Henry Ford Hosp. Med. Journal
Vol. 19, No. 4, 1971

Precipitable Hetero-Antibodies in Man to Animal Serum Proteins*

Relation to Immunoglobulin Status

Hajime Hayashi, Ph.D., ** **Gerald A. LoGrippo, M.D.** **
Mary Perry, ** **and Judene Mueller, M.S.** **

Immunologic studies show that man develops specific antibodies to a variety of serum proteins of bovine, sheep, goat, chicken, and rabbit origins as well as antibodies against cow's milk protein. The data in this report emphasize that these hetero-antibodies are more frequently found in individuals with immunological problems and/or chromosomal aberrations. The clinical significance of human hetero-antibodies against these animal proteins deserves closer clinical observation for an understanding of these immunological problems.

Precipitable antibodies in man to animal serum proteins are confusing factors in two major areas of clinical immunochemical analysis: (1) in quantitation of serum proteins with anti-protein sera induced in animals; and (2) in human sera prepared from plasma with bovine thrombin, particularly where human sera are used for the screening of Australia antigen and antibody.

This type of antibody was reported in three recent publications. Leikola et al[2] described these antibodies in individuals with immunoglobulin (Ig)-A deficiency and normal IgA levels against bovine IgM. Huntley et al[3] reported precipitable antibodies in humans with selective IgA deficiency against ruminant serum and milk proteins. Ammann and Hong[4] also reported "anti-antiserum antibody" as a cause of double rings in immunoglobulin quantitation.

In this report, convincing data are given that these antibodies are found not only in individuals with IgA deficiency, but also in individuals with normal and excessive IgA serum levels. Moreover, certain individuals possess these precipitable antibodies to serum proteins from animals other than ruminants, such as chicken and rabbit. The significance of these findings is discussed in terms of the confusing factors during immunochemical assay and clinical interpretation.

*Presented in part before the annual meeting of the American Association of Immunologists at Chicago, Illinois in April 1971.[1]

**From Division of Immunology and Virology, Department of Pathology.

Materials and Methods

Serum specimens from patients. More than 9,000 serum specimens submitted to the immunology laboratory for quantitative analysis of immunoglobulin determinations were subjected to this study during the past 2½ years. The data in this report are classified according to the immunoglobulin-A serum status: ie, deficiencies, normal levels, and excesses. The hetero-antibodies have been specifically identified in the tables.

Antigen sources. Fresh serum samples from bovine*, goats, sheep*, pigs, horses, chimpanzee, monkeys, guinea pigs, and hamsters were studied with each hetero-antibody found in human sera. Whole milk proteins from cows were stored frozen and used in this study for identification of the precipitable hetero-antibodies.

Immuno-assays. Quantitation of immunoglobulins (IgA, IgM, and IgG) was performed by a micro-double diffusion technique.[5,6] Our laboratory standards for immunoglobulins were: 30-135 mg% for IgA, 30-120 mg% for IgM, and 600-1400 mg% for IgG. The same micro-double diffusion method was used for the detection and identification of the hetero-antibodies. In addition, a two-dimensional immunoelectrophoresis technic (method of Laurell)[7] was also applied in these studies.

Fractionation of animal serum proteins. Immunoglobulin-M from bovine and chicken serum was fractionated on Sephadex G200 (method of Murphy et al[8]) and some modifications in the density gradients for ultra-centrifugation. The purity of the fractions was demonstrated by immuno-chemical assays by either micro-double diffusion[5,6] or immuno-electrophoresis.[9]

*Bovine and sheep sera were gifts from Dr. Gail Riegle, Michigan State University, East Lansing, Michigan.

Results

Characterization of hetero-antibodies. The precipitable antibodies found in certain patients were demonstrated with a variety of animal proteins from goat, sheep, and bovine sera as well as cow's milk. Major precipitable components from these animal sera were IgM, albumin, and several unidentified serum components (Figs 1 and 3). Certain patients also demonstrated precipitable antibodies either against chicken serum or rabbit serum proteins. However, these were different from those formed with ruminant proteins mentioned above.

Figure 1 demonstrates the actual precipitation patterns found with human antibodies and certain animal serum proteins. Patient's serum, (case #R-1449) containing hetero-antibodies, was placed in the center well and a variety of animal sera in the peripheral wells. Goat, bovine, and sheep serum proteins demonstrated antigenic similarity by their fusion at the lateral junctions of each precipitating band. These are not the same as the reactants demonstrated for chicken serum component.

Individuals with the described hetero-antibodies showed no precipitable reactions against the serum proteins from chimpanzee, horse, pig, dog, monkey, guinea pig, and hamster.

The number of precipitable reactants (specific human antibodies against animal proteins) in patient's serum varied from one to as many as

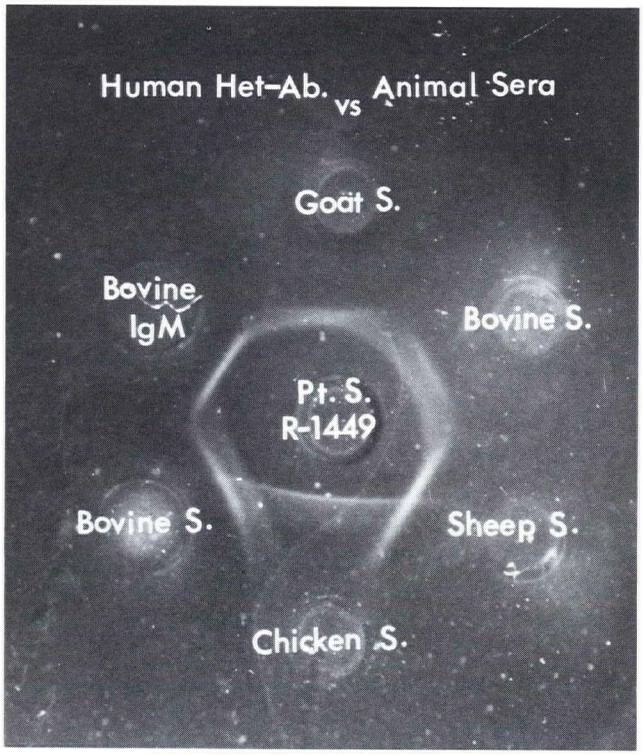


Figure 1

Patient's serum (Case #R-1449) containing hetero-antibodies was placed in the center well and goat, bovine, sheep, chicken, bovine sera and bovine IgM were placed in the peripheral wells in clockwise rotation. Notice that the precipitation line by patient's serum and chicken serum is not the same as lines by other ruminant animal sera.

ten different precipitable bands, as exemplified in Figure 2. Patient serum (case #BR-P-17) demonstrated at least eight different precipitable reaction bands against goat serum proteins. Figure 3 shows two patterns ("a" and "b") of a two-dimensional immuno-electrophoretic procedure (method of Laurell) of the same patient's serum. In both of these patterns, normal bovine serum was first electrophoresed in the horizontal direction in agar. Pattern "a" (on the left) was counter-phoresed vertically into the agar containing rabbit serum immunized with normal bovine serum proteins; whereas, pattern

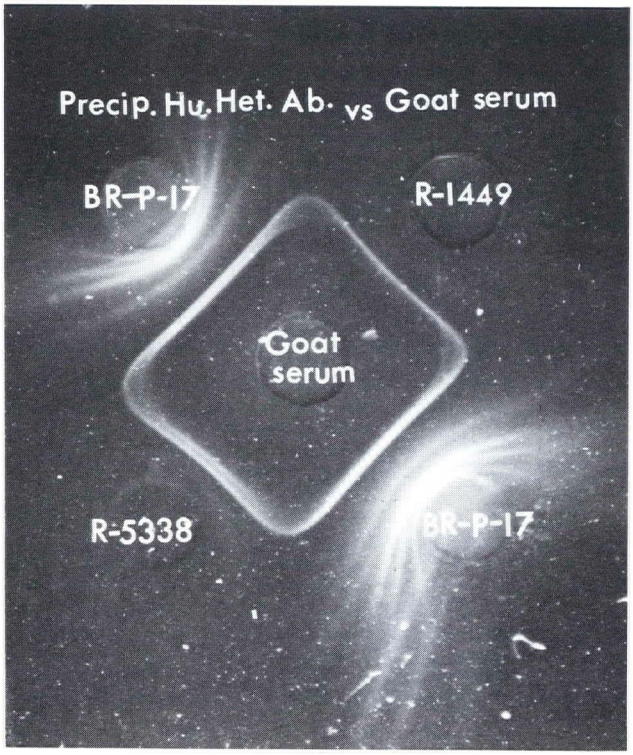


Figure 2

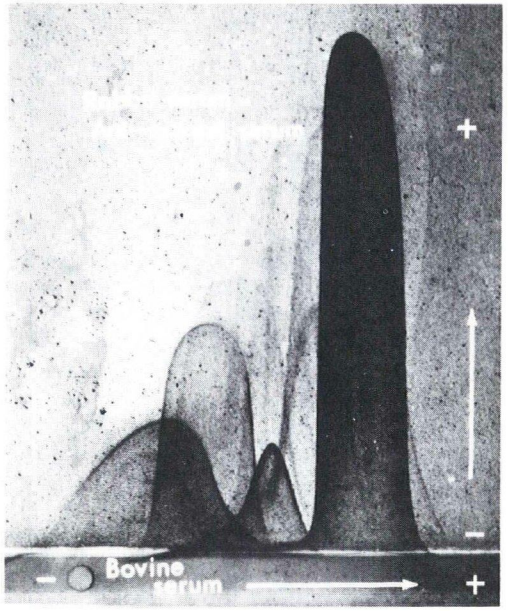
Goat serum was placed in central well and sera from three patients in the peripheral wells. Serum from case #BR-P-17 showed at least eight different precipitable bands with goat serum proteins.

"b" (on the right) was counter-phoresed vertically into the agar containing the patient's serum with hetero-antibodies. Both patterns demonstrated similar peaks of precipitable reactants from normal bovine serum. The patient's serum demonstrated similar antibodies against unaltered bovine serum proteins as those produced in the immunized rabbits.

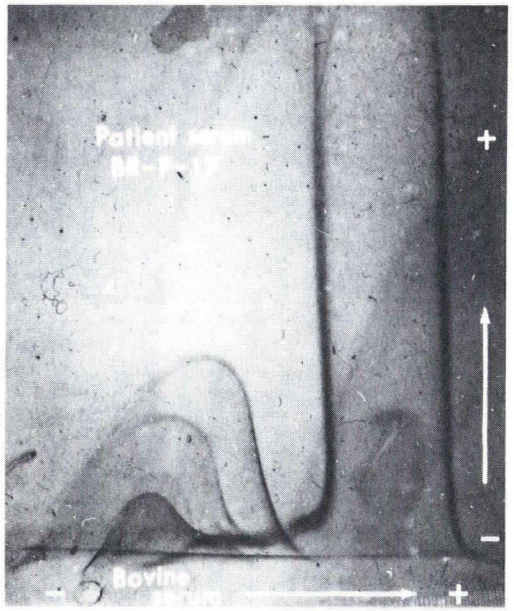
Clinical data:

Since 1968, 28 patients among more than 9000 individuals evaluated

HUMAN HETERO-ANTIBODIES VS BOVINE SERUM
(Two-Dimensional Electrophoresis-Laurell)



Pattern "a"



Pattern "b"

Figure 3

In both patterns "a" and "b", normal bovine serum was first electrophoresed in agar (horizontal direction). Pattern "a" was counter-electrophoresed vertically into the agar containing rabbit serum immunized with normal bovine serum. Pattern "b" was also electrophoresed vertically into agar containing the patient's serum with hetero-antibodies. Both patterns demonstrated similar peaks of multiple precipitable reactants from normal bovine serum proteins.

(0.31%) revealed hetero-antibodies during routine immunoglobulin and other serum protein determinations by our double immuno-diffusion technique. The hetero-antibodies are readily encountered because all specific antisera for human components are produced in animals and the micro-double diffusion technique readily reveals non-specific reactants. Table I shows that 11 of the 28 patients were found with IgA deficiency; 13 with a normal range of IgA values; and 4 with IgA excesses. All demonstrated antibodies to the serum proteins from ruminant animals (goat, sheep, and bovine). Three of 11 patients with IgA deficiency and 6 of 13 with normal IgA levels showed antibodies to cow's milk proteins. However, 6 of 11 patients with IgA deficiency demonstrated antibody to chicken serum. At present, only one precipitable reaction band has been observed between human serum (antibody) and chicken serum (antigen). From purified chicken serum proteins, the IgM component has been shown to be the only protein reacting with human antibody. Two patients (one with IgA deficiency and one with normal IgA level) demonstrated precipitable antibodies against rabbit serum. To our knowledge this is the first report showing human antibodies to chicken or rabbit serum proteins.

In Table II, 65 patients with varying degrees of IgA serum levels are correlated according to the number of individuals with or without hetero-antibodies. Seven of 13 patients (53%)

Table I

PRECIPITABLE HETERO-ANTIBODIES

IN PATIENTS WITH VARYING IgA SERUM LEVELS

IgA Level	Number of patients with hetero-antibodies against serum from:				
	Total	Ruminants*	Cow's Milk	Chicken	Rabbits
Deficiency	11	11	3	6	1
Normal**	13	13	6	0	1
Excess	4	4	0	0	0

28 positive/ 9000 tested (0.31%)

*The total numbers of patients tested showed hetero-antibodies to ruminant animals (bovine, sheep and goat origin). In addition, only some of these patients demonstrated antibodies to other animal proteins as the numbers indicated.

**Normal range for serum IgA: 30-135 mg%.

Table II

PRECIPITABLE HETERO-ANTIBODIES (Het-Ab) IN MAN
(Individuals with Deficient Serum IgA)

IgA Levels (mg %) *	No. of Patients	
	Total	With Het-Ab (%)
Negative (<0.05)	13	7 (53.8%)
Trace (0.05 - 0.9)	15	4 (26.7%)
Low (1 - 29)	37	0
Total	65	11

*Normal range for serum IgA: 30-135 mg%.

Table III

PRECIPITABLE HETERO-ANTIBODIES (Het-Ab) IN MAN
(Individuals with Normal and Excess Serum IgA)

IgA Levels	No. of Patients with Het-Ab	Clinical Entities
Normal *	6	Mental retard.
	3	Asthmatic children
	4	Infections
Excess	2	Australia antigenemia
	1	Sys. lupus eryth.
	1	Mult. myeloma (IgA)

*Normal range for serum IgA: 30-135 mg%.

with negative IgA levels (<0.05 mg%) showed hetero-antibodies in their sera. Four of 15 patients (26%) with trace IgA levels (0.5-0.9 mg%) showed hetero-antibodies and none of 37 patients with low IgA levels (1-29 mg%) possessed any hetero-antibody. It appears that the lower the IgA level, the greater the incidence of hetero-antibodies.

However, the data in Table III demonstrate that patients with normal and excessive IgA quantities also possess hetero-antibodies against the same variety of heterologous proteins. These are patients with basically immunological problems, such as childhood asthma, chronic infections, Australia antigenemia, systemic lupus erythematosis, and multiple myeloma of the IgA class. In addition, the mentally retarded group of children showed a relatively higher incidence of hetero-antibodies.

The mentally retarded children were studied further in an institution. A comparative study with IgA levels and hetero-antibodies between sera collected in 1962 (frozen stored sera) and freshly collected sera is shown in Table IV. In the 1970 study, 6 of 15 (40%) mongoloid children showed hetero-antibodies as compared to one out of 31 (3%) non-mongoloid children. The high difference is significant. In the 1962 serum study, an increased in-

Table IV

PRECIPITABLE HETERO-ANTIBODIES (Het-Ab) IN MAN

(Mentally Retarded Children)

Group	No. of Children Total	No. of Children With Het-Ab (%)	Year Studied
Institutional			
Mongoloid	15	6 (40%)	1970
Non-mongoloid	31	1 (3.2%)	1970
Mongoloid	40	15 (37.5%)	1962
Non-mongoloid	40	0	1962
Non-institutional			
Mongoloid	9	0	

cidence of hetero-antibodies in mongoloids was also found. Fifteen of 40 (37%) mongoloid children possessed these hetero-antibodies, but none of 40 other mentally retarded children showed these antibodies. Although the number is small, it is noteworthy that nine mongoloid children who were not institutionalized demonstrated none of these antibodies. The significance of these findings warrant further study.

Discussion

With the increased use and demand for animal antisera in the detection of human serum proteins by immunochemical methods of analysis, the hetero-antibodies in man are confusing factors that must be recognized for specific determinations. This is particularly important in assaying for IgA deficiency states, where undiluted serum is tested by single radial diffusion and immuno-electrophoresis techniques. This confusing factor has also been emphasized by Leikola and associates.[2] They reported that one patient who actually had no serum IgA gave a false value of 450 mg% of IgA by the radial diffusion test. The component being measured was actually a hetero-antibody. In marked contrast to the radial diffusion technique, the micro-double diffusion technique[5,6] (used in our laboratory for immunochemical assays) readily detects and differentiates these miscellaneous precipitable components in a routine manner.

Other current problems in immunochemical assay are the growing use of animal sera for the preparation of specific antibody against Australia antigen, and the practice of using bovine thrombin to human hemophiliac plasma containing Australia antibody. Both of these practices produce non-specific precipitable reactions between human hetero-antibody and animal sera.[1] The frequency of hetero-antibodies in man against animal proteins was found to be 0.3% in our study. This is practically the same incidence rate as that being reported for Australia antigenemia which varies from 0.1 to 0.5% in supposedly healthy populations.[10,11] All precipitable reactants must be specifically differentiated when animal sera is used in the production of antibody against the Australia antigen. Furthermore, the use of bovine thrombin must be avoided when human plasma is collected by plasmaphoresis for Australia antibody content.

Recent literature[2,3] emphasizes that IgA deficiency in man is correlated with antibodies to the IgM serum component from ruminant animals. Our data suggest that a fair percentage of patients with hetero-antibodies were found among those individuals with normal and excessive quantities of IgA serum levels. In addition, hetero-antibodies in man are formed not only against macromolecular IgM serum component, but also against a variety of serum components of smaller molecular sizes (Fig 3). Moreover, besides ruminants, chicken and rabbit serum proteins are also sensitizing to man. The question arises whether the hetero-antibodies are being stimulated by an IgA deficiency state or by an enzymatic deficiency of the intestinal tract which prevents digestion before assimilation of these animal proteins. The latter appears more plausible as a working hypothesis, since the presence of hetero-antibodies suggests a functioning IgG system with or without

IgA. Therefore, clinicians should be alerted to request hetero-antibody studies in patients with allergies and digestion problems as well as immunoglobulin deficiencies.

From our data, the hetero-antibodies are more frequently found in individuals with immunological problems and/or chromosomal aberrations. Closer clinical observation is needed for an understanding of these immunological problems and the significance of hetero-antibodies against these animal proteins.

REFERENCES

1. Hayashi, H., and LoGrippo, G. A.: Precipitable hetero-antibodies in man to animal serum proteins, abstracted in *Fed Proc* 30:248, 1971.
2. Leikola, J., et al: Antibodies to bovine IgM in IgA deficient and normal human serums, presented at 23rd Ann Meeting of Amer Assoc Blood Bank, San Francisco, Oct 1970.
3. Huntley, C. C., et al: Characterization of precipitating antibodies to ruminant serum and milk proteins in humans with selective IgA deficiency, *New Eng J Med* 284:7-10, 7 Jan 1971.
4. Ammann, A. J., and Hong, R.: Anti-antiserum antibody as a cause of double precipitin rings in immunoglobulin quantitation and its relation to milk precipitins, *J Immun* 106: 567-569, Feb 1971.
5. Sharpless, N., and LoGrippo, G. A.: A standardized immunochemical method for quantitative determination of the immunoglobulins in serum, *Henry Ford Hosp Med Bull* 13:55-77, Mar 1965.
6. Hayashi, H.; LoGrippo, G. A., and Perry, M.: Immunoglobulin levels in spinal fluid and saliva by direct immunochemical assay and microscopic measurements, *Henry Ford Hosp Med J* 18:263-274, Winter 1970.
7. Laurell, C.-B.: Antigen-antibody crossed electrophoresis, *Anal Biochem* 10:358-361, Feb 1965.
8. Murphy, F. A.; Aalund, O., and Osebold, J. W.: Physical heterogeneity of bovine gamma globulins: Gamma-1 M globulin electrophoretic heterogeneity, *Proc Soc Exp Biol Med* 117:513-517, Nov 1964.
9. Grabar, P., and William, C. A.: Méthode Permettant L'Etude Conjuguee Des Propriétés Electrophorétiques Et Immunochimiques D'Un Mélange De Protéines. Application Au Sérum Sanguin, *Biochim Biophys Acta* 10:193-194, 1953.
10. Hayashi, H., and LoGrippo, G. A.: Australia antigen, immunoglobulins, C-reactive protein in virus hepatitis, abstracted in *Bact Proc* 70:117-118, 1970.
11. Soulier, J. P. (ed): Symposium on virus hepatitis antigens and antibodies, Munich August 1970. The Australia antigen hepatitis associated antigen (HAA) and corresponding antibodies, *Vox Sang* 19:193-416, Sept- Oct 1970.