Search for Ferris's Fecal Antigen and Australia Antigen in Stool Samples Obtained During a Large Outbreak of Infectious Hepatitis (36290)

DAVID L. MADDEN, EARL B. MATTHEW, DALE E. DIETZMAN, JOHN L. SEVER, AND BENEDICT NAGLER

Section on Infectious Diseases, Perinatal Research Branch, National Institute of Neurological Diseases and Stroke National Institutes of Health, Bethesda, Maryland 20014; and Lynchburg Training School and Hospital, Lynchburg, Virginia 24505

Ferris *et al.* (1) recently reported the presence of a precipitating "fecal antigen" in stool extracts obtained from patients with infectious hepatitis. This antigen was detected in 90 of 220 (40.9%) of patients with hepatitis. Of the 220 patients, 13 had Australia (Au) antigen in their sera and 3 of these also had fecal antigen in stool extracts. He also detected this fecal antigen in 5 of 158 (3.1%) of nonhepatitis patients. The fecal antigen was not serologically related to the Au antigen, although electron microscopic studies showed a particle of similar size and morphology.

The results of our attempts to identify fecal antigens or antibodies in 30 patients from a large epidemic of infectious hepatitis are given below.

Materials and Methods. Description of the epidemic. A large outbreak of hepatitis occurred in the Lynchburg Training School and Hospital (LTSH) in Lynchburg, VA. This institution has 3600 mentally retarded children and 1600 employees. The epidemic began in Apr., 1970, and spread throughout the institution until by Sept., 1970, 565 of the patients were affected. Serial samples of serum and stool were obtained at all stages of the disease from patients on several of the highly affected wards. The hepatitis was not associated with an increase in the occurrence of Au antigen in serum from the infected patients. The epidemiology of this outbreak was described in detail (2).

Patients' specimens. From the more than 400 stool samples collected, 42 specimens from 30 patients were selected for this study. These specimens were chosen because they were available for well-defined periods of disease, as judged by serum glutamic pyruvic transaminase (SGPT) enzyme levels and clinical signs. Twenty-two patients had hepatitis, 3 of whom were Au antigen carriers. Of the remaining 8 patients, all had been exposed to the infection but did not develop clinical or laboratory signs of infection; 4 of these patients were Au antigen carriers. The sequence of sample collection is shown in Table I. The stool samples were collected in dry cups and frozen at —70° until examined about 3 months later.

Serum specimens, obtained from these same patients prior to the development of the disease, at the time of acute SGPT enzyme rise, and 1, 2, 3, 4, 6, and 8 weeks later, were studied. In each case an aliquot of each sera was inactivated at 56° for 30 min and stored at —20°.

A vial of the commercial gamma globulin administered to the patients and staff of LTHS (Hyland Laboratories, Costa Mesa, CA, Lot No. 0442H992C1 - Sep-8-72) during the epidemic was obtained and tested for the presence of Au antigen, Au antibody, Ferris' antigen, and antibody and antigens in the stool extracts obtained from the LTHS patients.

Preparation of stool extracts. Extracts were prepared by a method similar to that described by Ferris *et al.* (1) except that Eagle's minimum essential medium (EMEM) containing 100 units of Mycostatin, 0.04 mg of Achromycin, 4000 units of penicillin, and 0.4 mg of streptomycin/ml was used in place of the Hanks' solution which contained antibiotics. Briefly, a 20% suspension was made

TABLE I. Reaction of Stool Samples with Fecal Antibody Obtained from Dr. Ferris.

No. of patients	Diagnosis of disease	No. of samples	Time samples obtained: Before SGPT	At time of SGPT	Post-SGPT (2–3 weeks)
19 Au neg	8 Clinical	12	—	0/7[a]	0/5
	11 Subclinical	17	0/1	0/8	2/8
3 Au pos	1 Clinical	1	—	—	0/1
	2 Subclinical	4	—	0/2	0/2
4 Au neg	4 No disease	4	0/4	—	—
4 Au pos	4 No disease	5	0/5		

[a] One patient died shortly thereafter with acute yellow atrophy.

in the EMEM and clarified by centrifugation at 1400*g* for 15 min. The supernatant was further centrifuged at 26,000*g* for 20 min in a Serval RC-2B refrigerated centrifuge. The supernatant from the second centrifugation was used as the antigen and is referred to as the stool antigen. It was stored at —20°.

Reference reagents. Two fecal antigen samples and a specific antiserum produced in guinea pigs were kindly supplied by Dr. Ferris of Melbourne, Australia.

The Au antigen and antibody used in these studies were tested for specificity against known reference Au antigen and antibody provided by Dr. Robert Purcell, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD. The Au antigen had a titer of 1:4 using gel diffusion (gel) and a titer of 1:512 with complement fixation (CF) (3). The Au antibody was concentrated 3× by ammonium sulfate precipitation and had a gel titer of 1:2; it was highly anticomplementary in the CF test.

Serum from 14 people with hemophilia was obtained from Dr. H. Fallon and Dr. O. Roberts, University of North Carolina School of Medicine, Chapel Hill, NC. Six of the sera had varying levels of Au antibody; none had Au antigen.

Immunodiffusion precipitation test. Agarose-gel precipitation plates with a center well surrounded by 6 outer wells were obtained from Dr. Henry Bloom and Mr. Don Alger, Benton and Dickens Laboratories, Baltimore Biological Laboratories, Baltimore, MD. Each well contained 20 μl of fluid. The inoculated plates were incubated at room temperature in a moist container up to 10 days and were examined for the development of precipitation lines after 1, 5, and 10 days.

Results. The fecal antigen and homologous antisera provided by Dr. Ferris gave a clear, specific band (Fig. 1). As indicated in Table I, stool extracts obtained from 2 of the 22 LTSH patients with hepatitis gave a precipitation line when reacted with the antisera obtained from Dr. Ferris. One of the 2 reacting stools (called fecal antigen-1) gave an identity reaction with the Ferris fecal antigen (Fig. 1); the other (called fecal antigen-2) did not. In both cases, the antigen was found in stool samples obtained 2 or 3 weeks after

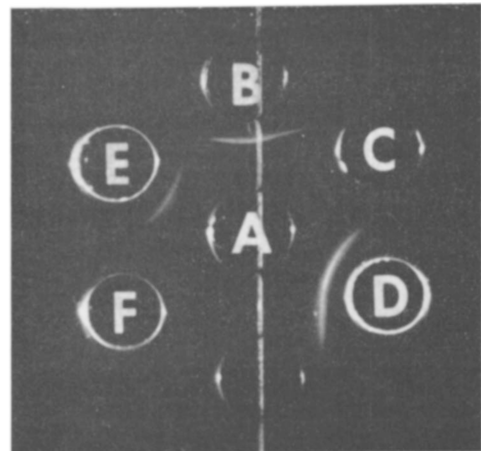

FIG. 1. Reaction of Ferris' fecal antigen and antibody with stool samples obtained from patients at Lynchburg: (A) Ferris' fecal antibody; (B) Ferris' fecal antigen; (C and D) fecal antigen-2; and (E and F) fecal antigen-1.

TABLE II. Reaction of Stool Samples Against Au Antigen, Au Antibody, Hemophiliac Sera, and Gamma Globulin.

Serum sample	Reaction against various stool samples					
	Ferris antigen	Lynchburg stool antigens				
		1[a]	2[a]	3[b]	4[c]	5[c]
Ferris antibody	+	+	+	—	—	—
1 Standard Au antigen	—	—	—	—	—	—
1 Standard Au antibody	—	—	—	—	—	—
6 Hemophiliac Au antibody	—	—	—	—	—	—
6 Hemophiliac no Au antibody	—	—	—	—	—	—
1 Hemophiliac no Au antibody	—	+	—	—	—	—
1 Hemophiliac no Au antibody	—	—	—	+	—	—
Gamma globulin	—	+	—	—	+	+

[a] Clinical disease.
[b] Au antigen carrier, no disease.
[c] Subclinical disease.

the initial evidence of disease was detected by a rise in the SGPT enzyme. These antigens were not detected in stool samples obtained from these same 2 patients 2 weeks earlier. Both patients had subclinical hepatitis as detected by weekly SGPT determinations; neither had jaundice, bile in the urine, nor changes in stool color or consistency. Both patients lived on the same ward which had an attack rate of 65%. No antigen was detected in 11 other stool samples obtained from patients on that ward during the same period. Neither patient had detectable Au antigen or antibody in their sera. No antigen which reacted with the antisera obtained from Dr. Ferris was detected in the stool samples from the other 20 diseased patients or from the 8 nondiseased patients.

Serial serum specimens from the patients with our fecal antigen-1 and -2 did not show precipitating antigen or antibody when tested with the reference antigen or antibody received from Ferris or against our fecal antigens-1 or -2. Additional sets of serial samples obtained from 8 patients did not have precipitating antibodies against fecal antigens-1 and -2. Serial serum specimens from these same 10 patients did not have precipitating antigen or antibodies against 5 other fecal antigens.

Neither the Au antigen nor antibody reacted with any of the stool samples, thus indicating that detectable levels of Au antibody or antigen were not present in these stools.

Serum from 2 of the hemophiliacs, each without Au antibodies (see Table II) reacted with different stool samples. One of the sera reacted with fecal antigen-1, however, it did not react with the reference antigen received from Ferris. The other serum reacted with a stool sample which was designated fecal antigen-3. The other 12 hemophiliac sera, including 6 with Au antibody, did not react with any of the stool samples.

The lot of gamma globulin used during the LTHS epidemic did not contain Au antigen or antibody. It did react with 3 fecal samples, one of which was fecal antigen-1; the other 2 fecal samples were designated antigens-4 and -5. The gamma globulin did not react with the Ferris reference antigen. The 3 precipitation lines formed were not identical.

Discussion. The present study was undertaken in an attempt to detect Ferris' fecal antigen in the stools of patients exposed to infectious hepatitis. Antigen was detected in 2 of 34 stool samples (6%) obtained from 22 patients with detectable hepatitis and in none of 9 stool samples obtained from 8 institutionalized individuals without detectable hepatitis. This low incidence is twice that reported by Ferris in nonhepatitis controls, but is quite different from the 40.9% which was reported from his patients with infectious hep-

atitis (1). Only 1 of the 2 antigens was identical with Ferris' antigen. Both antigens were detected at the peak SGPT enzyme rise, but not at the time of the initial enzyme elevation in one, or prior to it in the other. The low incidence of this antigen seems to indicate that it was not associated with the large outbreak of infectious hepatitis at the Lynchburg Training School and Hospital.

Neither Au antigen, Ferris' antigen nor Au antibody was detected in the 10 stool samples obtained from 7 patients who had Au antigen in their sera. No serological relationship between Au antigen and antibody and Ferris' fecal antigen and antibody was demonstrated. Cossart and Vahrman (4) reported that they did not find Au antigen in stool samples from patients with acute serum hepatitis. Grob and Jemelka (5) reported Au antigen with subsequent developing antibody in feces obtained from patients with acute Au antigen associated hepatitis. Krugman and Giles (6) have reported *per os* transmission via infected serum. Lander *et al.* (7) have suggested that in order to account for the high percentage of Au antibody carriers, as determined by the radioimmunoprecipitation test, nonparenteral transmission must occur. In order to fully understand the epidemiology of this disease, the important question of fecal Au antigen needs to be clarified.

Summary. A precipitating antigen, which reacted with the fecal antibody identified by Ferris, was detected in the feces of 2 of 22 patients with infectious hepatitis and in none of 8 patients who were exposed during the epidemic. Serial serum specimens from these patients and 8 other infected patients did not show precipitation reactions with these 2 fecal antigens or 5 other fecal preparations. Neither Au antigen nor Au antibody was detected in the stool samples from serum Au antigen carriers. No precipitating antibody was found among 14 sera from hemophiliac patients which would react with a significant number of stool specimens from patients in this epidemic. It is unlikely that the Ferris antigen was associated with this epidemic of infectious hepatitis.

The physicians, nurses, psychiatric aides, and laboratory personnel at the Lynchburg Training School and Hospital gave us great assistance in the collection of all specimens. Documentation of all aspects of the epidemic was provided us by the medical records and statistical personnel of the Institution. We acknowledge the technical assistance of Mrs. Suellen M. Kiger of Microbiological Associates Inc., Bethesda, MD, in performing the Australia antigen complement-fixation assays, and cataloging the numerous specimens.

---

1. Ferris, A. A., Kaldor, J., Gust, I. D., and Cross, G., Lancet **2**, 243 (1970).

2. Matthew, E. B., Madden, D. L., Dietzman, D. E., Newman, S. J., Sever, J. L., and Nagler, B., Presented: USPHS Commissioned Officers Ass., 1971.

3. Purchell, R. H., Holland, P. V., Walsh, J. H., Wong, D. C., Marrow, A. G., and Chanock, R. M., J. Infec. Dis. **120**, 383 (1969).

4. Cossart, Y. E., and Vahrman, J., Brit. Med. J. **1**, 403 (1970).

5. Grob, P. J., and Jemelka, H., Lancet **1**, 206 (1971).

6. Krugman, S., and Giles, J., J. Amer. Med. Ass. **212**, 1009 (1970).

7. Lander, J. J., Alter, H. J., and Purcell, R. H., J. Immunol. **106**, 1166 (1971).

Received Oct. 11, 1971. P.S.E.B.M., 1972, Vol. 139.