

Australia Antigen in a Closed Adult Population Monitored for Serum Glutamic Oxalacetic Transaminase

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A study of the presence of Australia antigen (Au/SH) was conducted over a period of 21 weeks among volunteer plasma donors living in a prison and being monitored for serum glutamic oxalacetic transaminase (SGOT). A good correlation was observed between the level of SGOT and presence of Au/SH, the latter being present in 33% of donors with SGOT values higher than 101 Karmen units and in 12% of those with SGOT values of 41 to 100 units. Furthermore, none of the 87 donors with all SGOT values below 40 was found positive for Au/SH. It should be noted, however, that single specimens only were tested from 72 of the 87 individuals. Au/SH was detected with equivalent efficiency by both agar gel precipitation and complement fixation procedures. Implications of these findings in the prognostication of hepatitis carrier state are discussed.

Numerous reports have corroborated the association of the Australia antigen (Au/SH) with viral hepatitis since the antigen was first reported by Blumberg et al. (2, 3) and by Prince (13). Blumberg's "Au" antigen is generally accepted as being similar, if not identical, to Prince's "SH" antigen. Although the relationship of Au/SH to serum hepatitis, infectious hepatitis, and possibly other conditions has not yet been completely resolved, the value of this antigen in screening blood or plasma donors is gaining recognition.

Both of the above authors reported the relationship of Au/SH to elevated transaminases [serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase]. In rare cases, Au/SH persisted for long periods of time, suggesting a carrier state of hepatitis. Other investigators have since pointed out the temporal relationships of liver function tests to Au/SH (4-7, 9, 10, 12, 16-18).

The purpose of this report is to present data on the correlation of SGOT, Au/SH, and clinical signs of hepatitis in a closed prison population continually monitored for SGOT levels.

MATERIALS AND METHODS

Study population. The study was carried out in a penal institution for males. The total population of this institution has varied from 1,531 to 1,718 over the last 3 years. Approximately half of these males, ranging in age from 18 to 60 years, donated plasma in a voluntary program. Viral hepatitis has not been a significant problem in this institution during the last several years, occurring only sporadically from time to time. Illicit use of drugs by inmates of this institution has been occasionally detected.

During the last 3 years, the men volunteering for donation of plasma have been monitored for SGOT values on a regular schedule consisting of one estimation at least every 4 weeks. Donations were accepted on a weekly basis from all those whose SGOT values remained below 40 Karmen units. Those whose values fell between 41 and 100 units were tested at weekly intervals for as long as these values remained above 40. Their donations, however, were accepted during this period at the discretion of the medical officer in charge; when the SGOT values were ≥101, acceptance of donations was stopped. In all cases, individuals were carefully observed for clinical signs of illness including any that might indicate viral hepatitis.

Monitoring of the donors also included the following: hematocrit, serum protein determination, protein electrophoretic pattern, and, when appropriate, bilirubin determinations.

Selection of donors for the study was based on the level of their SGOT values; preferentially, samples were requested from those with values of 40 or higher. An approximately equivalent number of individuals with SGOT values below 40 was included. Clearly, then, the survey does not represent a true cross section of the entire closed population.

Assays. The micro-Ouchterlony agar gel double-diffusion technique (AG) as modified by Prince (13) was used except that protamine was omitted after it was determined that it was not essential for the reaction to take place. Briefly, standard microscope slides
were layered with 3.0 ml of 0.9% agarose. A seven-well pattern was adopted consisting of a center well surrounded by six peripheral wells each 2 mm in diameter and 3 mm apart. For Au/SH testing, the antisera was placed in the center well; conversely, for detecting antibody to Au/SH, the antigen was placed in the center well. Test sera or plasmas were not inactivated before testing. For routine screening, each pattern contained an Au/SH-positive control reagent in the top outer well. Incubation was in moist chambers at room temperature (22 to 25°C). Precipitin lines generally appeared in 1 to 2 days but the slides were observed for 7 days.

All test sera (or plasmas) were first screened undiluted. Some were also tested twice-concentrated by refilling the wells about 1 hr later. Positive sera were titrated by serial twofold dilutions. The same procedure was applied to assays of antibody to Au/SH.

The complement fixation (CF) assay was performed by the modification of Kolmer-Boerner's test tube method described in Diagnostic Agents for Clinical and Laboratory Use (11). This modification uses 0.1 ml each of antigen, antibody, hemolysin and sheep cell suspension, and 0.2 ml of complement containing 2 exact units. These are determined in the presence of 2 units of antigen when the antibody is titrated and in the presence of 4 units of antibody when the antigen is titrated. The antigen, antibody, and complement mixtures are incubated overnight at 4°C. After addition of sensitized sheep erythrocytes, the mixtures are further incubated in a 37°C water bath until the complement and hemolysin controls are completely clear (10 to 20 min). A hemolysis not greater than 2+ is recorded as the titer end point.

The SGOT levels were determined as described by Karmen (8). Values of 40 Karmen units or lower were considered to be within normal limits, although values between 40 and 100 units can hardly be taken as evidence of clinical illness.

Reference reagents. Unaltered human sera or plasmas were used as sources of antigen and antibody. The house reference reagents produced lines of identity when tested with reference sera kindly provided by A. M. Prince, New York Blood Center, New York, N.Y., and by A. G. Redeker, Los Angeles County-University of Southern California Medical Center, Los Angeles.

A supply of Au/SH was obtained from two patients reported as infectious hepatitis cases. Antigen GR-Le (AG titer of 1:4) came from an acute hepatitis case in Athens, Greece; we thank R. Triantaphilli for this antigen. Plasma from donor 9 (see Table 3), with an AG titer of 1:32, was from a prisoner who later developed clinical hepatitis; this plasma was not anti-complementary and was therefore reserved for CF assays (CF titer of 1:512).

A plasma containing antibody to Au/SH was selected by screening 11 hemophilia patients, 4 of whom possessed antibody. Plasma KK, being anti-complementary, was used only in the AG test (titer of 1:4). Plasma Clarkson, being free of anticomplementary activity, was reserved for CF assays (titer of 1:32).

The reagents were stored frozen and were repeatedly thawed without evident loss of reactivity.

RESULTS

Au/SH, SGOT levels, and incidence of clinical hepatitis. The study covered a 21-week period during which 185 persons were examined and from whom 348 specimens were assayed for Au/SH. Of the 185, 39 contributed two or more specimens at various time intervals. Single specimens were obtained from 146 persons. Of the 185 persons, 15 with 99 specimens were positive for Au/SH by AG as well as CF procedures.

Antibody to Au/SH was not detected by AG in any of the 348 specimens tested.

Each donor was placed into one of three SGOT categories: 0–40, 41–100, or >101. Persons with multiple specimens were categorized according to the highest SGOT value recorded (Table 1). SGOT values were obtained on 169 of the 185 persons tested for Au/SH.

The overall impression gained from Table 1 is that a good correlation exists between SGOT values and the incidence of Au/SH. This correlation, however, is not absolute, as 67% of those with high (>101) SGOT values had no detectable antigen. Moreover, four of seven Au/SH-positive individuals in the middle category (41–100) and one of the eight in the >101 group had occasional SGOT levels below 40 in sequential samples (see Table 2).

Except for the samples mentioned above, no individual with a SGOT level below 40 was found to be positive for Au/SH. It should be noted, however, that single specimens were tested from 72 of the 87 individuals shown in Table 1. Up to nine serial specimens from 15 of 87 individuals also remained antigen-negative.

Special attention was paid to individuals in whom Au/SH was detected in the initial specimen. Collection of consecutive weekly samples was sufficiently complete to permit an evaluation of clinical hepatitis. The SGOT values and the incidence of clinical hepatitis for the 169 cases are shown in Table 2.

Table 1. Distribution of Au/SH among 169 persons in relation to SGOT values and clinical hepatitis

| Highest recorded SGOT | Au/SH-positive/total tested | Per cent positive | No. of cases of clinical hepatitis |
|-----------------------|-----------------------------|-------------------|-----------------------------------|
| 0–40                  | 0/87                        | 0                 | 0                                 |
| 41–100                | 7/58*                       | 12                | 1                                 |
| >101                  | 8/24*                       | 33                | 2                                 |

* One or more weekly sera from four of seven persons in the 41–100 group and one of eight in the >101 group showed SGOT values below 40 (see Table 2).
| Donor | Weeks |
|-------|-------|
|       | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    | 13    | 14    | 15    | 16    | 17    | 18    | 19    | 20    | 21    |
| 1     | 60/4  | 46/4  | 60/4  | 60/4  | 63/2  | 63/2  | 63/2  | 51/2  | 60/1  | 60/1  | 46/1  | 46/1  | 36/1  | 46/1  | 46/1  | 46/1  | 51/1  | 36/1  | 36/1  | 24/1  |
|       | 78/7  | 66/7  | 90/7  | 2/64  | 2/64  | 2/64  | 2/64  | 2/32  | 2/32  | 1/32  | 1/32  | 2/64  | 2/64  | 4/128 | 4/128 | 8/256 | 8/256 | 8/256 | 2/78  | 2/78  |
| 2     | 4/64  | 4/64  | 4/64  | 4/64  | 4/128 | 4/128 | 4/128 | 4/128 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 |
| 3     | 32/1024 | 32/1024 | 32/1024 | 32/1024 | 32/1024 | 32/1024 | 32/1024 | 32/1024 | 32/1024 | 32/1024 | 32/1024 | 32/1024 | 32/1024 | 32/1024 | 32/1024 | 32/1024 | 32/1024 | 32/1024 | 32/1024 | 32/1024 |
| 4     | 16/512 | 16/512 | 16/512 | 16/512 | 32/512 | 32/512 | 32/512 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 |
| 5     | 1/4   | 60/16 | 51/16 | 36/8  | 8/128 | 4/128 | 4/128 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 |
| 6     | 4/128 | 128/8 | 128/8 | 90/7  | 70/5  | 60/3  | 36/7  | 8/128 | 4/128 | 4/128 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 | 8/256 |
| 7     | 95/8  | 78/128 | 128/128 | 128/128 | 128/128 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 |
| 8     | 95/8  | 78/128 | 128/128 | 128/128 | 128/128 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 |
| 9     | 95/8  | 78/128 | 128/128 | 128/128 | 128/128 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 |
| 10    | 95/8  | 78/128 | 128/128 | 128/128 | 128/128 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 |
| 11    | 95/8  | 78/128 | 128/128 | 128/128 | 128/128 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 |
| 12    | 95/8  | 78/128 | 128/128 | 128/128 | 128/128 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 |
| 13    | 95/8  | 78/128 | 128/128 | 128/128 | 128/128 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 |
| 14    | 95/8  | 78/128 | 128/128 | 128/128 | 128/128 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 |
| 15    | 95/8  | 78/128 | 128/128 | 128/128 | 128/128 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 | 32/512 |

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* SGOT value
  AG titer/CF titer.
* Agarose well filled twice.
* Onset of clinical hepatitis.
of the persistence of antigenemia during the 21 weeks of the study in relation of SGOT levels. The findings on 15 antigen-positive individuals are summarized in Table 2.

Two or more samples were obtained from 12 of the 15 persons over a period of 21 weeks. In each of these, Au/SH was detected over the entire test period. The SGOT levels were consistently greater than 40 units in 7 of the 12 persons. The SGOT values of the other five persons occasionally decreased to within the normal range while antigenemia persisted without interruption.

As indicated in Table 2, the number of specimens received from each of the 15 antigen-positive individuals varied greatly; only one specimen was received from 3 of the 15, and 2 to 17 samples were obtained from the other 12.

Finally, the three cases of clinical hepatitis recorded during the time of this study occurred in donors with values of over 40 units. All three cases were also found positive for Au/SH.

**Correlation between AG and CF results.** Data accumulated thus far (Fig. 1) indicate a direct correlation between the results of the two assays. At first, a total of 93 sera were found positive by AG. When all 348 sera were retested by CF, 6 more were found positive by this test. However, when these six were retested for AG by doubling the concentration, they were also found positive. A 100% correlation between these two methods is clearly represented in Fig. 1. The only superiority of the CF test was in the higher titers obtained. The geometric mean of the AG results was 2 as compared to a geometric mean of 62 for CF, a 31-fold difference in titer between the two procedures. Of the 348 samples tested by CF, only 7 obtained from two donors were anticomple-

![Fig. 1. Comparison of agar gel (AG) and complement fixation (CF) assays on 99 Au/SH-positive sera. (*) Well in AG filled twice.](image)

**DISCUSSION**

In this study, 185 plasma donors who were monitored for SGOT on a regular schedule were tested for Au/SH. There were 87 individuals with SGOT values of 40 Karmen units or less; 58 persons with values between 41 and 100, and 24 with values $\geq 101$.

In those tested whose highest SGOT value was less than 40 units, none was found to have Au/SH in one or more samples tested. In the 41–100 unit group, 7 were positive for Au/SH as were 8 of 24 in the $>101$ group. During the 21-week period of the study, clinical hepatitis was recorded in two donors in the $>101$ group and one in the 41–100 group; these three were also positive for Au/SH. Thus, presence of Au/SH may be of greater prognostic value in clinical hepatitis (3 of 15) than an elevated SGOT value (3 of 82).

Although our findings do not prove that a combination of Au/SH testing and SGOT determination is a more sensitive approach to the detection of hepatitis carrier donors, it is conceivable that screening in this fashion would further reduce the risk of accepting blood donations from potential carriers. In any event, neither procedure at present will detect all hepatitis carriers, as already indicated for Au/SH by Gocke and Kavey (7) and for SGOT by Bang et al. (1). This latter procedure has the disadvantage, however, of also eliminating an appreciable number of noncarriers. It is likely that the AG procedure could be made to detect a higher percentage of Au/SH-positives, as indicated for example by our results with twice-filled agar wells.

From the results of this study it appears that AG and CF are equally effective in their ability to detect Au/SH. This conclusion conforms more with that of Purcell et al. (14) than with the findings of Shulman and Barker (15), who claimed for their CF procedure a considerably higher sensitivity than for the AG test. The AG test, however, offers the advantage of greater simplicity. Whatever the test method used, this study adds to the body of data already accumulated on the value of Au/SH testing as a potential marker of serum hepatitis carrier state.

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