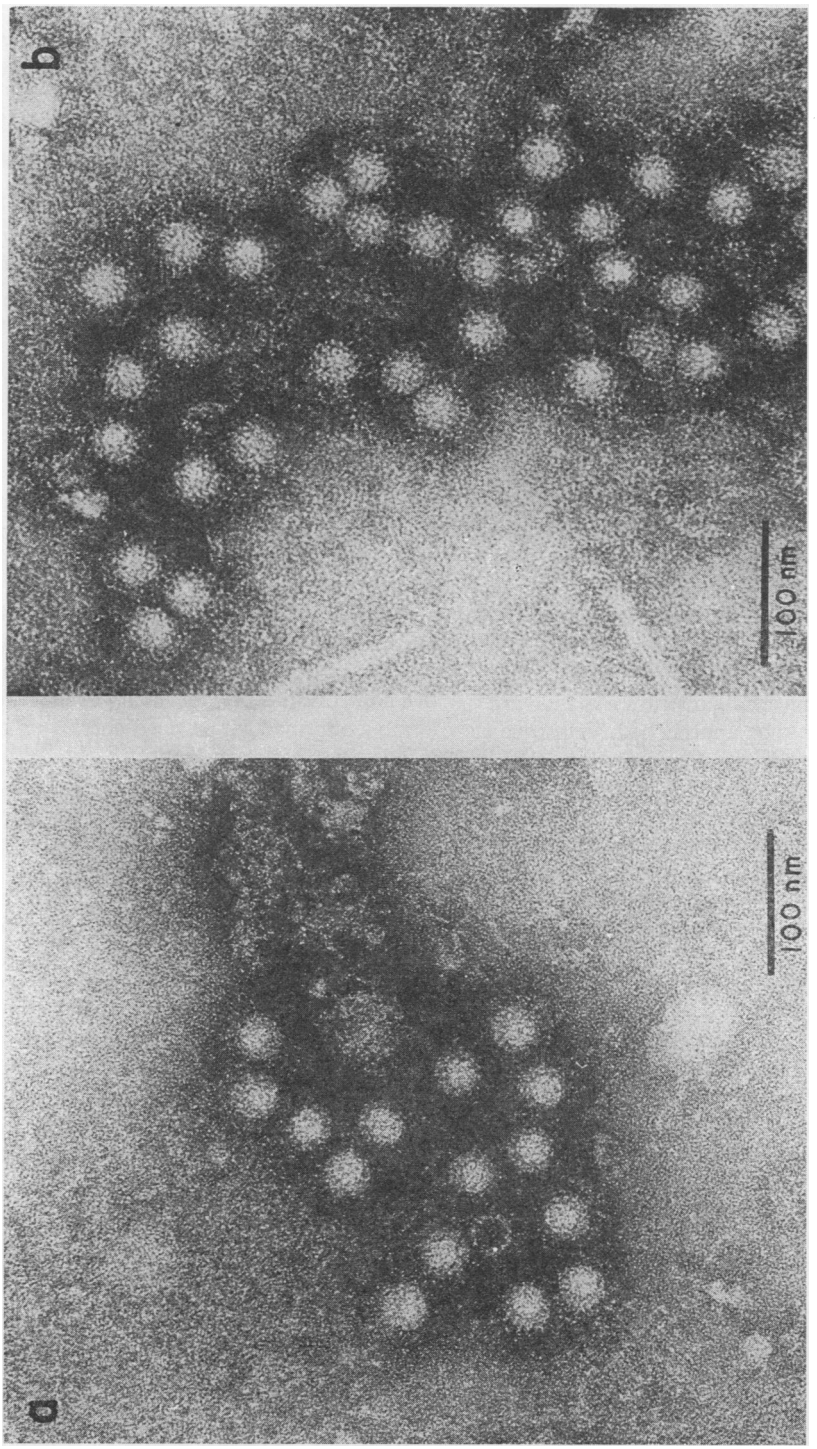

Fig. 1a and 1b. Immune electron micrographs of hepatitis A virus particles recovered from faeces in outbreaks of epidemiologically characteristic type A disease in Los Angeles, USA (1a) and in Rosario, Argentina (1b).

complement was then added, and the antigen–antibody–complement mixture incubated at 37°C for 40 min. Finally, dithiothreitol and red cells (1%) were added. Haemagglutination was complete after 90–120 min at room temperature and was scored on a scale of 0 to 4+ (end-point for titration = 2+).

The epidemic in Los Angeles occurred in a residence for handicapped children. Specimen collection yielded 36 faecal samples, of which 17 were weakly to strongly positive. We chose specimen HH-A for further work because of its high particle density (average 155 per grid square). The outbreak in Rosario involved four schoolchildren and three of their family contacts. We recovered HAV only from a contact whose serum alanine aminotransferase activity was slightly elevated (45 IU/litre) at that time. Fortunately, the specimen (R-A) was satisfactory (average particle count = 110 per grid square) for our studies.

In the antigenic comparison of two HAV strains, we used the standard serum (LA-1), three convalescent sera (HH-1, 2, and 3) from residents of the home for the handicapped, and serum (R-1) from a child attending the Rosario school.

RESULTS

Fig. 1a and 1b show particles obtained from the patients in Los Angeles and Rosario, respectively. The viruses in each outbreak had an average diameter of 27 nm; they were identical in shape, with a dense inner core and a capsid easily discernible when the particle was empty.

Fig. 2a and 2b show the results of determining buoyant density. The peak for the Los Angeles virus was 1.39 g/cm³, and that for the virus from Argentina was 1.36 g/cm³. We did not observe a difference in the proportion of empty particles in the two preparations to account for this difference.

A comparison of the serological reactivities of the Los Angeles and Rosario isolates is given in Table 1. The two sera gave identical titres by IAHA with standardized quantities of HAV from each of the two outbreaks.

DISCUSSION

The limitation of virus excretion in the faeces to the period of prodromal illness (*4*) makes difficult the recovery of HAV strains in different countries for comparisons of properties. We were fortunate to obtain appropriately timed faecal and serum

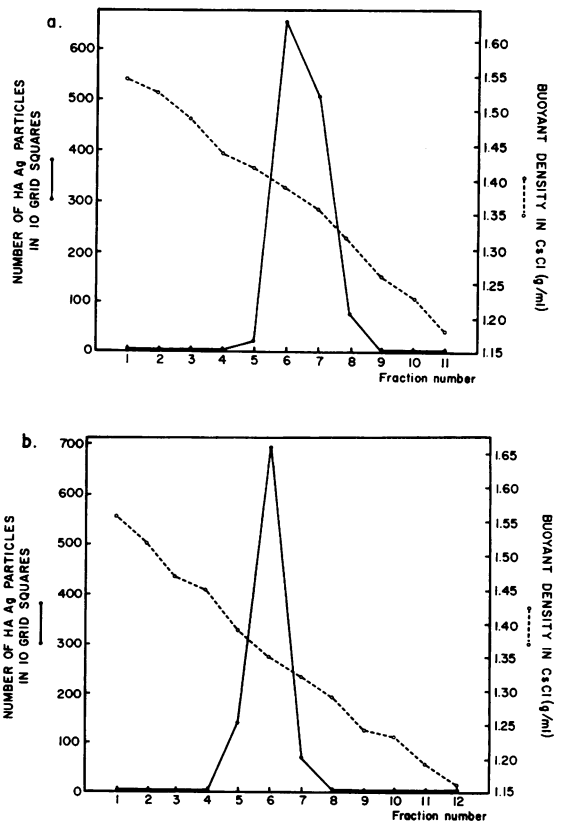

Fig. 2a and 2b. Buoyant density estimations of hepatitis A virus particles recovered in outbreaks of epidemiologically characteristic type A disease in Los Angeles, USA (2a) and in Rosario, Argentina (2b).

Table 1. Serological comparison by IAHA of hepatitis A viruses isolated in outbreaks in Los Angeles, USA and in Rosario, Argentina

Source of serum	Specimen [a]	Virus strain (reciprocal dilution)	
		Los Angeles (HH-A)	Rosario (R-A)
Los Angeles	LA 1	6400	6400
	HH 1	12 800	12 800
	HH 2	3200	3200
	HH 3	3200	3200
Rosario	R 1	12 800	12 800

[a] LA = Los Angeles; HH = Hope House (Los Angeles); R = Rosario.

samples that allowed us to study the viruses responsible for outbreaks in two widely separated areas. The Los Angeles and Rosario strains are physically very similar and differ only in buoyant density, which other workers have also found very difficult to reproduce. The differences between HH-A and R-A encountered by us are no greater than those observed by others for strains recovered within the United States (*2*, *5*, *6*).

Although the sera used for antigenic comparison were limited in number, we believe they were adequate to establish immunological identity. Similar studies should be continued as new strains become available from different parts of the world.

RÉSUMÉ

SIMILITUDE ENTRE DEUX SOUCHES DE VIRUS DE L'HÉPATITE A

Pour déterminer la similitude entre des agents responsables d'épidémies, caractéristiques au point de vue clinique et épidémiologique de l'hépatite de type A, épidémies survenues à Los Angeles (Etats-Unis d'Amérique) et Rosario (Argentine), on a examiné des échantillons par l'immuno-microscopie électronique.

Lorsque des suspensions fécales à 2% étaient incubées avec du sérum de convalescent, une adsorption sur des particules de 27 nm a été observée pour 17 des 36 échantillons en provenance de Los Angeles, mais pour seulement 1 sur 7 de ceux qui provenaient de Rosario. Les souches de Los Angeles et celles de Rosario étaient identiques morphologiquement, mais présentaient une densité de flottation de 1,39 et 1,36 respectivement. Il faut souligner que divers chercheurs ont obtenu des résultats inconstants lors de la mesure des densités de flottation.

A partir des deux souches étudiées, on a obtenu des échantillons dans des zones d'équidensité et on leur a appliqué l'épreuve d'immunoadhérence-hémagglutination; on a ainsi obtenu des titres identiques avec 4 sérums de convalescents de Los Angeles et 1 de Rosario. Cette comparaison antigénique permet de conclure que les souches de virus de l'hépatite A responsables des épidémies survenues dans ces régions tellement éloignées l'une de l'autre, sont nettement apparentées, sinon identiques.

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