Current Topic:

Hepatitis A Virus Infection

Summary of Discussion and
Statement of Opinions on Selected Issues
at an International Workshop

Guest Editor:

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Foreword

Since 1970, viral hepatitis has been the subject of many international meetings. Such conferences have provided the opportunity to exchange information about the technical details of new procedures and data that result from their application. New ideas or interpretations are also offered, but the time allocated to discussion is often inadequate to reconcile apparently discrepant observations. The latter circumstance is unfortunate because new findings assume importance in terms of their impact on our concepts, and our conceptional framework needs to be consistent with new findings.

The international workshop on hepatitis A virus infection, held in Athens, Greece, 17–19 November 1980, remains unique among the gatherings that have been held on viral hepatitis. Thus far, it is the only one devoted exclusively to hepatitis A virus and type A disease. This fact permitted much more adequate attention to the new information about this still important cause of morbidity in all areas of the world. More importantly, however, its focus was as much upon the meaning of observations as upon the observations themselves, and an attempt was made to stipulate the kinds of investigation necessary to fill the gap in our knowledge.

One other problem with some of the meetings on viral hepatitis has been the fact that the proceedings have been published as separate volumes. The extent to which libraries have funds to make such special purchases is limited, and is becoming more so in the economic climate that presently obtains throughout much of the world. Thus, much that is said is unavailable to many of those with an interest in keeping informed. It is for this reason that it seems particularly appropriate that the journal include a summary of the Athens workshop in this issue. It seems likely that it will remain a useful and accessible statement of what has been learned and what still needs to be investigated.

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Introduction

The International Workshop on Hepatitis A Virus Infection brought together persons actively engaged in the study of hepatitis A virus infection (HAV). They were joined by distinguished senior investigators who could contribute perspective and insights from their years of work with many other infectious agents. The task of these participants was to review available information concerning the nature of HAV, the methods for diagnosis of HAV infection, the virus's ecology and epidemiology, and the approaches to prevention. The goal was to compare and try to reconcile apparent discrepancies in findings, to promote greater uniformity in collection and presentation of data, and to encourage standardized procedures. There were four periods of discussion organized around a series of questions addressed with pertinent data and comments by all participants. Following these plenary sessions, small working groups considered specific questions and prepared statements that would represent the opinions expressed during the deliberations. At a final plenary session, the working groups' drafts were reviewed, modified to represent a consensus, and adopted as recommendations.

This report represents a summary of the scientific discussions, and concludes with the consensus statements.

Virology and Diagnostic Testing

Siegl summarized data from different laboratories concerning the structure, biochemical characterization, and stability of HAV (Table 1). The virion has a naked capsid structure, probably consisting of 32 capsomeres arranged according to the symmetrical requirements of a rhombic triacontahedron or a pentakis dodekahedron. The diameter is 27 to 28 nm, and the sedimentation coefficient is estimated to be 156 to 160 S. Five viral proteins are recognized (VP 0, 1, 2, 3, and 4), of which VP 1 and VP 3 appear to be partly exposed and VP 2 and VP 4 to be located inside the virion. The nucleic acid is a linear, single-stranded ribonucleic acid (RNA), estimated to consist of

6500 to 8100 nucleotides depending upon the method of study. HAV is stable in ether, in chloroform, and at pH 3. It is rather stable for several weeks at room temperature, and only slowly inactivated at 60 °C, but in one minute at 85 °C.

Deinhardt reported his group had cloned at least part of the HAV RNA in bacteria, and obtained a few cell clones that expressed some of the genes. He anticipated that further studies would allow detailed characterization of the HAV genome, and the isolation of the specific products of single genes. The important points to be examined are: antigenic identity of the HAV proteins, with which task monoclonal antibodies may be of help, the sequential appearance of various proteins during the reproductive cycle, and the functions of various products.

Most participants agreed that the data are adequate to classify HAV as a picornavirus.

It is evident that HAV has some, but not all of the characteristics of an enterovirus. Mosley pointed to several difference with epidemiologic implications:

(a) Replication in the oropharynx has not been demonstrated, although studies of this question have been limited. This is of particular interest because type A hepatitis usually has a seasonal peak in the late autumn or early winter months, in contrast to the summer peak in incidence of enteroviral infections.

(b) Intestinal replication has not been demonstrated, and it is presumed that progeny enter the host's gut by excretion in bile.

(c) The period of fecal excretion is relatively limited in duration when compared to that of the polioviruses. Purcell pointed to a difference in morphogenesis in that marmoset hepatocytes become filled with cytoplasmic vesicles containing HAV particles, a process not seen in enteroviral replication.

Complete cross-reactivity with available immunologic assays has been obtained among all HAV strains thus far isolated from cases in North and South America, Central Europe, the Mediterranean area, and the USSR. A single report of antigenic differences remained unconfirmed. Deinhardt suggested that some variations may be found when monoclonal antibodies induced by various strains become available. The participants generally agreed, however, that any differences that may be found are

Table 1: Characteristics of HAV as reported by G. Siegl. Summary of data from several laboratories.

Virion

Naked, spherical particles; capsid probably consisting of 32 capsomeres which are arranged according to the symmetrical requirements of a rhombic triacontahedron or a pentakis dodekahedron.

Diameter		27–28 nm	
Sedimentation coefficient		156–160 S	
Density in CsCl (g/ml)	dense particle 1.38–1.44	mature virion 1.33–1.34	immature particles 1.32–1.33

Proteins (daltons)[a]

VP 0		39,000 ± 1,000
VP 1	30,000–33,000	30,000–33,000
VP 2	24,000–27,000	24,000–27,000
VP 3	21,000–23,000	21,000–23,000
VP 4	7,000–14,000	7,000–14,000

Nucleic acid

Type	RNA
Configuration	single-stranded, linear
Sedimentation coefficient[b]	32.5 S
Molecular weight	2.25×10^6 [c] $- 2.8 \times 10^6$ [d]
Number of nucleotides	8,000–8,100
Poly (adenylic acid)	40–80 nucleotides
Polarity	positive
Translation strategy	probably monocistronic

Stability

Organic solvents ether, chloroform, Freon		stable
Acid	pH 3	stable
Heat	60 °C, 60 min	stable
	60 °C, 12 h	partially inactivated
	85 °C, 1 min	inactivated
Formalin	1:4,000, 35–37 °C, 72 h	inactivated
	1:350, 20 °C, 60 min	partially inactivated
Chlorine	0.5–1.5 mg/l, 5 °C, 60 min	partially inactivated
	2.0–2.5 mg/l, 5 °C, 15 min	inactivated
Sodium hypochlorite	10 mg/l, 20 °C, 15 min	inactivated
Potassium permanganate	30 mg/l, 20 °C, 15 min	incativated
Chloramine T	1 g/l, 20 °C, 15 min	not inactivated
Peracetic acid	300 mg/l, 20 °C, 15 min	not inactivated

[a] In preliminary experiments the structural disposition of these polypeptides in the capsid was studied by measurement of their relative susceptibility to iodination. It was concluded that the VP 1 and VP 3 polypeptides are partially exposed. Other polypeptides, VP 2 and VP 4, appear to be located inside.
[b] Under non-denaturing conditions.
[c] *Calculated on the basis of sedimentation under non-denaturing conditions.*
[d] From electrophoretic mobility of completely denatured molecules.

likely to be minor. The point is particularly important because the major cross-reactivity among strains indicates that a vaccine prepared from any otherwise appropriate isolate should be equally effective in all parts of the world. Purcell cautioned, however, that there could temporarily be questions on this point because a non-A, non-B (NANB) agent recently recognized in India produces disease having a pattern that epidemiologically resembles that of HAV. The pathogenesis of type A hepatitis also needs further study. It is not clear whether HAV replication itself results in destruction of hepatocytes. In cell culture systems, HAV does not produce a cytopathic effect. It is possible, therefore, that cellular mechanisms may be responsible for hepatocellular necrosis in the infected human. Pertinent to the host's subsequent recovery, Frösner reported that treatment with antibody to HAV (anti-HAV) of HAV-infected continuous cell cultures failed to diminish the amount of intracellular HAV antigen. He suggested, therefore, that cellular immunity could also be important in the resolution of HAV infection.

HAV has been experimentally transmitted to some species of marmosets and to chimpanzees. Apparent anti-HAV reactivity has been demonstrated in sera from many of the more extensively studied non-human primates, but these antibodies may be evoked by cross-reacting antigens of non-human viral variants. Such cross-reactivity is known between human and non-human enteroviruses. Feinstone reported that primates of several species (rhesus, patas, owl, african green, cebus, and woolly monkeys) developed anti-HAV following intravenous inoculation of HAV, but only rhesus monkeys have had seroconversion after oral inoculation.

He suggested that specificity of anti-HAV presumably due to natural infection be checked by use of immune electron microscopy (IEM) with HAV from human stools, which would permit one to see whether antibody adheres to the viral particles.

Feinstone also expressed the opinion that even if HAV is infective for some of the species in which anti-HAV has been found, they probably are not very useful as models of type A hepatitis in human because none develops detectable biochemical abnormalities, morphologic changes, or virus excretion in the feces.

It has become possible to propagate HAV in a number of cell cultures. HAV strains isolated directly from human feces, and also those passed as many as 30 times from marmoset to marmoset, have been grown in primary marmoset kidney cells, fetal rhesus kidney cells, diploid human embryonic cells, and two continuous lines derived from human hepatocellular carcinomas. With respect to the last systems, it is of interest that growth occurs only in lines carrying components of the hepatitis B virus (HBV) genome, although it is not clear that HBV-induced changes in the cells are responsible for the susceptibility. Specific host cell receptors for HAV have not been identified; in established in vitro infection Siegl observed direct spread of virus from cell to cell.

HAV replication in cell culture is detectable only by intracellular immunofluorescence; HAV antigens have not been demonstrable in the medium. Tests for HAV interference with multiplication of superfecting coxsackie A9, coxsackie B3, poliovirus 3, and echovirus 30 have all been negative. HAV does not hemagglutinate erythrocytes from the mouse, rat, chicken, pigeon, rabbit, pig, marmoset, or human.

Multiplication of HAV in all cell cultures is initially extremely slow, but more rapid growth cycles and higher yields of virus are achieved in some systems by serial passage. The mechanisms responsible for the initially slow virus replication are undefined, and need to be studied. The highest yields have been obtained from the continuous hepatocellular carcinoma lines.

Virus strains grown for several passages in fetal rhesus monkey cell cultures seem to lose their virulence for marmosets, which suggests that it will be possible to develop an attenuated live HAV vaccine.

Laboratory procedures to document that a clinical illness is due to HAV were discussed. The schematic summary of Frösner (Figure 1), derived from study of serial specimens from several hundred patients, was accepted as an appropriate statement of the general experience.

The diagnosis of type A hepatitis can sometimes be made by demonstration of HAV particles or viral antigen (HA Ag) in feces. The amounts in stools are highest, however, during the two weeks prior to onset of symptoms, and diminish rapidly thereafter. Approximately half of patients are positive for HA Ag at the onset
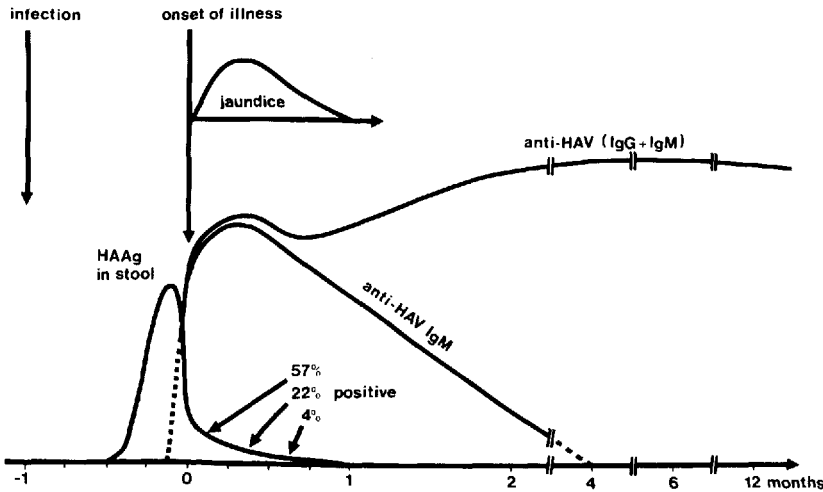
Figure 1: Model of serology of clinical hepatitis A infection according to Frösner.

of symptoms, but only one-quarter by the second week of illness, and a very small proportion during the third and fourth weeks. It is a minority of patients, therefore, that has detectable amounts of of HA Ag in the feces by the time the diagnosis is usually conjectured, and negative tests do not disprove HAV infection. It was agreed that testing of fecal specimens is not a useful approach to diagnosis.

Chronic HAV excretion does not seem to occur. Even in cases in which the disease follows an abnormally protracted course of hepatic abnormalities (up to two or three months), prolonged HA Ag excretion has not been found.

Immunoglobulin M (IgM) anti-HAV appears in serum early in the course of type A hepatitis, reaches an easily detectable level by the time the diagnosis is usually suspected, and decreases rapidly in titer during convalescence. Immunoglobulin G (IgG) anti-HAV reaches high titer in convalescence, and persists for many years or throughout life in the majority of persons experiencing infection.

Radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), and other tests that detect both IgM and IgG are unsatisfactory for diagnosis in the acute phase even if one attempts to use them to demonstrate a change in level. High titers of anti-HAV IgM are reached so quickly early in the illness that it is often impossible to demonstrate a significant increase. This limitation does not apply to complement fixation or immune adherence

hemagglutination (IAHA) because these tests detect only anti-HAV IgG, but they have the disadvantage of requiring acute and convalescent specimens and thus entail a delay in diagnosis.

All participants agreed that for diagnosis of acute cases of type A hepatitis, RIA or ELISA tests that are designed to detect only high levels of anti-HAV IgM are ideal and should be used. Nordenfelt and colleagues found staphylococcal protein A absorption was unreliable for this purpose because of failure to remove immunoglobulin A (IgA) that may persist in serum for two years or more after acute infection.

To detect past HAV infection, the tests for anti-HAV IgG are those that are appropriate. For epidemiologic surveys, the three procedures likely to be used are RIA, ELISA, and IAHA. The participants considered that all were approximately equal in their sensitivity. Mosley, however, cited an experience with sera from Gambia in which the RIA test was positive in a significantly higher proportion of persons tested than was IAHA.

RIA is technically easy to carry out and is highly reproducible. It requires a gamma counter, which may be expensive and difficult to service in developing countries. Its reagents, with their shelf-life of only 30 days, also pose problems in remote areas. Finally, some countries have restrictions upon the importation of isotopes with half-lives as long as 125I, and safe disposal of radioactive materials may be a cause for concern. ELISA has the advantage of

reagents that are stable for as long as on year, and a photometer is the only equipment needed. With a proper balance of reagents, a positive result can be distinguished from the negative by the unaided eye, but an automated reader is desirable for large-volume work such as epidemiologic surveys. IAHA similarly needs little equipment, and is usually the least expensive of these tests for the laboratory. It requires, however, careful standardization, and purified HA Ag is not available to most facilities.

IgA anti-HAV can be demonstrated in serum during and after acute phase of illness, and in feces during convalescence. Tests of either serum or feces for specific antibodies of this class, however, have not been found useful for routine diagnosis, but may be useful in studying mechanisms of immunity. Specific immunoglobulin D is also detectable in serum during type A hepatitis, but the assay is not clinically helpful.

Ecology of Hepatitis A Virus

Ecology is defined as: "The branch of biology dealing with the relations between organisms and their environment". It is particularly concerned with those environmental factors that determine the ability of an organism to survive and influence the relative and absolute size of its population.

For an agent such as HAV, presumably dependent upon its human host for its perpetuation, ecologic and epidemiologic behavior would seem to be synonymous. If we look at these words in terms of strict etymologic derivation, however, one can infer a difference in scientific approach to this and other agents similarly dependent on man. Epidemiology is concerned with factors determining the impact of an infectious agent on humans; ecology, on the other hand, looks at how the agent utilizes humans, their habits, and their habitats to progenerate. The latter frame of reference, if pursued systematically, sometimes permits recognition of gaps in our knowledge of which we would otherwise be unaware; we may investigate characteristics that would otherwise go unnoticed.

The first question asked in this workshop session was whether man is the only natural host for HAV? The question assumed not only

susceptibility of an animal, but also sustained animal-to-animal transmission without the necessity of re-introduction by direct or indirect contact with man.

It was pointed out that attempts at experimental induction of type A *disease* in various nonhuman primates began in the 1940's but were judged unsuccessful or equivocal for many years. Epidemiologic observations in the 1960's, however, permitted recognition of susceptibility to *subclinical* infection with HAV of *several* families and genera of non-human primates. Repeated examples of transmission to humans from chimpanzees newly imported into the United States were found at that time. In addition, other non-human primates were associated with human cases in such a way as to suggest that they were the source for the latter; these included the Colombian woolly monkey, the Celebes macaque, and the gorilla. In some instances it appeared that there also had to be primate-to-primate transmission in captivity. The data concerning chimpanzees, however, strongly suggested that these animals acquired their infections from man after capture.

Several investigators presented data on the frequency of anti-HAV in sera from non-human primates that had not been experimentally exposed. Most of the animals, however, had been held in zoos or laboratories for relatively long periods, so that infection in captivity rather than in the wild again seemed likely. Deinhardt and colleagues reported that 13 of 49 (27 percent) newly-captured grass monkeys from five locations in Africa were anti-HAV reactive. They also cited the work of Dr. Martin Smith, who found anti-HAV reactively in all four animals in one group of newly-captured baboons. Studies at the Max von Pettenkofer Institute with baboons, however, had not been consistent with infectibility.

Under any circumstance, members of the workshop agreed that even specific IgG reactivity without IgM positivity among animals caught in the wild would not be evidence that sustained transmission occurs there among nonhuman primates. Animals living in relatively close proximity to man could be infected by contaminated food, water, or fecal deposits. Observations of Dr. Alfred M. Prince were cited. Working at a research facility in Liberia, he found that most chimpanzees in the colony there were initially non-reactive for anti-HAV.

HAV infection manifested by seroconversion occurred as an epizootic, however, after the animals were exposed to water contaminated with human feces. Finally, even if infection in the wild leads to subsequent transmission within a colony, the limited period of excretion may result in a dead end for the virus.

Even if infections of non-human primates in the wild does occur, it was the general opinion that they are not epidemiologically important (i.e. they do not contribute significantly to the occurrence of HAV infections among humans). It also seemed probable that non-human primates are ecologically unimportant to the survival of the virus.

Fox asked whether HAV may replicate in non-human vertebrates other than primates. He was particularly interested in those that are frequent within human communities and have an interactive role with humans; pets and domestic animals would be epidemiologically the most likely. Gust reported anti-HAV reactivity in coprophagic dogs in the South Pacific, but not in pigs or other animals tested. Purcell mentioned briefly collaborative work on an equine hepatitis, and the fact that during its course anti-HAV seroconversion was observed in one instance.

The second question in the area of HAV ecology concerned survival under various environmental conditions, and any estimate of the infectious dose. Obviously, the more stable the agent, and the lower the amount of viable particles required to produce infection, the greater the ecologic advantage. With further development of cell culture systems, so that even small amounts of HAV can be recovered, stability can be studied much more easily. Balayan pointed out that studies with purified virus may not be applicable to stability of HAV in nature where it could be protected by a protein matrix. Advantage should be taken of any situations in which contaminated food responsible for outbreaks becomes available for viral recovery and titration.

Frösner cited a study of an extremely isolated population in the highlands of Papua-New Guinea whose custom is to defecate on the ground quite near to their villages. Even though they had no identifiable contacts with the outside, 16 of 17 young children were anti-HAV positive. This finding could indicate that HAV persists in fecal deposits for long periods.

The amount of HAV in sewage is dependent upon the number of infected persons contributing to the system and the amount of dilution. Balayan, using an ELISA obtained low-level but fluctuating positivity in one suburb of Moscow, and intermittently borderline values on three occasions in two other towns. Dr. F. Blaine Hollinger was cited as having observed a higher than expected incidence of anti-HAV among sewage workers. Because HAV would be highly dilute in sewage, the latter finding implies that low levels of HAV are infective. That is also true of waterborne epidemics, although the etiology of these occurrences needs to be reevaluated in view of a possible NANB agent transmitted by the fecal-oral route in India.

Gust reported that endemicity of HAV infection on isolated South Pacific islands may in part be due to persistence of the agent in mollusks. The indigenous population of one atoll studied defecates directly into a lagoon at one end of the reef, and the contaminated sea water is carried by tides over the area of shellfish harvesting. Thus, only a few children may be infected at any one time, but the agent survives.

Mosley questioned whether HAV would persist in mollusks in areas in which the seas are warm throughout the year. He cited the work of Dr. Theodore Metcalf, who found that oysters in New Hampshire waters retain viable enteroviruses through the winter months when the animals are inactive, but rapidly clear their digestive tracts when the water temperature increases to an appropriate level in the spring. If bivalves are continually active in feeding, HAV would have to be present in the water for at least a few hours every one to several days, which seems unlikely with a small population.

Transmission by blood was generally felt to be rare because of the briefness of the viremia. The possible exception, cited by both Iwarson and Mosley, is among parenteral drug abusers who share needles and syringes. Such drug abuse is as frequently a background in type A as in type B cases in some urban areas. It is recognized, however, that fecal-oral transmission could also account for transmission among such individuals because their hygienic habits are often poor and the circumstances under which they share drugs could facilitate spread by contact.

The only direct observations on the infective dose have been the titration at Willowbrook

State School in New York by Krugman and co-workers. Their estimate for a serum pool from the acute phase containing MS-1 strain was 100 infectious doses per ml of serum. No data on fecal titrations were presented. Krugman felt that in that institution the most important factor was close exposure sustained for one week or more.

The third question was how the members of the Workshop reconciled evidence that serial transmission from person to person during the relatively brief period of fecal excretion is the only mechanism of propagation, with the concepts of endemicity in small, relatively isolated populations, and hyperendemicity in similar as well as larger and contiguous populations.

Mosley stated that "endemicity" implied to him that the agent persisted in some part of a population at all times over prolonged periods without the necessity of re-introduction, and found no difficulty with applying that meaning to most usages in the literature on HAV infection. The major exception is in articles on type A hepatitis in institutions for the mentally disabled, to which situation the term is often applied without an apparent consideration of the possibility of intermittent re-introductions from the outside community. He found a much greater problem with usage of "hyperendemic", which seems to be applied pejoratively to epidemiologic situations in which there is a high level of experience with HAV under conditions of poor sanitation.

Horstmann and Fox pointed out that the term hyperendemic is used in two different ways: (a) To indicate that almost all persons in a population have evidence of experience with the agent in question, usually by a young age; or (b) to mean that a large proportion of susceptible persons or groups entering the situation or locale to work or live become infected within a relatively short period of time. Fox suggested that more precise definitions for either the former or latter usage could be formulated, e.g. a 95 percent prevalence of anti-HAV by five years of age, or a 25 percent attack rate among susceptibles during their first year of residence. He did not feel, however, that such exactness was necessary; Krugman was of the same opinion.

Ben-Porath pointed to a recent example of hyperendemicity. In the indigenous population of southern Lebanon, it is known that most persons acquire experience with HAV by four years of age. When United Nations contingents entered that area in the late 1970's, only French troops were not given immune globulin (IG) for prophylaxis of HAV infection, and this was the only contingent to experience the disease. Several hundred cases were seen among French soldiers.

Three individual investigators reported studies of institutions for the mentally retarded in Europe, the United States, and Australia. In all three situations, the pattern of HAV infection was one of stable levels of seropositivity until an introduction or re-introduction produced an outbreak. Clearly, in these institutions HAV was neither endemic or hyperendemic, and the point was made that endemicity should not be assumed until it is demonstrated. Krugman agreed that epidemiologic conditions in facilities for the retarded varied. He indicated that at Willowbrook State School in the 1960's, there was a rapid increase in the population of the institution to a total of some 6000 residents, and at the same time a high rate of turnover with some 100 admissions per month. Later study after tests for anti-HAV became available showed that 97 percent of residents during this period had had experience with HAV by the end of two to three years. This situation among residents was accompanied by a high incidence of infection of staff members, indicating widespread circulation of HAV in the school. After admissions were stopped at the end of 1969, HAV infection was no longer a problem among new members of the staff. Thus, the earlier situation clearly could be described as hyperendemic.

Villarejos referred to information from his long-term studies of viral hepatitis in two rural districts of Costa Rica. In one district, he examined the movement of type A hepatitis from household to household and from village to village, as well as in the town of San Ramon, the commercial and social center in that mountainous area. He found that the town tends to have a small number of cases more or less continuously. In the surrounding villages, however, HAV infections occur as periodic clusters separated from each other by months to years. The initial case in any village usually gives a history of having visited within one incubation period either San Ramon, or a village where cases are occurring. Figure 2
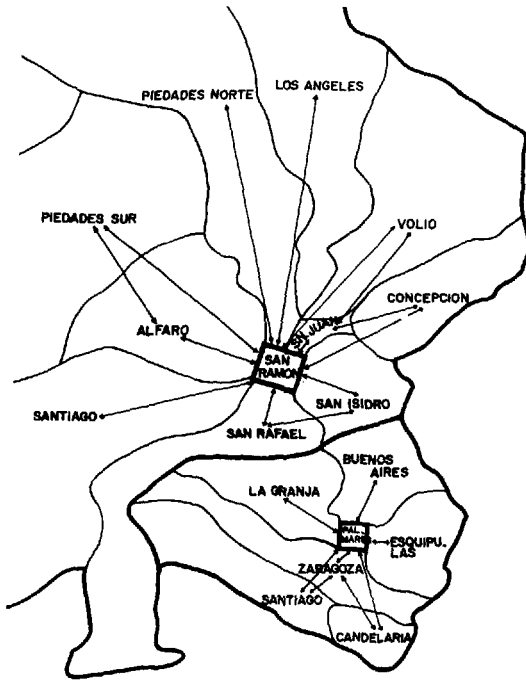
Figure 2: Study of spread of hepatitis within two rural districts in Costa Rica.

illustrates such radial distribution out from, and back into, San Ramon. Thus, the district's center, along with the surrounding villages, constitute an extended ecologic zone for maintaining endemicity of HAV. Although the specific prevalence of anti-HAV increases more rapidly in Costa Rica than in developed countries, Villarejos did not feel that the term hyperendemic was truely appropriate.

The fourth question considered was whether persons who had previously experienced HAV infection but lost detectable anti-HAV could become re-infected, and whether detectable anti-HAV was protective against re-infection. If either or both occur, and such persons excrete HAV, then the potential for amplification in the community is greatly enhanced. That occurrence would obviously be to the ecologic advantage of the agent, and would help explain endemicity, and especially hyperendemicity.

Both Szmuness and Frösner mentioned studies in which they compared anti-HAV levels at intervals of 10 and 12 years, respectively, in follow-up assessments of persons observed earlier. Both found decreases in titer, but the differences suggested only slow declines.

In cross-sectional studies, two patterns of anti-HAV prevalence are seen: (a) In developed countries, the proportion with positivity increases through late childhood and early adulthood, being highest in persons 40 years of age or over; and (b) in developing countries, the proportion is usually lower in older than younger adults. The latter point was well-illustrated by Feinstone's data from Kenya and those of Szmuness and co-workers from Senegal. Feinstone also found that titers of anti-HAV declined with increasing age, and pointed out that one would not anticipate this occurrence in areas in which seroconversion in early childhood suggests continuous circulation of the agent.

Mosley presented data from Costa Rica which he had analyzed in collaboration with Villarejos and colleagues. Prospective follow-up of 980 members of 230 households in which a clinically recognized case occurred revealed 303 associated infections (either co-primary or secondary). The ratio of inapparent infections (anti-HAV seroconversion by IAHA) to hepatitis (clinical symptoms or aminotransferase abnormality) was 1.3 : 1 among persons less than 20 years of age, but 28 : 1 among adults 20 years of age and over. This unexpected finding seems most likely explained by re-infection of persons with previous experience who had lost detectable antibody. Testing for specific IgM and IgG showed that the two adults with overt hepatitis had IgM predominance, and the 57 adults with inapparent infection, with one exception, had IgG predominance. From these data one would conclude that persons who have lost detectable anti-HAV can be re-infected to an extent sufficient to restimulate a specific IgG response.

Frösner had encountered only one instance of re-infection evidenced by a significant increase in existing anti-HAV levels; that person was an older woman who had provided nursing care for two members of her family with acute disease. Hadziyannis, on the other hand, did encounter increases in specific IgG of some adults during an epidemic among children in two Greek villages. Only a few low level aminotransferase elevations were observed among adults.

Horstmann concluded this part of the discussion by remarking that re-infection occurs in many viral diseases — e.g. rubella and poliovirus infection — so that its occurrence in HAV infection would not be surprising. The data suggest, however, that symptomatic re-infections are very uncommon.

The fifth question asked was how large a contiguous population is necessary to sustain HAV if serial transmission is the only mode of transmission. A subsidiary question was whether the size of the population varies by geographic region — e.g. between arctic communities and tropical islands.

Skinhoj opened the discussion by pointing to the periodic epidemics in Greenland, during which almost everyone acquired anti-HAV, followed by disappearance of the virus until re-introduced. Immunity sufficient to prevent clinical manifestations was definitely persistent. The experience in the Faroe Islands has been somewhat similar. Even though their hygienic standards are much higher than those in Greenland, with most families living in single-household units, they have also had periodic, large-scale epidemics. Anti-HAV patterns show a similar age-specific curve, indicating no infections occur in the intervals.

Purcell indicated that his findings for the South Pacific islands were similar to those of Gust. The prevalence of anti-HAV suggests continuous and high level exposure of the populations. One exception was Ponape, where no one under age 20 was anti-HAV-positive. An epidemic of type A hepatitis swept through the South Pacific in the 1960's, following which virtually all persons tested had evidence of experience. Little is known of that epidemic, but its occurrence is suggestive of the situation in other islands that had similarly susceptible pre-epidemic populations.

Mosley suggested that travel by the indigenous population using their own open boats could mean that many of the island groups constitute an ecologic unit, with introductions occurring continuously. All members of the Workshop agreed that more information is needed.

The final question of this session concerned the extent to which "special" populations had been excluded as potential reservoirs for HAV. The populations discussed that could be dismissed on the basis of available data include the mentally retarded, normal infants, and persons immunocompromised by chronic renal disease. Krugman suggested that immunocompromised infants should be examined because of their known tendency to chronic enteroviral infection, but no observations on this group were known.

Epidemiology of Hepatitis A Virus Infection

Fox opened the session by disagreeing with Mosley's distinction between ecology and epidemiology when the organism is a pathogen and man is the only (or an essential) host. He feels that the ecology of an organism is inseparable from its epidemiology, which he defines as the discipline that seeks to describe and explain the occurrence in man both of infection with the organism and of the related disease.

The initial question concerned morbidity and mortality data as rough but possibly useful indices of the trend in HAV prevalence in various countries and regions of the world. Papaevangelou pointed out that the completeness and reliability of the desired information about type A hepatitis has varied greatly from the 1940's to the present according to the general understanding among physicians of the nature of the disease. During and after World War II, it was common (and perhaps moderately accurate at that time) to classify cases of viral hepatitis as "infectious" (type A) in etiology when there was no history of percutaneous exposure to blood transfusion or a demonstrably hazardous inoculation. After 1968, when it became possible to identify cases of type B hepatitis serologically, the designation "type A hepatitis" was generally used for cases without the "appropriate" epidemiologic background that were also seronegative if tested for hepatitis B surface antigen. This perseveringly negative definition of type A disease remained unsatisfactory, as became clear in the mid-1970's when NANB infections were found to exist. Although most cases of NANB hepatitis in the United States and Western Europe have had exposures more suggestive of type B than type A hepatitis, one form of NANB infection may be spread by the fecal-oral route. Diagnosis, therefore, must be based on specific laboratory procedures in both non-fatal and fatal cases.

In limited areas, the majority of clinical cases are now characterized serologically and reported. Müller estimated that 75 percent or more of cases of viral hepatitis in Hannover during the period from 1975 through 1979 were reported to health authorities. Most were also serologically evaluated. Similarly, reliable information is available or services are being developed for other parts of Northern Europe, and selected areas in the United States, Costa Rica, and Australia.

On the other hand, in many developing countries, and in even a few developed ones, viral hepatitis of whatever etiology is not included among the notifiable diseases. If viral hepatitis is reportable, there may be no provision in the coding of cases for etiologic differentiation. Whatever the basis for diagnosis at present, the incidence of what is reported or recognized as type A hepatitis varies markedly from country to country and region to region. Even more fundamental than laboratory testing is the availability of physicians to see and diagnose any form of viral hepatitis. In many countries, the priorities in utilization of very limited resources dictate that attention be devoted to medical problems that much more frequently result in serious disability and death. This situation will change only slowly.

It has been suggested that for many countries mortality data are more reliable than morbidity reports because there is usually a legal requirement that the cause of death be certified by a physician. Krugman indicated the extreme infrequency of this outcome in children, and the possible contribution of other health problems in fatal cases among adults. He felt that mortality figures are probably less meaningful as indicators of trends than any morbidity reports that may be available.

The participants generally agreed that comparisons based on reported cases or deaths must be made with great caution.

The second question concerned long-term secular trends in the incidence of HAV infection in different parts of the world. In the absence of usable morbidity data, it is necessary to rely upon the patterns observed in serologic surveys. Although not all areas of the world have yet been covered, the information available is already quite extensive.

Feinstone described three patterns of anti-HAV prevalence that may be found:

(a) In the least developed countries, the proportion with antibody reaches high levels in early childhood, remains high through early adulthood, and declines modestly in older age groups.

(b) In better developed countries, there is a relatively linear increase in anti-HAV prevalence through late childhood and early adulthood, with a modestly higher level in older age groups.

(c) In the most highly developed countries, the proportion is very low until mid-life, and then abruptly increases to levels in the range of 30 to 70 percent. These findings suggest that HAV has continued to be highly endemic in the least developed areas, to be declining in better developed countries, and to have reached low levels in the last 20 to 30 years in the most highly developed countries.

Müller presented his observations on HAV infection in Hannover based on serologic surveys made in 1965 and 1975. The curve of prevalence in the latter survey has shifted to the right by ten years which was compatible with the observation made by Gust for Melbourne. In Hannover, both the 1965 and 1975 surveys showed that almost all persons born before 1936 had had HAV infection, likewise approximately half of those born between 1936 and 1945, and about ten percent of those born after 1945. Iwarson showed data for Sweden which indicated that the decline in incidence to the present very low levels began in the early part of the century.

Villarejos reported that surveillance of type A hepatitis in the San Ramon-Palmares districts of Costa Rica indicated a decline in incidence in the 14-year period of observation. Cyclic fluctuations still occur, but the trend has been downward.

Participants were invited to evaluate the relative contributions to the decline in HAV prevalence of three factors: (a) improved sanitation and housing; (b) extensive pre- and post-exposure immunoprophylaxis; and (c) early hospitalization to minimize transmission to contacts.

Although causality could obviously not be completely established, participants agreed that the decline in incidence has closely paralleled improved conditions of living that include better sanitation, less crowding, and the improved hygiene that probably accompanies better education. Iwarson stated that the proportions

of Swedish households in recent decades with an indoor water closet were: 1940–49, 22 percent; 1950–59, 40 percent; 1960–69, 66 percent; and 1970–79, 87 percent. Villarejos' data for the San Ramon-Palmares districts showed that the decline in type A hepatitis correlated with an increase in the number of homes with running water and electricity.

IG has been very widely used for post-exposure prophylaxis in the United States since the late 1940's, and extensively in some parts of the USSR for both pre-seasonal passive immunization of school children and post-exposure prophylaxis. It has not been routinely used, however, in Scandinavia, where the decline in incidence has been the most marked. Balayan felt he did not have adequate data to comment about the trend in incidence in the USSR, but he did not feel that IG would have influenced the amount of HAV circulating in the population or done any more than delay the age at which infection occurred.

Routine hospitalization of all suspected cases of viral hepatitis to decrease the amount of transmission to contacts has been widely practiced in Eastern Europe since the 1950's. Participants again called attention to the fact that the most marked declines in incidence have been in areas in which only a small proportion of cases are hospitalized. The recent data showing maximal excretion prior to the onset of first symptoms place this approach even further in question.

The proportion of clinically overt cases attributable to HAV infection was considered, but the question was complicated by the frame of reference. It was clear that the answers were related to a consideration of all reported cases or only hospitalized cases, and also to the age group. Mosley, for example, stated that 98 percent of pediatric cases in Los Angeles were type A hepatitis, but only 25 percent of hospitalized adult cases. Müller found only 20 percent of all hepatitis cases in Hannover to be due to HAV, but his data showed that over 80 percent of cases in persons under ten years of age were type A disease. In countries in which type A hepatitis remains a common infection, however, HAV accounts for a majority of cases in both children and adults. Villarejos stated that 70 percent of all cases of overt hepatitis in Costa Rica are still due to type A.

Several participants provided information on the epidemiologic factors responsible for type A cases in areas of low incidence. Favero emphasized that in the United States, day-care centers, especially those accepting diapered children, have become foci for transmission from child to child, and then to siblings and parents at home. The marked increase in the numbers of such centers and the children enrolled in them in the 1970's have made this an occurrence of epidemiologic significance. Both Frösner and Müller have found that in Germany the children of foreign workers introduce the infection into kindergartens and schools when they return from visits to their home countries.

Among adults in Scandinavian countries, drug addiction is the background of many of the cases, which sometimes cluster in the form of outbreaks. Szmuness called attention to the frequency with which type A hepatitis can be related to promiscuity among male homosexuals in the United States. He found the attack rate among a prospectively studied group to be 6.4 percent in a 21 month period.

The ratio of subclinical to clinical infections was also considered. It has sometimes been estimated to be as high as $10:1$ to $100:1$, the latter ratio being based largely on the frequency of anti-HAV positivity among persons who do not recall having had an icteric illness at any time in their life. Mosley cited two prospective studies in the United States and one in Yugoslavia in which the ratio in children five years of age or older was $2:1$ or less. Skinhoj made comparable findings in the same age group in Greenland, but found a much higher ratio in children under five. That would also be the conclusion to be derived in studies of day-care centers in the United States. Krugman suggested that the ratio may depend not only on age, but also the size of the inoculum.

Discussion then turned to the relative importance of vehicles such as water and food. The participants agreed with the statement of Gust that outbreaks attributable to either are dramatic but of little real importance in the total epidemiologic picture.

Control and Prevention of Hepatitis A Virus Infection

The session opened with a consideration of ways to control water-borne, food-borne, and person-to-person transmission.

The role of untreated supplies of drinking water, especially in developing countries in which almost all persons acquire HAV infection in early childhood, remains undefined. No data were available to indicate whether water is more important as a means of infection than personal contact or environmental contamination in village or poorly sanitated urban settings. According to Purcell's observations, HAV may not have the role in water-borne epidemics that it has appeared to have in India; that water-borne outbreaks do not occur in developing countries with very high levels of immunity has not been established.

In relation to treated public supplies in developed areas, Bancroft called attention to a WHO Technical Report in which a group concerned with human viruses in water suggested that very low levels of contamination (one infectious unit per 20 liters) permitted by presently accepted methods of treatment may be responsible for enteric infectious in as much as 18 percent of users each year. Presumably, HAV cases in towns and cities emptying sewage into surface streams could be responsible for introducing HAV infection into communities downstream. Mosley stated that the testing of this concept by an investigation in Peoria, Illinois, in 1962 demonstrated that almost no cases of type A hepatitis occurred in the part of that city supplied by treated surface water. Iwarson pointed out that in Northern Europe and the United States, the incidence of type A hepatitis has remained at very low levels of endemicity during recent years when the amount of sewage polluting rivers has been steadily increasing. Thus, the possibility that low levels of HAV are surviving treatment and causing outbreaks seems unsupported by either the epidemiologic pattern trends or the incidence of HAV infection.

Food-borne outbreaks of type A hepatitis are recognized most commonly in developed countries. This fact results from several circumstances: (a) the proportion of susceptible adults is relatively high; (b) services which might become aware that an epidemic exists are largely limited to developed countries; and (c) many more resources for investigating cases epidemiologically and implicating food(s) as a vehicle are available in developed countries. Most recognized occurrences are caused by contamination of one or several items by food-handlers in the late phase of incubation. Such outbreaks are preventable only if the fact that exposure has occurred (e.g. to a child in the household) becomes known prior to the time when excretion of HAV would begin, and the person is excluded from work. Contamination of foods during growth, harvesting, or shipment may not produce a sufficient number of cases in any given locale to alert public health authorities to the problem. The difficulty in such instances may be even greater if contaminated fruit or vegetables are supplied to consumers in frozen form, and if this occurs over a prolonged period. In the latter instance, cases may be scattered not only geographically, but also in time. Nonetheless, some instances of food-borne transmission can be documented; several recent epidemics in Europe, for example, seem to have been due to contaminated strawberries.

Person-to-person transmission appears to be responsible for the vast majority of cases of type A hepatitis. The frequency of unrecognized HAV infections, both anicteric and subclinical, makes control by limiting contacts very unlikely to succeed. In families, HAV is usually introduced by children, and most other susceptible members of the household become infected subsequently.

The excretion of HAV is maximal prior to the onset of symptoms, and most instances of transmission occur prior to diagnosis. Thus, there is no reason to impose special measures (e.g. separate handling of eating utensils) upon other members of the household. There was also general accord that *hospitalization for the purpose of limiting transmission in the community* is useless. Only in institutions in which the residents cause gross environmental contamination may segregation of cases contribute even modestly to control.

Most persons who develop type A hepatitis do not require hospitalization for diagnosis or management. In some cases, however, this measure may be needed. When it is, difficulties may occur because of apprehension on the part of hospital authorities and staff about the

potential hazard of transmission to healthcare personnel and/or other patients.

The workshop's participants reported on three types of observations pertinent to the risk of nosocomial HAV infections: (a) the frequency with which hospitalized patients are readmitted with a second episode of icteric disease within a compatible interval, or members of the staff become ill; (b) the prevalence of anti-HAV in hospital personnel compared with an appropriately matched sample of persons in the general community; and (c) the incidence of anti-HAV seroconversion in patients and/or staff members after an acceptable interval.

Gust commented that review of experience at the Infectious Disease Hospital in Melbourne had demonstrated only two cases in personnel, both of which could be related to overt fecal contamination by incontinent patients. Three instances in which patients probably became infected all involved drug addicts whose exposure may have been due to continuation of their illicit habit. Chiaramonte reported that she and her colleagues surveyed 504 hospital employees in Northern Italy, and found no increased prevalence compared to age-matched controls; Skinhoj's experience in Copenhagen was similar. Frösner and co-workers in prospective serologic studies found no instances of HAV transmission from adult cases hospitalized for type A disease, but did see an outbreak attributable to infection of a child exposed to another child hospitalized for an unrelated problem. Papaevangelou's prospective study in Athens showed no instances of patient-to-patient transmission on a ward for hepatitis cases. Thus, there was agreement that it is unnecessary to limit care of patients with type A hepatitis to infectious disease hospitals or isolation wards where special precautions can be strictly enforced. In addition, it was generally felt that enteric precautions (e.g. routine use of gloves and gown) are not necessary unless there is fecal incontinence. Gust, however, expressed reservations about this last point, and called attention to the possibility that the paucity of nosocomial HAV infections could be due to the measures now considered no longer necessary.

With regard to passive immunization to prevent type A hepatitis, the major issues concerned the standardization of IG preparations, the correlation of anti-HAV titers with efficacy of protection, and the frequency and possible duration of passive-active immunity.

Gerety reviewed data of the Bureau of Biologics in the United States concerning trends through time in anti-HAV titers of lots used in that country. The concern is that in areas in which the incidence of HAV infection has dropped to low levels, the amount of antibody in plasma pools derived from donors in the general population may decline. Gerety's survey, however, showed no decline in 200 lots from six manufacturers in the United States released between 1967 and 1977. He raised the question whether fragmentation in some older batches may have contributed to higher titers in such material, and expressed the belief that lots with a level of 1:500 as measured by RIA are probably effective, judging by general experience.

Hilleman stressed the variability in titers among lots he and his associates had tested, and the fact that the level for minimal potency in the presently recommended doses is unknown. He believes manufacturers should "guarantee" a given titer in their product.

The participants generally held the view that there was no longer any reason to accept IG preparations containing whatever level of anti-HAV happens to be in the plasma pool used for its preparation. Minimal acceptable concentrations need to be established in terms of an international unitage, and that fact stated on the label of each vial. The possibility was discussed that a "hyperimmune" preparation might be efficacious for post-exposure prophylaxis in the later part of the incubation period, but no consensus could be reached because no evidence concerning that possibility is available.

Iwarson placed emphasis upon the need of IG prophylaxis for anti-HAV-negative travellers to developing countries. Experience with Swedish nationals indicates that the areas posing the highest risk are the Middle East, Southeast Asia, and the interior parts of Africa. For prolonged stays, repeated doses are necessary. Data of Dr. O. Weiland was cited according to which passive-active immunization was seen only once among 615 Swedish soldiers serving for six months in Cyprus.

The last topic discussed was the possible development of, and need for, active immunization against HAV infection. Bancroft pointed out that the basis for an inactivated vaccine had already been laid by Hilleman and his associates.

The latter passaged the CR326 strain of HAV in marmoset, harvested antigen from liver, inactivated if with 1 : 4000 formalin for four days, obtained an anti-HAV response in susceptible animals, and showed they were protected on challenge. If a suitable supply of HAV antigen becomes available, it is reasonable to assume that a similar inactivated vaccine can be used in humans.

Attention was focused upon the possibility of an attentuated HAV vaccine when the agent is adequately adapted to an appropriate cell culture system. The data indicate no late sequelae from HAV infection, so that a live-virus vaccine should be quite safe in this regard. Several participants remarked that the low incidence of type A hepatitis in developed countries at the present time, the low fatality rate, and the lack of long-term consequences will limit the extent to which HAV vaccine will be used. All agreed, however, that appropriate applications for selected groups will readily be found, and that all possible haste in development is desirable.

Consensus Opinions on Selected Issues

After the general discussion sessions summarized above, a final series of questions was posed to the participants, and responses were formulated by small groups. These reports were presented at a final plenary session and amended as seemed necessary to reflect the consensus of opinion among workshop participants. The recommendations concerning policy, programmes and research goals that are given in the following statements reflect the collective scientific judgments of the group about what should be done. It was recognized that these opinions could be changed by new information, and that their applicability would be limited in many countries by available resources and the possibly greater importance of other health problems.

Question 1. *What changes, within practical limits, should be made in the International Classification of Disease (ICD) to permit more accurate notifications of morbidity and mortality attributable to type A and other forms of hepatitis?*

Answer. Unfortunately, the true incidence of overt viral hepatitis in the world is unknown. It is recognized that there is considerable under-reporting in most countries. Wide variations in completeness of reporting are due to differences in reporting systems, access to health services, and epidemiologic and laboratory services for investigation and diagnosis. In addition, there is sometimes hesitation among physicians to report because of the threat of restrictive measures that might follow notification. Nevertheless, type A hepatitis is a major cause of morbidity in developed countries, and is likely to become increasingly important in developing countries as levels of hygiene improve and the age at infection rises. Therefore, the frequency with which the disease occurs, and the trend in incidence, need to be defined for the purpose of planning preventive measures.

In most countries in which viral hepatitis is a notifiable disease, cases are classified as a single nosologic entity. The last edition of the ICD (1977) specifies the following subcategories under rubric 070:

Viral hepatitis A with hepatic coma.
Viral hepatitis A without hepatic coma.
Infectious hepatitis.
Viral hepatitis B with hepatic coma.
Viral hepatitis B without hepatic coma.
Serum hepatitis.
Other specified hepatitis with hepatic coma.
Other specified hepatitis without hepatic coma.
Unspecified hepatitis with hepatic coma.
Unspecified hepatitis without hepatic coma.
Viral hepatitis unspecified.

This classification is obviously cumbersome and includes outdated terms. It is hoped that the next revision of the ICD will provide a simplified and contemporary set of designations. For the interim, it is suggested that countries adopt the following categories that are compatible with present knowledge and laboratory capabilities.

Viral hepatitis, type A.
Viral hepatitis, type B.
Viral hepatitis, non-A, non-B.
Viral hepatitis, type unspecified.

Cases should be individually reported rather than as collective numbers seen within a specified time (e.g. each week). Specific laboratory diagnosis of the etiologic type is desirable, but it is recognized that this is now feasible only to a limited extent, even in countries in which

testing services are available. The epidemiologic background will undoubtedly continue to be used on a presumptive basis, even though there is evidence that it is notably unreliable in sporadic cases. Whenever clusters or groups of cases are investigated by health authorities, an etiologic classification should be attempted even if laboratory confirmation cannot be obtained.

For mortality data, the complexities of certifying the the cause of death, and the many inaccuracies in diagnosis, make estimation of cause-specific rates meaningless in most countries.

There should be continuing attention to the adequacy of classification systems in use, and increasing reliance on laboratory procedures.

Question 2. *With regard to tests for specific etiologic diagnosis, are there relative priorities among cases of overt hepatitis for utilization of these procedures?*

Answer. Wherever appropriate laboratory capabilities exist, tests for specific diagnosis of hepatitis due to HAV, HBV and, by exclusion, NANB viruses should be used. The benefits include: (a) a better definition of prognosis in individual cases; (b) a basis for management of contacts; (c) improved accuracy of reporting; (d) definition of epidemiologic patterns, and (e) definition of the need for vaccines.

In addition to overt cases, testing to identify susceptible persons may be desirable as a guide to IG prophylaxis: (a) among members of closed populations in which HAV infection is readily transmitted, and for personnel that provide services to them; (b) for frequent travellers from areas of low risk to countries with a high level of infection, especially when an extended stay is planned.

Question 3. *With regard to tests for specific etiologic diagnosis, what procedures should be recommended for routine use, taking into consideration specificity, sensitivity, cost, and availability?*

Answer. For detection of anti-HAV in serum, both RIA and ELISA are recommended for their specificity and sensitivity. For diagnosis of acute cases, modification is needed so that only the high level of IgM associated with the

acute phase of infection gives a positive result. Either RIA or ELISA is suitable for epidemiologic surveys, as is IAHA for laboratories able to obtain HA Ag; IAHA has the advantage of low cost.

The immuno-electron microscopy (IEM) test for HAV in feces is an important research tool, but is not practical as a diagnostic procedure. The same is true for RIA and ELISA procedures adapted to fecal specimens, but IEM is felt to be more meaningful because it detects viral particles rather than HAV antigens. Routine diagnosis, however, is easily accomplished by serologic procedures.

The development of a sensitive cell line for culture of HAV will be very important for studies of the pathogenesis and epidemiology of HAV, but it may not be practical for routine diagnostic purposes because of the time required to obtain a result.

Question 4. *Maximum usefulness of any serologic survey to define the epidemiologic patterns of HAV infection in a population requires that its results can be readily compared to those of other surveys in different populations. To assure such comparability, what recommendations can be made concerning the characteristics to be defined, the age groupings to be used, and the methods of testing to be preferred?*

Answer. To attain comparability in serologic surveys in different populations, the following recommendations are made:

(a) Population samples should be similar in age distribution, socioeconomic status, and level of hygiene, even within a given country. Other variables, where identified, should also be used in describing populations. These include environmental sanitation, number of occupants per room, population density, educational level, cultural pattern, and occupational exposures.

(b) All age groups should be tested, and it is suggested that estimating the age by which 50 percent of the population have developed anti-HAV may be a useful index. In developing countries, therefore, the emphasis must be upon young children, with no more than two to three years of life being represented in reports of surveys of this group. The WHO Technical Report on Serum Banks and Multi-

purpose Serologic Surveys (No. 454) is recommended as a guide.

Sampling specifically designed on the basis of what is known about HAV epidemiology is most desirable but such surveys are costly and difficult to do. Where sera are already available, they can be useful for HAV testing. Such samples may be found in the WHO Serum Bank, as well as the national public health laboratories and infectious disease research institutes of various countries. When existing collections of sera are tested for anti-HAV, it is very important to include in the report all available information concerning the original reason the collection was made, the method of sampling used, and pertinent characteristics of the area and the population.

Question 5. *Can the terms "endemic" and „hyperendemic" be appropriately applied to hepatitis A virus infection? If so, what are their definitions?*

Answer. The term "endemic" implies that an agent is able to sustain itself in a population on an indefinite basis without reintroduction. For example, it has been well-demonstrated that in relatively isolated communities such as arctic and subarctic towns and villages, HAV is not capable of sustaining itself indefinitely because there are no chronic infections. On the other hand, in South Pacific islands approximately comparable in size, serologic surveys show continuing infection in young children. The latter occurrence is probably due to the fact that social exchange, trade, and tourism result in inter-island transmission. Thus, the term "endemic" is perhaps not appropriate for any one island, but may be applicable to groups of islands as ecologic entities. The fact that sanitation in such areas is often very poor permits social interactions to result in spread after reintroduction much more easily than would occur under better hygienic conditions. There have been a few reported instances, however, of apparent endemicity in small, almost completely isolated communities, attributed to HAV persistence in a heavily contaminated environment; the exact mechanism remains to be explained in such situations. In some highly developed areas where HAV was formerly endemic, such as Scandinavia, occurrence of the agent is now essentially limited to re-

introductions. Because of a high socio-economic level, very few secondary cases follow except in certain population subgroups (e.g. male homosexuals and drug addicts).

"Hyperendemicity", as a subcategory of endemicity, is a frequently used but ill-defined term. Most commonly, it implies that a very high proportion of individuals in the population, especially young children, have serologic evidence of experience with HAV; it also suggests that the agent is being transmitted more or less continuously. Hyperendemic has long been used to describe areas in which clinical hepatitis, especially with jaundice, is rare, but risk for susceptible visitors is high. It would be preferable to describe this situation quantitatively by stating the known or presumed age-specific prevalences of anti-HAV.

Question 6. *There is an apparent discrepancy between the low frequency with which clinical hepatitis is recalled by persons with a positive test for anti-HAV, and the relatively low subclinical to clinical case ratios ($< 2 : 1$) in studies of epidemics in the United States and Europe. How can one reconicle the presumed contradiction?*

Answer. The subclinical to clinical ratio undoubtedly varies substantially with the age group, and perhaps with the size of the infecting dose. Nonetheless, it is probable that many persons have clinical illness in which the diagnosis is not even suspected except in an investigational setting.

Question 7. *What applications of cell cultures should be given priority as the sensitivity of these systems is improved?*

Answer. Cell culture now shows promise as a means of detecting infectious HAV, and hopefully can be substituted for experimental animals. The sensitivity of such cultures in comparison with established models in the marmoset and chimpanzee, however, has not yet been established. When this stage is reached, several questions should be investigated: (a) the shedding of HAV by patients and experimentally infected animals; (b) the pathogenesis of type A hepatitis in both man and experimental animals; and (c) possible non-human sources of HAV infections.

The time between infection and the beginning of fecal viral excretion (latent period) should be defined in relation to onset of disease. For icteric illnesses, a common reference point is the first appearance of dark urine. In anicteric disease, the onset of first symptoms is useful, and in subclinical cases the initial elevation of enzymes must suffice. Suitable groups for study would include persons exposed in a common source outbreak and close contacts of patients.

Also to be suggested is the relationship between excretion of infective HAV and detectability of fecal HA Ag.

To explore possible modes of transmission other than the fecal-oral route, saliva, pharyngeal swabs, blood, and other body fluids should be studied.

In studies of pathogenesis, cell culture will help identify the site(s) of HAV replication. The animals of choice for such studies at present are the several susceptible species of marmosets, but additional models may be found among the families of the suborders monkeys and apes because various non-human primates have been shown to have anti-HAV reactivity.

Infective HAV should be sought in sewage (concentrated by established methods), in bi-valves, and in other vehicles suspected of being responsible for infection. Such investigations will contribute to better understanding of the ecology of the virus.

Question 8. *Given a case of illness clinically consistent with viral hepatitis, what precautions should be observed initially to minimize transmission of the agent from the case? What precautions should be maintained for how long when (or if) it is evident (or very probable) that the agent is HAV?*

Answer. Most cases of acute viral hepatitis of all etiologic types can be adequately cared for at home and do not require hospitalization. Those patients who are hospitalized with this diagnosis, including type A hepatitis, can be managed in general-care hospitals and do not require admission to special infectious disease units.

For many years two categories of isolation have been used for patients hospitalized with hepatitis: *enteric precautions* and *blood precautions*. In the past, both sets of procedures were applied to all cases because of inability to diagnose the etiologic form. It is now appreciated that by the time a person ill with type A hepatitis is hospitalized for that disease, the level of HAV in stools is low. There is the possibility that HAV could be transmitted by direct contact with feces, but the precautions used when handling excretions of *any* patient are sufficient for persons with a presumed or documented diagnosis of type A disease. The emphasis should be on blood precautions because all three etiologic forms can be transmitted by its introduction through broken skin or across mucous membranes.

Extraordinary measures are not warranted. These have sometimes included placing hepatitis patients in private rooms regardless of age or fecal continence; routinely requiring all staff to wear gowns and gloves for casual patient contact; and the use of high level disinfectants (e.g. formaldehyde or gluteraldehyde) for general housekeeping.

Specific guidelines pertinent to infection control strategies and use of gloves, masks, gowns, etc., have been published (Favero, M. S., Maynard, J. E., Leger, R. T., Graham, D. R., Dixon, R. E.: Guidelines for the care of patients hospitalized with viral hepatitis. Annals of Internal Medicine 1979, 91: 872–876).

Question 9. *What recommendations should be made concerning presently available IG preparations in the prophylaxis of HAV infection?*

Answer. It would be preferable to be able to discuss dosage of IG in terms of international units, and WHO and other appropriate agencies should work toward this goal. In the meantime, it is suggested that manufacturers state on the label of each lot a titer and the method of titration (e.g. 1:200 by RIA). At present, suggestions concerning dosage can only be based on recommendations derived from monitored experience, such as those of the Advisory Committee on Immunization Practices in the United States.

In areas in which the population has a high rate of susceptibility, all household contacts of a case should receive an appropriate dose of IG as soon as possible after recognition of the index case. The use of IG more than two weeks after exposure is not indicated, however, because the protective efficacy in the last half of the

incubation period is low. Persons other than household members should be given prophylaxis only if they have very close contact with an infected person.

IG should be considered for persons from areas of low HAV endemicity when they travel to, or live in, an area for which epidemiologic evidence indicates that HAV infection is acquired with a significant frequency. It is suggested that at least 2.0 ml (or 0.03 ml/kg) be given to adults, and repeated every three to four months if exposure continues. An alternative procedure is to give a larger initial dose (0.06 mg/kg) and repeat that amount after four to six months. General experience has suggested that with most preparations these doses are protective. Persons working in an institutional setting may need IG when HAV infections are occurring, depending upon epidemiologic circumstances. With tests for anti-HAV usually available, however, testing for past acquisition of active immunity will undoubtedly demonstate that many persons to whom repeated doses of IG are given for pre-exposure protection do not need this measure.

In the event of an extensive common vehicle outbreak, a community-wide programme of IG prophylaxis is not indicated if clinical cases have already begun to occur. On the other hand, if epidemiologic evidence suggests that the vehicle has continued to be contaminated, so that some specifically exposed persons may have newly acquired infections, an attempt at passive protection may be worthwhile.

Question 10. *When and if an effective HAV vaccine becomes available, what public health priority should be assigned to its use, considering the lack of chronicity, the low case fatality rate, and, in many countries, the declining incidence of the disease? How, if at all, would such priority be affected by the type of vaccine – i.e. an inactivated compared with a live-virus preparation?*

Answer. The need for a safe, effective hepatitis A vaccine is recognized by all participants. A major point favoring vaccine development is the observation that all strains of HAV are so closely related antigenically that one strain should protect susceptibles in all areas of the world. To be useful, either type of HAV vaccine should have an acceptably low level of side effects, and produce long-lasting immunity with little decline in anti-HAV levels in later life. An effective vaccine could have a major impact on the elimination of epidemics of type A hepatitis in high-risk populations.

Several populations can be identified as potential recipients of a hepatitis A virus vaccine. Additional epidemiologic data, however, are required to define these completely and to recognize other possibly appropriate groups. In areas with high rates of HAV infection, vaccination, to be effective, would have to be carried out before the ages in which risk is maximal. This probably should be no earlier than 10 to 12 months of age because of the possible inhibitory effect of maternal anti-HAV.

In areas of low incidence the following high-risk groups should be considered for immunization: (a) Individuals going for short-term or long-term visits to areas of known hazard, including vacationers, business people, academic and scientific investigators (especially anthropologists and other field workers), members and staff of the diplomatic corps, and military personnel; (b) residents and employees of institutions in which conditions promote the transmission of HAV, such as homes for the developmentally disabled and prisons; (c) children and employees at day-care centers and pre-school nurseries (which have become increasingly important as foci for epidemics of type A hepatitis); (d) persons in various occupational groups who are at increased risk of HAV exposure such as health-care workers, plumbers, and sanitation workers; (e) persons working at jobs that provide the opportunity for large-scale transmission to others, such as food-handlers; (f) persons whose social behavior facilitates transmission, such as male homosexuals and drug addicts; and (g) during epidemic waves of type A hepatitis, persons in communities in the path of geographically advancing infection.

The feasibility of producing an inactivated vaccine that provides protection against experimental inoculation has been demonstrated in marmosets using a heat/formalin-treated antigen derived from marmoset liver. This prototype inactivated vaccine conferred complete protection against challenge with live virus. The demonstration of HAV groups in cell culture systems already certified for vaccine production has increased the likelihood

that either a killed or attenuated live-virus vaccine can be produced for human use.

Several conditions must be met before a vaccine can be accepted for non-experimental use:

(a) Manufacture of an inactivated vaccine will require techniques for production of large amounts of potent, stable antigen.

(b) Any HAV strain selected for a live virus vaccine must have demonstrable genetic stability as measured by specific serologic and/or in vitro markers.

(c) If there are extra-hepatic intestinal or lymphatic sites of HAV replication, an ideal vaccine would be one that has replication confined to these tissues.

(d) There needs to be much better understanding of humoral and cell-mediated immune responses to HAV, including definition of the antigen(s) which induce protective immunity and of other antigens (if any) which may contribute to liver injury.

Priorities for use of hepatitis A vaccine can only be set by public health authorities of individual countries and will depend on their perceptions of type A hepatitis as a threat to particular groups or the general population. Nevertheless, it can be predicted that the availability of a safe and effective vaccine at reasonable cost could lead to a marked reduction in economic hardship in persons in the workforce, and to reduced morbidity from an infectious disease that is important throughout the world.

Question 11. *What are the important gaps in our knowledge of the characteristics of HAV itself?*

Answer. There is convincing evidence that HAV shares many characteristics of picornaviruses. The available information, however, does not allow final taxonomic classification. Studies should include the following: (a) detailed analyses of HAV RNA and its comparison with the genomes of accepted picornaviruses; (b) further detailed analysis of the structural proteins, including the mechanism of their synthesis and their presence in various types of HAV particles; (c) additional information on the origin, composition, and function of virus particles that vary in their buoyant densities; (d) resistance of HAV to various pyhsical and chemical treatments with respect to in-

fectivity, antigenicity, and structural integrity; (e) host-cell-virus interrelations; and (f) genetic markers of viral attenuation.

Question 12. *What are the important gaps in our knowledge of the pathogenesis of infection?*

Answer. The most important questions concerning pathogenesis of HAV infection and type A hepatitis include: (a) possible portals of entry other than oral (e.g. the conjunctival mucosa); (b) the site of primary replication; (c) the mechanism of entry into the blood; (d) whether viremia is cell-free or cell-associated; (e) sites of subsequent replication other than liver; (f) the mechanism(s) of hepatocellular necrosis; and (g) whether there is shedding other than in the feces, and the tissue sources of HAV excreted by each mode demonstrated; (h) the mechanism of suppression of viral replication; (i) whether HAV can persist in one or more tissues; and (j) whether there can be recrudescent shedding, or even disease.

Question 13. *What are the important gaps in our knowledge of the mechanisms of post-infection immunity?*

Answer. The questions to which priority should be given include the following: (a) the development and role of cell-mediated immunity in disease and in body defense; (b) the deveopment and role of humoral and secretory antibodies in disease and recovery; and (c) the persistence and importance of various classes of humoral and fecal antibody.

To find answers to these questions will require observation of infection with wild HAV in appropriate animal models, and of attenuated infection in humans. There is an urgent need for additional non-human primate models of HAV infection and disease. Meanwhile, the establishment and maintenance of primate colonies, particularly breeding colonies of marmosets and chimpanzees, should have the highest priority.

The non-systematic observations of anti-HAV reactivity in some non-primate animals should be verified with respect to specificity, and the susceptibility of such animals should be re-evaluated accordingly.

Question 14. *What are the important gaps in our knowledge of the ecology of HAV and the epidemiology of HAV infection?*

Answer. The most important question is how HAV manages to perpetuate itself so that it can be most effectively attacked by all available approaches. If there is not an important non-human natural host, then there must be a continuing chain of transmission from person to person. The circumstances permitting such persistence, however, remain to be defined. Perpetuation would be favored by prolongation of the links in the chain, such as continued shedding, recrudescent shedding, and/or persisting survival in a contaminated environment.

Question 15. *Considering the research tools that may be available, and the relative importance of the knowledge to be gained, what types of studies appear especially promising?*

Answer. Attention should be called to the careful epidemiologic studies in progress in Costa Rica. Similar investigations should be undertaken in communities with differing levels of endemicity to determine the important mechanisms of transmission, and patterns of transmission within households and communities.