Coproantibodies in Hepatitis A: Detection by Enzyme-Linked Immunosorbent Assay and Immune Electron Microscopy

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A collection of 104 fecal specimens from 45 patients with hepatitis A, 14 patients with hepatitis B, 10 patients with non-A, non-B hepatitis, 6 patients with diseases other than hepatitis, and 18 healthy adults were studied for the presence of secretory immunoglobulin A and immunoglobulin M to hepatitis A virus by solid-phase enzyme-linked immunosorbent assay and immune electron microscopy. Specific fecal antibody was found only in patients with hepatitis A. Of 54 specimens from patients with hepatitis A, only 10 (18.5%) possessed detectable levels of fecal antibody, and each of these was collected within 10 days from the onset of dark urine. All 10 fecal specimens contained hepatitis A-specific secretory immunoglobulin A, and 4 were also positive for hepatitis A-specific immunoglobulin M. Four of the 10 antibody-positive specimens also contained hepatitis A virus particles which could be shown by immune electron microscopy to be coated with specific secretory immunoglobulin A. Since specific fecal antibody was not detected in all the patients with hepatitis A that were studied, it would appear to have limited diagnostic value, although its detection is evidence of recent infection.

Hepatitis A is one of the most common and most widespread infections of humans and is caused by a 27-nm naked icosahedral virus which is almost certainly a member of the genus Enterovirus within the family Picornaviridae (3, 4, 13, 20, 23). Whereas considerable information is available on the nature of the humoral antibody response to infection with hepatitis A virus (HAV), to date there have been no reports of the detection of coproantibodies in this disease and their relationship to fecal shedding of the virus. This information is clearly important, as it is now recognized that secretory immunoglobulins play a significant role in protection against many viral infections, particularly those of the respiratory tract and the gut.

This communication describes the development of enzyme-linked immunosorbent assay (ELISA) and immune electron microscopy (IEM) techniques for the detection of hepatitis A-specific coproantibodies and the detection of this class of antibodies in the feces of patients with acute hepatitis A.

MATERIALS AND METHODS

Patients. Five groups of subjects were studied: 45 patients with acute hepatitis A, 14 patients with acute hepatitis B, 10 patients with non-A, non-B hepatitis, 6 patients with diseases other than acute viral hepatitis, and 18 healthy laboratory staff.

All patients were admitted to Fairfield Hospital for Communicable Diseases, Melbourne, Australia, between June 1978 and February 1979. At least one fecal specimen and several serum samples, including paired acute- and convalescent-phase sera, were collected from each patient and sorted at −20°C until tested. In all cases, the diagnoses were made by specialist physicians on the basis of clinical assessment aided by laboratory tests.

Diagnostic criteria. A patient was considered as having hepatitis A if HAV was detected in fecal specimens collected during acute illness (14, 15), if a rising titer of anti-HAV was demonstrated between paired sera (12), if hepatitis A-specific immunoglobulin M (IgM) was detected in acute-phase sera (11, 12), or if any combination of these was observed. Tests for HAV, anti-HAV, and hepatitis A-specific IgM were performed by solid-phase radioimmunoassay (SPRIA) and ELISA as described previously (9, 11, 12, 14). All sera were also tested for hepatitis B surface antigen by SPRIA (Australia II, Abbott Laboratories, North Chicago, Ill.) and found to be negative. The illness in all 45 patients with hepatitis A ran a typical course without complication or sequelae.

A diagnosis of hepatitis B was established if hepatitis B surface antigen was detected in acute-phase sera but absent from sera collected during the convalescent phase of illness. Hepatitis B surface antigen was detected by SPRIA as described above, and all positive results were confirmed by specific neutralization (5).

Patients with acute hepatitis in whom there was no serological evidence of infection with HAV, hepatitis B virus, Epstein-Barr virus, cytomegalovirus, or herpes simplex virus were classified as having non-A,
non-B hepatitis. Tests for infection with Epstein-Barr virus, cytomegalovirus, and herpes simplex virus were performed by standard techniques.

Illnesses other than acute viral hepatitis included alcoholic liver disease, cholelithiasis, and cholecystitis. Laboratory staff included in the study had no observable symptoms 3 days before and 3 days after specimen collection.

**Fecal extracts.** Fecal specimens were collected from the study group, held at 4°C for 4 to 6 h, and then frozen at −20°C to minimize protease digestion of fecal immunoglobulins. All specimens positive for occult blood (Hematest, Ames Division, Miles Laboratories, Australia) were excluded. Fecal extracts were then prepared as 10% (wt/vol) suspensions in phosphate-buffered saline (PBS, pH 7.4) containing 0.1% (wt/vol) sodium azide. After high-speed homogenization of the suspension, coarse particles were removed by centrifugation at 2,000 × g for 30 min at 4°C. The supernatant fluid was further clarified by centrifugation at 20,000 × g for 30 min at 4°C using a type 30 rotor in a Beckman model L5-65 preparative ultracentrifuge. The resulting supernatant fluid was then freeze-dried (Edwards Freeze Dryer, model EF03, England) and stored at 4°C until testing. This procedure increases the yield of fecal antibody (7). Specimens were then reconstituted with distilled water to give the required dilution of the original starting material.

**Reagents. (i) Coating antibody.** The antibody used to coat microtiter plates for the ELISA was prepared from serum collected from an adult male, 4 months after admission to the hospital with serologically confirmed hepatitis A. The IgG component was purified from 10.0 ml of serum by precipitation with ammonium sulfate and ion-exchange chromatography on diethylaminoethyl-cellulose (DE-52, Whatman, England) as described by Purcell et al. (21, 22). Puriﬁed IgG was stored at −20°C.

(ii) **Hepatitis A antigen.** Hepatitis A virus was detected in feces by SPRIA and IEM (14, 22) and then puriﬁed as described previously (3, 10). Briefly, this puriﬁcation protocol involved preparation of a 20% (wt/vol) extract of the feces in PBS, clariﬁcation by low-speed centrifugation, pelleting of the virus, extraction with chloroform, and, ﬁnally, agarose gel ﬁltration on Sepharose CL-2B (Pharmacia Fine Chemicals, Uppala, Sweden).

For ELISA, one sample of puriﬁed virus was treated with an acid buffer to remove any immunoglobulins bound to the virion surface. Puriﬁed HAV in PBS (pH 7.4) was pelleted at 240,000 × g for 2 h at 4°C in an SW60 Ti rotor using a Beckman model L5-65 preparative ultracentrifuge, the supernatant ﬂuid was carefully removed, the pellet was suspended in 0.1 M glycine-hydrochloride (pH 2.8) and incubated at 37°C for 2 h, and the virus was pelleted again as above and then suspended in PBS (pH 7.4).

(iii) **Conjugates.** Anti-human IgM (mu-chain speciﬁc) and anti-human IgA (alpha-chain speciﬁc) prepared in goats were obtained commercially (Hyland, Travenol Laboratories, Melbourne, Australia), and normal goat serum was obtained from the Commonwealth Serum Laboratories, Melbourne. Rabbit anti-human secretory component and normal rabbit serum were also obtained commercially (Dako-Immunoglobulin Ltd., Denmark). Portions of all sera were absorbed several times with polymerized normal human IgG.

The immunoadsorbent polymer was prepared by cross-linking puriﬁed human IgG (Commonwealth Serum Laboratories; Cohn Fraction V, further puriﬁed by ion-exchange chromatography as described above) with glutaraldehyde (Taab, Reading, England) using the method of Avrameas and Ternynck (1).

After adsorption, the IgG component of each serum was separated by ammonium sulfate precipitation and ion-exchange chromatography as described above, and conjugated with horseradish peroxidase (RRZ approxi mately 3.0; Sigma type VI; Sigma Chemical Co., St. Louis, Mo.) by the method used by Nakane and Kawaoi (16).

(iv) **Antisera.** As well as the above antiserum, goat anti-human IgG (gamma-chain speciﬁc) was obtained commercially (Hyland, Travenol Laboratories, Melbourne); all were used in an immunoglobulin/anti-immunoglobulin reaction to test for antibody-coated HAV by IEM (see below).

**ELISA.** (i) The test. The method used was described previously (11, 14). The optimal dilution of reagents used was determined by checkerboard titra tion. The substrate was prepared by dissolving 100 mg of o-phenylenediamine (British Drug Houses, England) in 10 ml of methanol; 2 ml of this solution was then mixed with 98 ml of distilled water and 200 μl of 3% (vol/vol) H2O2 and used immediately.

The wells of polystyrene microtiter plates (Cooke Engineering Inc., Alexandria, Va.) were coated with a 1:1,000 dilution of the human convalescent hepatitis A IgG in 0.85% (wt/vol) saline and incubated for 4 h at room temperature. After washing three times with PBS containing 0.05% (vol/vol) Tween 20 (PBS-T), the wells were ﬁlled with 1% (wt/vol) bovine serum albumin (Commonwealth Serum Laboratories, Melbourne) in saline and left to stand at 4°C overnight. After washing three times with PBS-T, 50 μl of puriﬁed acid-treated HAV suspended in PBS was added to each well, and the plates were incubated at 4°C for 16 h. After washing six times with PBS-T, 50 μl of the test fecal specimen, diluted 1:5 in PBS (pH 7.4), was added to each well and incubated at 37°C for 90 min. The samples were then aspirated, each well was washed nine times with PBS-T, 50 μl of peroxidase conjugate (normal goat serum, goat anti-human IgM, goat anti-human IgA, normal rabbit serum, or rabbit anti-human secretory component, all diluted 1:300 in PBS-T) was added, and the plates were incubated at 37°C for 90 min. Finally, after the conjugate was aspirated and each well was washed six times with PBS-T, 50 μl of the test fecal specimen, diluted 1:5 in PBS (pH 7.4), was added to each well and incubated at 37°C for 90 min. The samples were then aspirated, each well was washed nine times with PBS-T, 50 μl of peroxidase conjugate (normal goat serum, goat anti-human IgM, goat anti-human IgA, normal rabbit serum, or rabbit anti-human secretory component, all diluted 1:300 in PBS-T) was added, and the plates were incubated at 37°C for 90 min. Finally, after the conjugate was aspirated and each well was washed six times with PBS-T, 50 μl of the substrate was added, and the plates were incubated in the dark at room temperature for 30 min. The enzyme substrate reaction was stopped by the addition of 50 μl of 8 N H2SO4. The color produced in each well was recorded visually, using a 0 to 3+ scale: O, no color reaction; 1+, a light to medium color; 2+, a medium to strong color; 3+, a very strong color reaction. A 1+ difference in reading was considered significant (11, 14). To measure any nonspeciﬁc binding to the IgG-coated plates, each fecal specimen
was also tested on a control plate to which PBS-T had been added in place of the purified acid-treated HAV. A specimen was scored positive for fecal antibody if a 1+ or greater color reaction was detected on the test plate while the control plate was negative for color. All positive specimens were then titrated out in PBS (pH 7.4) by decimal titration to determine the endpoint.

In all testing, substrate and conjugate controls were also included on each microtiter plate. For hepatitis A-specific IgM determinations, positive and negative serum controls were included (11). All testing of fecal specimens was performed and read under code, and each specimen was tested twice in duplicate.

Since human IgG was used to coat microtiter plates for the solid-phase ELISA, it was not possible to test specimens for hepatitis A-specific IgG using an anti-human IgG peroxidase conjugate.

(ii) Specific conditions. (a) Hepatitis A antigen. In preliminary testing of the goat anti-human IgA and rabbit anti-human secretory component peroxidase conjugates, HAV purified by agarose gel filtration using Sepharose CL-2B produced unacceptably high background levels. To reduce this, column-purified HAV was further treated with 0.1 M glycine-hydrochloride (pH 2.8) as described above and then suspended in PBS (pH 7.4). This further procedure yielded HAV suitable for the ELISA.

(b) Conjugates. Since commercially available anti-human IgM, IgA, and secretory component antisera often contain traces of anti-human IgG reactivity, immunoadsorption with the human IgG polymer before conjugation was found necessary. After adsorption, the conjugates did not react with the IgG-coated wells.

The peroxidase-labeled normal goat and rabbit IgG conjugates demonstrated no reactivity with any of the test samples or reagents used in the ELISA.

The activity and specificity of the anti-human globulin peroxidase conjugates were tested by reacting them with a plate to which chromatographically purified human IgG, IgM, or IgA (Cappel Laboratories Inc., Cochraneville, Pa.) had been added to varying dilutions (28). The results of these experiments revealed that both the goat anti-human IgM and IgA conjugates were specific and would react when between 5 and 10 ng of homologous immunoglobulin per ml had been used to coat the plates (28).

IEM. (i) Antibody in fecal specimens. For detection of anti-HAV by IEM, 0.5 ml of purified, acid-treated HAV as used in the ELISA was reacted with 0.1 ml of a 1:5 dilution of fecal extract. After incubation at 37°C for 1 h and then overnight at 4°C, the reaction mixtures were centrifuged at 100,000 x g for 90 min. Supernatant fluids were carefully removed, and the deposits were suspended in PBS (pH 7.4). Samples of these pellets were then negatively stained with 4% phosphotungstic acid (pH 7.4) and examined immediately in a Philips EM301 electron microscope at a plate magnification of 57,000. All specimens were examined under code on EM400 mesh grids.

Additional samples (0.1 ml) of the IEM pellets in PBS (pH 7.4) were also reacted with 50 μl of a 1:10 dilution of the following reagents: normal goat serum, goat anti-human IgG, goat anti-human IgM, goat anti-human IgA, normal rabbit serum, rabbit anti-human secretory component, and PBS; samples were then processed for electron microscopy as described above.

(ii) Antibody on fecal-derived virus. Two other fecal specimens containing HAV (Table 1, 709A and 743) were purified by agarose gel filtration using Sepharose CL-2B as described previously (3, 10). HAV was detected in each purified specimen by SPRIA and IEM (14). No other virus-like particles were visualized by IEM.

To test for the presence of antibody bound to the virus, each specimen was then reacted with normal goat serum, goat anti-human IgG, goat anti-human IgM, goat anti-human IgA, normal rabbit serum, rabbit anti-human secretory component, and PBS and processed for electron microscopy as described above.

RESULTS

Antibody in feces. (i) Immunological studies. A total of 104 reconstituted fecal extracts from patients with liver disease and from healthy controls were tested for the presence of hepatitis A-specific IgM and secretory IgA by ELISA using acid-treated HAV as antigen. HAV-specific antibody was found only in fecal specimens from patients with hepatitis A (Table 1). Of 54 specimens obtained from the 45 patients, 10 (18.5%) were positive. These 10 specimens were collected from nine patients. Four of the 10 specimens containing fecal antibody were also positive for HAV by SPRIA and IEM. Fecal antibody was detected up to 10 days after the onset of dark urine. Within this time interval, 10 of the 43 specimens (23%) collected were positive for fecal antibody (Fig. 1). Classification and titrations of the specific immunoglobulin for the 10 positive fecal specimens are shown in Table 2. In one patient, two specimens were collected 4 and 9 days after the onset of dark urine. The first specimen (709A) had high levels of specific secretory IgA and was positive for HAV, whereas the second specimen (709B) had only very low levels of specific secretory IgA and was negative for HAV. Both specimens were negative for hepatitis A-specific IgM. The remaining eight specimens contained hepatitis A-specific IgA, but four were negative for specific secretory com-

| Table 1. Detection of hepatitis A-specific coproantibody in the study group |
|-----------------------------|------------------|-----------------|-----------------|
| Group          | No. of patients | No. of specimens | No. positive for fecal antibody |
| Hepatitis A    | 45              | 54              | 10              |
| Hepatitis B    | 14              | 14              | 0               |
| Non-A, non-B hepatitis | 10              | 11              | 0               |
| Non-hepatitis  | 6               | 7               | 0               |
| Healthy        | 18              | 18              | 0               |
IgA (Fig. 2B) and rabbit anti-human secretory IgA, IgM, and anti-human IgG, rabbit anti-human secretory component, normal goat and rabbit sera, and PBS were reacted separately. After incubation, the complexes were examined in the electron microscope.

In the PBS and normal goat and rabbit serum controls, HAV was seen only as isolated particles or in pairs directly touching each other in a manner identical to that shown in Fig. 3A. When reacted with goat anti-human IgA, large complexes of HAV were visualized, and there was a significant amount of antibody within each complex (Fig. 3B). Similarly, when the two specimens of purified HAV were reacted against the rabbit anti-human secretory component, complexing of HAV was again observed, but the complexes were smaller and the amount of antibody within the complexes was less than observed with the goat anti-human IgA reaction (Fig. 3C). No complexing of virus was observed when the specimens were reacted with the goat anti-human IgG and IgM antisera.

One of the purified specimens of HAV was then treated with 0.1 M glycine-hydrochloride (pH 2.8, readjusted in pH 7.4), and the experiment described above was repeated. After this acid treatment, no complexing of HAV was observed with any of the antisera or control sera. It would appear that secretory IgA does not aggregate virus, but rather coats it.

### DISCUSSION

This present study has demonstrated a specific antibody response, particularly secretory component. Four of the eight specimens contained hepatitis A-specific IgM, and in three of these HAV was also detected.

(ii) **IEM studies.** IEM was also used to detect fecal antibody. Three specimens (specimens 709A, 710, and 722; Table 2) in which specific secretory IgA was detected by ELISA were reacted with the acid-treated preparation of HAV and processed for IEM. In all three specimens, no complexing of HAV was observed. However, individual particles with a "fuzzy" halo around their periphery, as well as particles touching each other in clusters, were visualized (Fig. 2A). These IEM pellets were then reacted separately with goat anti-human IgA, anti-human IgM, and anti-human IgG, rabbit anti-human secretory component, normal goat and rabbit sera, and PBS and reexamined by electron microscopy. Complexing of HAV was observed in all three preparations after reaction with goat anti-human IgA (Fig. 2B) and rabbit anti-human secretory component (Fig. 2C). The size of the complexes and the amount of antibody involved varied in each of the three specimens, but correlated with the ELISA titers in Table 2, i.e., 722 ≤ 710 < 709. Complexing of HAV was also observed when the IEM pellet of specimen 722 was reacted with goat anti-human IgM. However, the complexes visualized were mixed aggregates of virus plus other particulate material presumably derived from the fecal specimen 722 itself. No complexing of HAV was observed after the reaction of the IEM pellets of 709A and 710 with goat anti-human IgM, nor with any of the three fecal IEM pellets after reaction with goat anti-human IgG, normal goat and rabbit sera, or PBS.

### Antibody on fecal-derived virus: IEM studies

Two specimens of feces (709A and 743) collected 4 and 5 days after the onset of dark urine were purified as described in Materials and Methods and examined by direct electron microscopy. There was minimal background debris, and HAV particles were found only as single entities over each grid square (Fig. 3A). A very faint halo could be discerned around some particles. Both specimens of purified HAV were then reacted separately with goat anti-human IgA, IgM, and IgG, and with rabbit anti-human secretory component, as well as normal goat and rabbit sera and PBS. After incubation, the specimens were then examined in the electron microscope.

In the PBS and normal goat and rabbit serum controls, HAV was seen only as isolated particles or in pairs directly touching each other in a manner identical to that shown in Fig. 3A. When reacted with goat anti-human IgA, large complexes of HAV were visualized, and there was a significant amount of antibody within each complex (Fig. 3B). Similarly, when the two specimens of purified HAV were reacted against the rabbit anti-human secretory component, complexing of HAV was again observed, but the complexes were smaller and the amount of antibody within the complexes was less than observed with the goat anti-human IgA reaction (Fig. 3C). No complexing of virus was observed when the specimens were reacted with the goat anti-human IgG and IgM antisera.

One of the purified specimens of HAV was then treated with 0.1 M glycine-hydrochloride (pH 2.8, readjusted in pH 7.4), and the experiment described above was repeated. After this acid treatment, no complexing of HAV was observed with any of the antisera or control sera. It would appear that secretory IgA does not aggregate virus, but rather coats it.
IgA, in the feces of some patients during acute hepatitis A. This antibody was also found to be firmly attached to the surface of virions purified from feces as well as coexisting in feces containing HAV.

Fecal antibody, when detected, does not appear to persist for very long after the onset of dark urine. In general, the earlier the specimen was collected, the higher the titer of specific coproantibody. The disappearance of hepatitis A-specific secretory IgA in patient 709 was quite dramatic. Within 5 days, the virus-specific titer had dropped 100-fold. The duration of the fecal antibody response found in hepatitis A differs from that observed in other viral infections, e.g., poliovirus (8) and echovirus 14 (18). In these infections, virus-neutralizing coproantibody can be detected up to 40 days after the shedding of virus in feces has ceased. It is possible that this discrepancy reflects the different techniques used, or, alternatively, the variations could reflect a fundamental difference in the pathogenesis of the diseases. However, it is possible that we are detecting the tail-end of a specific secretory IgA response and that specimens collected earlier in the illness, i.e., before or at the onset of dark urine, might contain specific coproantibody. During processing of the fecal specimen, a large portion of immunoglobulins are lost as a result of nonspecific binding to the solids or specific binding to virus. Hence, the estimation of immunoglobulins was limited to detection of those that were unbound and in excess of those that were specifically bound.

Antibodies in feces are readily degraded by intestinal proteases generating antibody fragments that are capable of some weak antigen binding in the ELISA (6, 8). In this study, there

Fig. 2. (A) Two HAV particles visualized after reaction of the purified acid-treated virus with fecal extract and processed for IEM. Fine strands can be seen around the particles. (B) An immune complex formed after reacting the IEM pellet shown in (A) with goat anti-human IgA. (C) An immune complex of HAV formed after the reaction of the IEM pellet shown in (A) with rabbit anti-human secretory component. The complex is smaller and has less antibody in the complex than that observed in (B). Bars, 100 nm.
was some evidence that this was occurring. Four specimens were positive for hepatitis A-specific IgA but negative for specific secretory component, implying that IgA fragments and not intact 11S secretory IgA were binding to the hepatitis A antigen used in the ELISA. This phenomenon could also account for the IEM observation that fecal antibody did not by itself agglutinate HAV, and only by further reacting the IEM pellets with monospecific anti-immunoglobulin antisera did aggregation of HAV occur.

The coexistence of specific coproantibody and HAV in feces was observed in four of the positive specimens, and two preparations in HAV purified from feces were found to have secretory IgA attached to their surface. Since antibodies bound to the virus surface appear to be more resistant to enzyme digestion (2, 26), acid dissociation tests should be incorporated into the purification protocols of HAV derived from feces if the virus is to be used in biochemical and serological investigations. This finding could have importance for current serological tests employing HAV purified from feces, since specificity and high background levels have been a problem with hepatitis A serology (11, 14). Because feces collected after the onset of dark urine have been shown to contain antibody-coated virions, acid dissociation may prolong the time after infection during which virus may be recovered from feces. This observation has also been found in infections with poliovirus (8), hepatitis B virus (17), and, more recently, human rotavirus (26, 27). Thus, secretory IgA in the intestine binds to the virus surface firmly, covering the virions and assisting in their elimination. This could account for the very brief shedding of HAV in feces after the onset of dark urine (15).

The ELISA test used in this study could not detect hepatitis A-specific IgG because the wells were coated with human convalescent-phase hepatitis A IgG. The IEM test for fecal antibody did not detect any specific IgG in the three specimens (709A, 710, 722) tested, nor was any specific IgG found on the surface of the two preparations of purified HAV. An unexpected finding involved the detection of hepatitis A-specific IgM in four of the positive specimens. The significance of this antibody in feces is unclear. Since all fecal specimens tested were occult blood negative, the IgM is unlikely to be an overflow from the systemic response, even though significant levels can be detected in serum at the stage of the illness when these fecal specimens were collected (11, 12). Interestingly, the antibody distribution around the particles is again not uniform. Bars, 100 nm.

**Fig. 3.** (A) A direct electron micrograph of HAV from specimen 709A after purification as described in the text. There is negligible background debris, and fine strands can be discerned on the virus particles. (B) An immune complex of HAV formed after reaction of specimen 709A (shown in [A]) with goat anti-human IgA. Most particles are coated with antibody, but it is not uniformly distributed over all the particles. (C) An immune complex of HAV found after reaction of specimen 709A (shown in [A]) with rabbit anti-human secretory component. The particles are not as heavily coated with antibody as in (B), and
a study by Porter et al. (19) of intestinal immunoglobulins in the local immune response to *Escherichia coli* O somatic antigens in gnotobiotic pigs revealed an initial early response of IgM immunocytes in the lamina propria, soon after which IgA cells predominated.

Because immunoglobulins in the alimentary tract are subject to degradation by intestinal enzymes, the concentrations of specific coproantibody in feces cannot accurately reflect the levels at the surface of the intestinal mucosa. Secretory IgA plays an important role in the intestinal resistance to many virus infections (18, 24, 25). Therefore, it needs to be established whether or not the secretory IgA detected in feces neutralizes HAV in vivo and what role this antibody plays in immunity to reinfection. This knowledge will be important for vaccine strategy when live attenuated vaccine becomes available.

Finally, since specific fecal antibody was not detected in all patients with hepatitis A studied, it appears, at this stage, to have limited diagnostic significance, although its detection is certainly a specific marker of acute infection. Improvements in extraction of fecal antibody lost in nonspecific binding might increase its frequency of detection.

**ACKNOWLEDGMENTS**

This work was supported by grants from the National Health and Medical Research Council of Australia.

We are most grateful to the medical, nursing, and laboratory staff of Fairfield Hospital for their cooperation. We also thank Noreen I. Lehmann for assistance with the radioimmunoassay and Ann M. Stratton for excellent technical assistance.

**LITERATURE CITED**


