by adjacent classes of equal width, each containing almost 0.15 of the total, as inspection of a normal probability table will confirm. The observed distribution is radically different for each of the five loci; indeed, adjacent positions empty in a number of cases. We must conclude that the neutral hypothesis is incorrect: instead, the number of allelic states equivalent or nearly equivalent to the best one is very few.

These observations, drawn from geographically and phyletically diverse hosts, include numerous sets of 10 to 20 clones, each set being taken from a single fecal sample. The variation within most sets is great enough to suggest that recombination takes place regularly within hosts. Returning to the major consideration, one can compile the data according to a very conservative procedure, in which each mobility class is counted only once per set. The prominence of the most common mobility class remains striking. This being the case, the selectionist hypothesis is favored.

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Hepatitis A: Detection by Immune Electron Microscopy of a Viruslike Antigen Associated with Acute Illness

Abstract. *Spherical 27-nanometer particles were visualized in stools obtained from hepatitis A patients in the acute phase of the disease. The particle was serologically specific for this disease, and every hepatitis A patient tested demonstrated a serologic response to this antigen. The findings suggest that it is the etiologic agent of hepatitis A.*

The detection of hepatitis B antigen (Australia antigen) in the serums of certain hepatitis patients and the discovery of its association specifically with hepatitis B (serum hepatitis) provided a diagnostic tool making possible the serologic identification of this important disease (*1*). Attempts to find a virus or antigen in the serums of patients with hepatitis A (infectious hepatitis) have been unsuccessful, possibly because the viremic stage of hepatitis A appears to be of short duration and of low magnitude. In contrast, there is epidemiologic and experimental evidence that feces of patients with hepatitis A are infectious from approximately 2 weeks before until 2 weeks after onset of clinical symptoms (*2*). We, therefore, examined stool specimens from patients with hepatitis A for viruslike antigens, using the technique of immune electron microscopy (IEM) (*3*). This method was used successfully in our laboratory for the detection in a stool filtrate of a viruslike agent (Norwalk) and also for the demonstration of its association with acute infectious nonbacterial "Norwalk" gastroenteritis (*4*). We report here the detection of a morphologically similar viruslike particle that is serologically associated with hepatitis A infection.

Stool specimens (as 20 percent saline extracts) were supplied, under code, by Dr. D. Gibson. They consisted of specimens obtained before inoculation or during acute illness from each of four

Table 1. Antibody to antigens associated with hepatitis A, hepatitis B, and Norwalk gastroenteritis in paired serums from patients with these diseases. Antibody to the hepatitis A antigen was detected by immune electron microscopy (IEM) and was rated on a 0 to 4 scale, depending on the amount of antibody coating the particles. Antibody to the gastroenteritis antigen was determined in the same way. Antibody to hepatitis B antigen was measured by radioimmunoassay (RIA). The first serum of each serum pair was obtained prior to exposure, except in the case of four serums which were obtained during the acute phase of illness (acute). The second serum of each pair was obtained approximately 1 to 6 months after the onset of illness. Serums were tested by IEM at an initial dilution of approximately 1 : 10. Serums were tested by RIA at a dilution of 1 : 4 or 1 : 10. Abbreviations: NT, not tested; —, negative; +, positive.

Patient No.	Antibody to indicated antigen in first and second serum samples					
	Hepatitis A antigen		Hepatitis B antigen		Norwalk gastroenteritis antigen	
	First	Second	First	Second	First	Second
	Experimental hepatitis A (MS-1), New York					
1	0	1–2	—	—	NT	NT
2	0	1–2	+	+*	NT	NT
	Experimental hepatitis A (MS-1), Illinois					
3	0	3–4	—	—	1	1
4	0	3–4	--	—	1	1
5	0	1–2	...	—	NT	NT
6	0	3–4	..	—	NT	NT
	Naturally acquired hepatitis A, Massachusetts					
7	0–1 (acute)	3–4	—	—	NT	NT
8	0 (acute)	3	—	—	NT	NT
9	0 (acute)	3	—	—	NT	NT
	Naturally acquired hepatitis A, American Samoa					
10	0	2	—	—	NT	NT
11	0	3	—	—	NT	NT
12	1–2 (acute)	3–4	—	—	NT	NT
	Naturally acquired hepatitis B					
13	0	0	—	+	NT	NT
14	0	0	—	+	NT	NT
	Experimental nonbacterial gastroenteritis					
15	0	0	—	—	1	4
16	2–3	2–3	—	—	1	4

* No antibody increase.

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adult volunteers who were inoculated either orally or parenterally with the MS-1 or MS-1–derived strain of hepatitis A virus (*5*). Each stool was further diluted to a 2 percent concentration and passed through a 450-nm Millipore membrane filter. The stools were examined by IEM, with minor modifications of the technique of Kapikian *et al.* (*4*). We took this previously described approach in the hope that virus particles, if present, would be aggregated by specific hepatitis A antibody, so that they could be observed even if present only in low titer.

We found small viruslike particles measuring approximately 27 nm in diameter in two of the four stool specimens from subjects in the acute phase of the disease, after the stool suspensions had been incubated with the serum of a convalescent patient (*5*). No particles were observed in the stool specimens of the four subjects before they had been inoculated. These particles stood out clearly from the surrounding matter and could be readily differentiated from other numerous, spherical objects normally found in stool samples because the former occurred either as single particles heavily coated with antibody or in groups aggregated by antibody (Fig. 1, A and B).

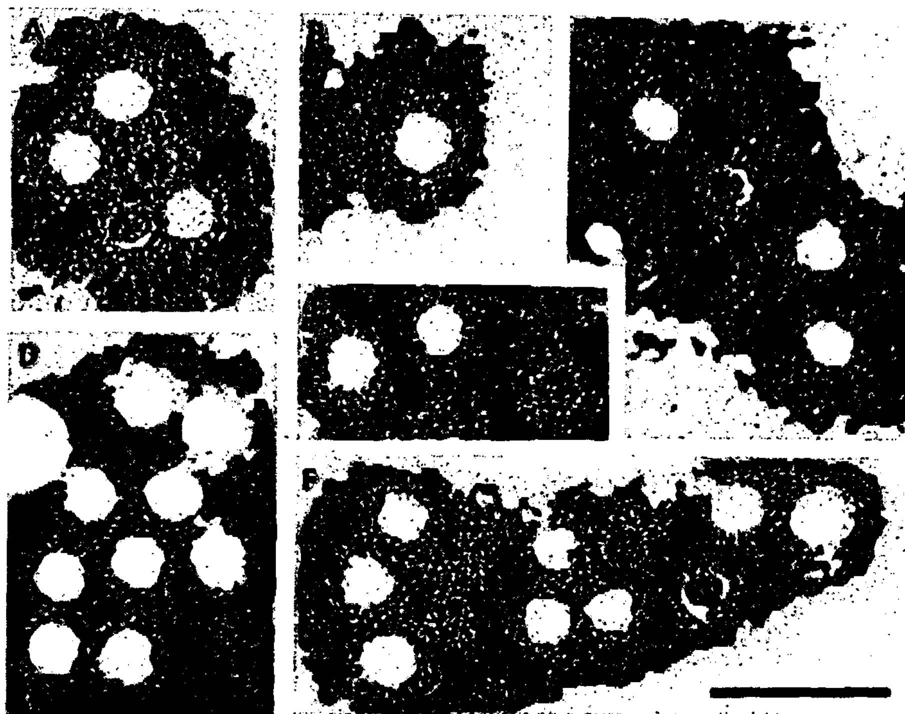

Fig. 1. A representative sample of hepatitis A viruslike particles observed after 0.9 ml of the 2 percent stool suspension was incubated with 0.1 ml of diluted serum, and then centrifuged and prepared for electron microscopy. The particles appeared to have cubic symmetry and resembled picornaviruses or parvoviruses morphologically. (A and B) An aggregate (A) and a single particle (B) observed after incubation of the stool filtrate with a serum from a convalescent patient from the Illinois study. The aggregate is composed of three "full" and two "empty" particles heavily coated with antibody. The single particle (B) is also heavily coated with antibody. The quantity of antibody in this serum to the hepatitis A antigen was scored as 3 to 4. (C and D) Aggregates after incubation of the stool filtrate with the serum of a patient convalescing from hepatitis contracted during the naturally occurring outbreak in Massachusetts. This serum was rated 3 to 4 also. (E and F) Aggregates and particles after incubation of the stool filtrate with immune serum globulin; they are heavily coated with antibody. [The bar is equivalent to 100 nm and applies to (A) through (F).]

We used the stool filtrate derived from a stool specimen obtained during the acute phase, which had detectable particles, to examine several groups of serums for antibody to this viruslike antigen by IEM. The relative concentration of antibody in each serum specimen was estimated by scoring the amount of antibody coating the particle on a 0 to 4 scale as judged by electron microscopic examination. The rating of 0 indicated that we could not identify particles coated with antibody. A 4 rating indicated that single particles or particles in aggregates were nearly obscured by antibody. Ratings of 1, 2, and 3 indicated presence of antibody but in lesser amounts than that rated 4. A change of 1 in antibody rating between paired serums was considered significant. Each antibody evaluation of paired serums was made by two observers on coded specimens in order to eliminate the possibility of biased interpretation. We rated a serum negative for antibody only after examining at least five good quality electron microscope grid squares.

All six experimentally infected hepatitis A individuals from the previously described New York and Illinois studies (*5*) developed serologic evidence (IEM) of infection with the 27-nm hepatitis A viruslike particle (Table 1). Since in both experimental challenge studies the material that contained hepatitis A virus was derived from the same source (MS-1), it was conceivable that the positive serologic responses in these six individuals might have been responses to an unknown adventitious agent in the original MS-1 inoculum which had no relationship to hepatitis A virus. However, this is most unlikely because we also demonstrated a significant increase in antibody to the hepatitis A antigen in paired serums of six individuals from naturally occurring outbreaks of hepatitis A in Massachusetts in 1969 and American Samoa in 1972 (*6*) (Table 1 and Fig. 1, C and D). None of the serums from hepatitis A patients prior to their exposure to the disease contained detectable antibody to the hepatitis A antigen, whereas two of four serums obtained during the acute phase of illness did contain such antibody at low levels, suggesting that the antibody response had already begun.

In experiments designed to determine whether a serologic relation existed between the hepatitis A antigen and hepatitis B antigens we found the following: (i) none of the 12 hepatitis A patients had a serologic response to hepatitis B antigen by radioimmunoassay (*7*); (ii) both individuals with hepatitis B demonstrated a serologic response to hepatitis B antigen by radioimmunoassay but did not develop antibody to the hepatitis A antigen as judged by the IEM technique; and (iii) neither guinea pig hyperimmune serum to hepatitis B antigen nor guinea pig serum to the "core" of the Dane particle, thought to be the hepatitis B virion, reacted with the hepatitis A antigen as judged by IEM (*8*). Thus we were unable to detect a relation between the hepatitis A antigen and hepatitis B antigen.

The hepatitis A antigen morphologically resembles the acute infectious nonbacterial gastroenteritis antigen, that is, Norwalk (*4*). Furthermore, despite intensive efforts, virologists have been unable to isolate either hepatitis A

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adult volunteers who were inoculated either orally or parenterally with the MS-1 or MS-1–derived strain of hepatitis A virus (5). Each stool was further diluted to a 2 percent concentration and passed through a 450-nm Millipore membrane filter. The stools were examined by IEM, with minor modifications of the technique of Kapikian *et al.* (4). We took this previously described approach in the hope that virus particles, if present, would be aggregated by specific hepatitis A antibody, so that they could be observed even if present only in low titer.

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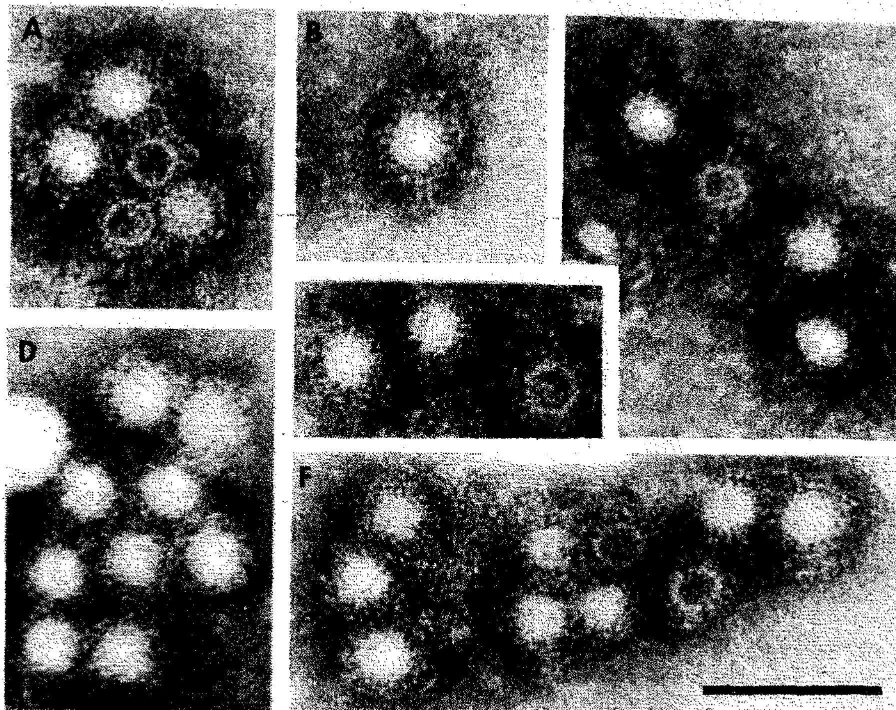

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The hepatitis A antigen morphologically resembles the acute infectious nonbacterial gastroenteritis antigen, that is, Norwalk (4). Furthermore, despite intensive efforts, virologists have been unable to isolate either hepatitis A

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or Norwalk viruses in tissue culture. Preliminary evidence suggests that the gastroenteritis antigen is the etiologic agent of "Norwalk" gastroenteritis and belongs to the class of viruses known as parvoviruses (*9*); studies by Provost *et al.* (*10*) suggest that the hepatitis A virus is also a parvovirus. We could not demonstrate a serologic response to the Norwalk gastroenteritis antigen with paired serums from two patients with hepatitis A, nor could we demonstrate a rise in antibody to hepatitis A antigen with paired serums from two volunteers infected with the gastroenteritis agent (Table 1). Thus, the two antigens do not appear to be serologically related.

In a previous study a virus was recovered from cell cultures inoculated with specimens from the Illinois volunteers (*11*). This virus was subsequently shown to be a parvovirus and to be related to the latent rat virus complex of agents (*12*). Although this isolate was most likely a contaminant unrelated to hepatitis A, the possibility of its association with the hepatitis A antigen was examined with guinea pig hyperimmune serum to the Kilham strain of latent rat virus and particulate antigen of rat virus grown in cell culture (*13*). In IEM studies done under code, a convalescent-phase hepatitis A serum from the Illinois study did not react with rat virus antigen but did with its homologous hepatitis A antigen; and the hyperimmune rat virus serum did not react with the hepatitis A antigen but did with its homologous rat virus antigen. Thus we demonstrated that the hepatitis A antigen was not related to rat virus and, in addition, confirmed the lack of an etiologic relationship of rat virus to hepatitis A.

Cross *et al.* reported particulate fecal antigens thought to be related to hepatitis A infection. However, one of these was smaller (15 to 25 nm) than the hepatitis A antigen (27 nm) whereas the other was larger (40 to 45 nm), and the latter appeared to be serologically related to hepatitis B antigen (*14*). Reagents are not now available to determine whether the hepatitis A antigen described here is antigenically related to the fecal antigens described by Cross.

Commercial immune serum globulin protects against or modifies hepatitis A illness. To determine whether such preparations contained antibody to the hepatitis A antigen, we tested two lots of immune serum globulin, prepared by different manufacturers: one was rated as being strongly positive for such antibody (3 to 4) (Fig. 1, E and F), and the other was found to have a moderate quantity (rated 2) of antibody. Testing for antibody to hepatitis A antigen may prove useful in the standardization of potency of immune serum globulin to be used in the prevention of hepatitis A infection.

Our data suggest that the 27-nm particle visualized in a stool filtrate derived from a patient with hepatitis A may be the etiologic agent of this disease. In addition, the discovery of this viruslike antigen in the stools of patients with hepatitis A, and the development of a serologic technique with which to detect antibody to it, provide, for the first time, a means of diagnosing and studying hepatitis A.

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Probit Transformation: Improved Method for Defining Synchrony of Cell Cultures

Abstract. *Cell numbers can be converted to probits that are used to compare the degree and timing of synchronized cell cultures. Data from synchronous cultures of* Chlorella pyrenoidosa *have been analyzed by this method by means of a readily available computer program. The method can be used with any logical system that generates normal sigmoidal data.*

The use of synchronous cultures of algae has been of value in numerous investigations of photosynthetic activity, pigment synthesis, nucleic acid synthesis, and changes in metabolism [reviewed in (*1*)]. A problem often encountered with synchronous cultures of microorganisms has been the quantitative comparison of the synchrony of different cultures, because the cell counts, when plotted against time, describe a sigmoidal curve rather than a straight line. In describing synchrony it is not sufficient to describe the growth conditions, moreover, because even when similar procedures are used the synchrony often varies between experiments. To circumvent this problem, some investigators used parameters that were a measure of the time required for completion of cell division (*2*). These numbers are not easily obtained and do not have the required precision. Engelberg (*3*) later introduced a more quantitative method. In the improvement introduced by Spencer *et al.* (*4*), the change in cell number was monitored every ½ hour and the resulting curve analyzed statistically. This method has the advantage of increased precision, but the necessity of making frequent cell counts for each culture at specific time intervals makes it impractical for routine use.

A simpler solution follows from the fact that the change in the rate of cell

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