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Seroepidemiologic Investigations of Human Hepatitis Caused by A, B, and a Possible Third Virus[1] (39432)

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The recent development of the immune adherence (IA) assay (1-3) for antibody against human hepatitis A together with established assays for hepatitis B antigen and antibody have made the conduct of seroepidemiologic investigations of these diseases possible. Evidence for a possible third agent other than type A or B and causing epidemic hepatitis in man has been presented (4). This report summarizes additional results of seroepidemiologic studies of hepatitis in human subjects in Costa Rica and in the United States.

Materials and methods. Human sera. The subjects in Costa Rica were residents of a community in which endemic hepatitis A and B occur and this has been under intensive epidemiologic investigation during the past 8 years (5, 6). The subjects in the United States were persons in the open community (Table I, Pennsylvania, high socioeconomic) and persons from selected populations (Table II), including plasmapheresis donors and employees or residents of institutions for the mentally retarded.

Serologic tests. The IA test for hepatitis A antibody was carried out as previously described by us (1), following the procedure of Mayumi *et al.* (7) as used for assays for hepatitis B antibody. A microtiter procedure was used, and each component was added in 0.025-ml vol. CR326 strain of hepatitis A virus, partially purified from the livers of infected *Saguinus mystax* (marmosets) served as antigen (1); 2-4 units were used in antibody assays. Complement was serum from Hartley-strain guinea pigs and was used at 1:50 to 1:100 dilution. Complement was stored at −70°. Human sera to be assayed for antibody were heated at 56° for 30 min and were assayed in serial twofold dilutions starting at a dilution of 1:5. The antigen-antibody mixtures were incubated at 37° for 1 hr. Complement was then added with thorough mixing. This mixture was further incubated at 37° for 40 min. Dithiothreitol solution (7) was then added to all wells with thorough mixing. This was followed by the addition of suitable human O, Rh-negative erythrocytes (1% suspension). This was thoroughly mixed and set for 3 hr at room temperature. Antibody titer was read as the highest dilution of serum (prior to addition of other reagents) at which complete erythrocyte agglutination was seen.

Tests for hepatitis B antigen and antibody and the biochemical tests for liver function were done as reported earlier (4). All sera were stored at −20° until assayed.

Results. Hepatitis A antibody distribution. The distribution of hepatitis A antibody, according to age, among persons in the open population in the United States and Costa Rica is shown in Table I. The Pennsylvania sera were from persons in high socioeconomic groups in the Philadelphia area who had been subjects in investigations of immune response to various vaccines other than hepatitis. The Costa Rican sera were from persons in the open community who were selected at random. It is seen that none of the persons in Pennsylvania who were less than 20 years of age had antibody against hepatitis A. Ten percent of the young adult group (20-29 years) had antibody, and this increased to approximately 40-50% in persons of middle age. By contrast, 20% of persons 1 year of age sampled in Costa Rica had hepatitis A antibody and this was increased to roughly 45% by age 2 to 3 years. By 10 years of age, 90% or more

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TABLE I. AGE DISTRIBUTION OF HEPATITIS A IA ANTIBODY IN PERSONS IN OPEN POPULATIONS, PENNSYLVANIA AND COSTA RICA

Age (years)	Proportion seropositive			
	Pennsylvania (No.)	(%)	Costa Rica (No.)	(%)
1	0/18	0	4/20	20
2	0/17	0	10/20	50
3	0/16	0	8/20	40
4-6	0/22	0	38/60	63
7-9	0/16	0	48/60	80
10-14	0/18	0	19/20	95
15-19	0/20	0	19/20	95
20-29	2/20	10	18/20	90
30-39	6/21	29	18/20	90
40-49	10/17	59	18/20	90
50+	5/12	42	16/20	80

TABLE II. OCCURRENCE OF HEPATITIS A IA ANTIBODY AMONG PERSONS FROM SELECTED POPULATIONS IN PENNSYLVANIA

Population	Age (years)	Serostatus	
		Positive/total	Positive (%)
Adult residents at Hamburg State School and Hospital	21-51	9/10	90
Employees at Laurelton State School and Hospital	19-63	19/30	63
Plasma donors, Philadelphia	≥18	29/50	58
Child residents at St. Joseph's Home, Scranton, and Hamburg State School and Hospital	1-8	0/20	0

of the persons in Costa Rica had experience with the hepatitis A virus.

The presence or absence of antibody among persons in the United States was further investigated (Table II) in subjects who were institutionalized, who were employees of institutions, or who were paid donors of plasma. The highest rate for antibody presence was among adult residents of the Hamburg State School and Hospital. Adult employees of a similar institution also showed a high frequency of previous experience with the virus as did donors. None of 20 young children (1-8 years of age) in two institutions for the mentally retarded had had experience with this agent.

Family epidemics of hepatitis, Costa Rica. Table III presents the results of investigations for hepatitis A and B in outbreaks in seven families in Costa Rica. Within the time periods shown, hepatitis A alone occurred in one family (033), hepatitis B alone in another (039), and both hepatitis A and B occurred in the five other families. Hepatitis A never occurred in persons with preexisting hepatitis A antibody, and hepatitis B did not occur in persons who had preexisting antibody against hepatitis B. Once introduced into a household, both hepatitis A and B appeared to be highly contagious, usually involving one to several susceptible members of the family within a period of a few months. Five cases of hepatitis occurred that did not develop antibody against hepatitis A or B virus and were possibly caused by agent(s) (hypothetical hepatitis C virus) other than the two known agents (4).

Table IV shows that the illness was clinically apparent in roughly half of the hepatitis A and B cases. The diagnosis in subclinical illness was based on significant elevations in transaminases and other biochemical determinations as well as antibody seroconversions. Four of the five cases of hepatitis in which serological evidence for hepatitis A and B was lacking showed subclinical disease and the fifth had anicteric hepatitis.

Table V shows that hepatitis A occurred in persons of very young age and that all but one of the 25 cases had occurred in persons less than 13 years of age (median age, 4 years). By contrast, hepatitis B occurred in persons of a wide range of ages, almost half being in persons 13 years or older. The cases of hepatitis other than A or B were distributed throughout the age spectrum. The difference in age distribution for hepatitis antibody in the families and the general population (Table I) was likely due to the fact that the family groups in whom epidemics occurred were necessarily selected for a high proportion of initially susceptible persons.

Discussion. Hepatitis A shows striking contrast in age distribution for antibody between persons in the open population groups that were studied in the United States and in Costa Rica. The chance for early infection of hepatitis A is high in Costa Rica and infection may occur in half the population by 2 years of age. By contrast, infection of half the population of high soci-

TABLE III. CLINICAL AND LABORATORY FINDINGS IN FAMILY OUTBREAKS OF HEPATITIS IN COSTA RICA

Family		Preexisting hepatitis antibody		Onset of hepatitis			
No.	Age of member	A	B	A	B	Other	Hepatitis
033	8	0	0	12/21/66	–	–	Clinical
	8	0	0	12/27/66	–	–	Subclinical
	9	0	0	1/5/67	–	–	Subclinical
	4	0	0	1/12/67	–	–	Subclinical
	2	0	0	1/12/67	–	–	Clinical
	11	0	0	2/1/67	–	–	Clinical
	10	0	0	2/7/67	–	–	Subclinical
	1	0	0	2/21/67	–	–	Subclinical
	48	+	0	–	–	–	–
	37	+	0	–	–	–	–
039	11	+	0	–	1/2/67	–	Clinical
	10	+	0	–	1/30/67	–	Clinical
	14	+	0	–	2/3/67	–	Clinical
	36	0	0	–	5/28/67	–	Clinical
	3	0	0	–	7/5/67	–	Subclinical
	33	+	0	–	/67–74	–	Subclinical[a]
	8	0	0	–	/67–74	–	Subclinical[a]
	9	+	0	–	–	–	–
	6	0	0	–	–	–	–
	4	0	0	–	–	–	–
	3	0	0	–	–	–	–
065	3	0	0	3/27/67	–	–	Clinical
	5	0	0	4/13/67	–	–	Subclinical
	1	0	0	4–5/67	–	–	Subclinical[a]
	39	+	0	–	6/8/67	–	Subclinical
	13	+	0	–	6/8/67	–	Subclinical
	32	+	0	–	–	–	–
	11	+	+	–	–	–	–
	9	+	+	–	–	–	–
	8	+	+	–	–	–	–
236	9	+	0	–	2/17/68	–	Clinical
	32	+	0	–	4/1/69	–	Clinical
	4	0	0	8/25/68	–	–	Clinical
	3	0	0	10/2/68	–	–	Subclinical
	28	+	0	–	–	–	–
	10	+	0	–	–	–	–
	7	+	0	–	–	–	–
	5	+	0	–	–	–	–
056	9	+	0	–	3/2/67	–	Clinical
	4	0	0	4/20/67	–	–	Clinical
	5	0	0	5/2/67	–	–	Subclinical
	37	0	0	/67–74	–	–	Subclinical[a]
	2	0	0	/67–74	–	–	Subclinical[a]
	40	+	0	–	–	–	–
	14	+	0	–	–	–	–
	13	+	0	–	–	–	–
	10	+	0	–	–	–	–
	7	+	+	–	–	–	–

TABLE III—*Continued*

Family		Preexisting hepatitis antibody		Onset of hepatitis			
No.	Age of member	A	B	A	B	Other	Hepatitis
330	9	0	0	4/6/68	–	–	Clinical
	7	0	0	5/9/68	–	–	Clinical
	12	0	0	5/13/68	–	–	Clinical
	6	0	0	6/3/68	–	–	Clinical
	2	0	0	6/17/68	–	–	Clinical
	6	+	0	–	2/15/69	–	Subclinical
	36	+	0	–	–	4/16/68	Subclinical
	16	+	0	–	–	4/16/68	Subclinical
	12	+	0	–	–	4/16/68	Clinical
	5	0	0	–	–	5/14/68	Subclinical
008	15	+	0	–	10/11/66	–	Clinical
	7	+	0	–	12/13/66	–	Subclinical
	6	+	0	–	8/28/67	–	Subclinical
	2	0	+	8/20/67	–	–	Clinical
	4	0	0	8/25/67	–	–	Clinical
	3	0	0	8/28/67	–	–	Subclinical
	2	0	+	–	–	11/15/66	Subclinical
	34	+	0	–	–	–	–
	29	+	0	–	–	–	–

[a] No illness reported. Assumed to be subclinical. Diagnosis based solely on seroconversions.

TABLE IV. SEVERITY OF ILLNESS IN HEPATITIS CASES (TABLE III)

	Hepatitis type (No. persons)		
Hepatitis	A	B	Non A, Non B
Clinically evident	13	8	1
Subclinical	12	8	4

TABLE V. DISTRIBUTION ACCORDING TO AGE OF HEPATITIS CASES (TABLE III)

	Hepatitis type (No. persons)		
Age (years)	A	B	Non A, Non B
1–3	9	1	1
4–6	7	2	1
7–9	5	4	0
10–12	3	2	1
13–16	0	3	1
32–39	1	4	1
Total	25	16	5
Median age	4.0	10.5	12.0

oeconomic level in the United States does not occur until middle age. It may well be that hepatitis A may occur at a younger age in low socioeconomic groups. Adult residents of institutions in the United States and their attendants show a far greater incidence of antibody against hepatitis A than do their counterparts in the open population. Additionally, paid volunteer donors of plasma in the United States (often persons of relatively low socioeconomic standing) also show a relatively high level of previous experience with the agent. Interestingly, young children sampled in two institutions in Pennsylvania were free of antibody against this agent.

The presence of circulating antibody against hepatitis A or B appeared to afford immunity against their homologous agents in studies in family epidemics in Costa Rica. Hepatitis A, known to be of greater epidemic potential than hepatitis B (8), occurred on the average in persons at a younger age than did hepatitis B. Once introduced into the family, hepatitis B and especially hepatitis A viruses appeared to be capable of substantial spread among susceptible members of the group. The disease tended to be very mild (subclinical) in roughly half the subjects and clinically apparent in the other half, whether the illness be due to hepatitis A or B. Four of five patients not classified as A or B exhibited subclinical disease; the fifth case was clinical

but anicteric. Of these five patients, three had preexisting hepatitis A antibody and a fourth had preexisting hepatitis B antibody. None developed detectable hepatitis B antigenemia. None showed a boost in antibody titers against hepatitis A or B in convalescence. Additional information concerning these five cases tentatively classified as hepatitis C has been presented in Villarejos *et al.* (4), cases 1 and 4–7.

Summary. Seroepidemiologic studies were made of normal subjects in populations in the United States and Costa Rica and in family outbreaks of hepatitis in Costa Rica. Hepatitis A affected a majority of children of very young age in Costa Rica, while such experience in persons of high socioeconomic status in the United States did not occur before middle life. Persons of low socioeconomic status (paid plasma donors) and residents and attendants of institutions for the mentally retarded showed a far greater incidence of hepatitis A antibody than did their counterparts in the open community. Hepatitis A and B epidemics occurred in families in Costa Rica with rapid spread to other susceptible members of the group. The disease was clinically apparent in roughly half the cases, whether the responsible agent be hepatitis A or B. Five cases of nonhepatitis A or B (hypothetical hepatitis C) were found and all but one of them were subclinical.

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