Auto-agglutination in albumin

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SYNOPSIS An example of the ‘albumin’ auto-agglutinating phenomenon is reported. This was found in the serum of a man dying from carcinoma of the stomach with liver secondaries. It is suggested that the serum factor is of a protein (probably globulin) nature, and may have been produced by the diseased liver.

In spite of the fact that innumerable blood grouping and cross matching tests have been carried out using albumin as a suspending medium, only a few instances have been recorded of an auto-agglutinin active only in albumin (Weiner, Tovey, Gillespie, Lewis, and Holliday, 1956; Weiner and Hallum, 1957; Moore, Linins, and McIntyre, 1959; Griffiths, 1962; Crowley and Hyland, 1962).

The patient’s serum in this anomaly agglutinates his own, and all other red cells, when suspended in an albumin medium. Weiner et al. (1956) appropriately described this as a ‘diagnostic pitfall for the unwary’. The nature of this abnormal serum constituent is not known. Crowley and Hyland (1962) adduced evidence suggesting that it is of a protein nature but the sera hitherto described have had varying physical properties. We report a further case because of its rarity, its clinical association, and its diagnostic importance, and provide further evidence as to its possible nature.

CLINICAL HISTORY

The patient was a man 72 years old when first admitted to hospital in May 1963. He complained of abdominal pain of over one year’s duration, with more recent diarrhoea and weight loss. Barium meal showed the presence of a gastric neoplasm. At operation a small prepyloric neoplasm was found with widespread secondary deposits in the liver. A palliative partial gastrectomy was performed. Histological examination confirmed that the lesion was an adenocarcinoma. The patient’s blood was group 2d using only saline techniques but no blood was crossmatched or transfused. The patient was readmitted in September 1963, on account of urinary retention of seven hours’ duration. Prostatectomy was performed on 23 September.

Difficulty was experienced in obtaining compatible blood. All suspensions of the patient’s serum with bovine albumin agglutinated all donor cells. These samples were compatible when tested by the indirect antiglobulin test (in which saline was the suspending medium). Three pints of blood compatible in saline and by the indirect antiglobulin technique were transfused on 28 September. The patient was febrile before and after the transfusion. Thirty to 45 minutes after the start of transfusing the third pint of blood he developed back pain and rigors. However, he had had a similar attack on the day before transfusion. No evidence of incompatibility was found between donor and recipient’s blood (other than auto-agglutination in albumin).

Histological examination of the prostate showed benign adenomatous hyperplasia. After operation, his general condition continued to deteriorate, with increasing emaciation, jaundice, and ascites, until he died on 24 December.

INVESTIGATIONS

Haemoglobin was 13·9 g. % on first admission, falling to 7·0 g. % after prostatectomy but was restored to 14·6 g. % following blood transfusion.

The serum acid phosphatase level was 1·4 K-A units, blood urea 70 mg. per 100 ml. and the one-stage prothrombin ratio 1·5.

Serum proteins were 5·5 g. % (4·1 g. % albumin) on 20 May 1963 and 6·2 g. % (2·22 g. % albumin) on 1 October. Cellulose acetate electrophoresis gave α1 globulin 0·31 g. %, α2 globulin 0·97 g. %, β globulin 0·60 g. %, and γ globulin 2·10 g. %.

BLOOD GROUPING AND CROSSMATCHING The patient was grouped as group O Rh (D) positive on 17 May 1963. All cells were set up in saline. However, when crossmatching (minor and major) on 28 September 1963 it was found that all tubes containing the patient’s serum plus albumin showed agglutination. When tested in the presence of bromelase (Bromogen, Exogen Ltd., Glasgow) no agglutination occurred.

Further tests were performed as follows:—

The indirect antiglobulin test on the patient’s serum was negative. This applied to the cells matched before transfusion; pooled O D-positive cells; as well as a panel

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of known genotype, O R1Rz, O R1R1, O R2R2, O R0, of
known Kell, Duffy, Kidd, Lewis, P, M N S, Lutheran,
and Sutter groups (Knickerbocker Pancell Series
no. 354).

The patient’s serum agglutinated all group O test cells
when tested in an albumin suspension.

No irregular antibody could be detected when checked
at the Regional Blood Transfusion Centre.

The albumin agglutinating property of the patient’s
serum was found with 30%, 20%, and 15% bovine
albumin but not with 10%. The reaction proceeded
equally well at 37°C., 22°C., and 4°C. The property
was present in the patient’s serum up to a 1 in 8 dilution when
tested at 37°C., but was active at 1 in 32 dilution after
standing overnight at 4°C. The agglutinating property
was unaffected by heating at 58°C. for 30 min., or
storage at −20°C. for three months. The agglutinating
property was still present after being allowed to react
over two days with four changes of group O cells, and
could not be eluted from agglutinated cells. If agglutinated
red cell masses were dispersed, they could be further
agglutinated on repeated exposure to the patient’s serum.

No haemolytic activity could be demonstrated in fresh
or stored serum.

Saline-suspended cells were weakly agglutinated after
prolonged standing at 4°C.

The supernatant after precipitation of 0.5 ml. serum in
10 ml. of 5% trichloroacetic acid showed no agglutinating
activity.

The AutoAnalyzer was used to fractionate the serum.
When serum and an equal quantity of normal saline were
taken from the donor stream, strong agglutinating
activity remained, as in the patient’s serum. On the other
hand when the recipient stream was taken after dialysis
with a double volume of normal saline, no agglutinating
activity remained. This procedure clearly demonstrated
that the abnormal constituent was of a large molecular
pattern, probably of a protein nature.

Dr. G. Franglen, of St. George’s Hospital, London,
examined this patient’s serum in an attempt to detect any
abnormal protein. All methods used, including immune
-electrophoresis, yielded normal results.

**DISCUSSION**

The serum described in this report is essentially
similar to those previously described, although
differences were found in various tests.

Examples of this condition come to light as a
result of difficulty experienced in blood grouping and
crossmatching. The nature of the difficulty will not
be appreciated unless saline suspensions are also
used. The anomaly does not appear to be of immuno-
logical significance, in that the patient’s red cells are
not sensitized to an antiglobulin serum, and the red
cells are not directly agglutinated by antiglobulin
serum. The absence of agglutination with bromelase-
treated cells helps to differentiate this phenomenon
from rouleaux formation.

The optimum temperature found for this activity
has varied in different reports: 37°C. (Weiner et al.,
1956); equally strong at 4°C., 20°C., and 37°C.
(Moore et al., 1959); and three sera with differing optima (Crowley and Hyland, 1962). No difference
between 4°C., 22°C., and 37°C. could be detected in
our patient’s serum on straight testing, but standing
overnight at 4°C. increased the titre of serum activity
from 1/8 to 1/32. The activity is independent of complement and stable on storage. Weak agglutina-
tion was found in saline after prolonged standing at
4°C., in which respect this resembled the serum
described by Moore et al. (1959) and was attributed
to a naturally occurring anti-H.

The serum titre of activity found in reported cases
has varied from 1/4 to 1/128. Weiner et al. (1956)
stated that the sera studied by him and his associates
did not show this agglutinating property with 17% human albumin. We only used bovine albumin, and
found that agglutination occurred with 15% bovine
albumin but not with 10% solution. Crowley and
Hyland (1962) found 1% commercial bovine or
human albumin sufficient to elicit the phenomenon
with all of their three reported sera.

Albumin auto-agglutination does not appear to have
any disease association. The property may be acquired or impermanent. The previously reported
sera were found in association with peptic ulceration,
pregnancy, haemorrhage, and hepatitis. We are
not able to state at what stage of our patient’s illness
this anomaly developed. But there is, at least, a
reasonable presumption that the abnormal serum
constituent could have been associated with neo-
plastic involvement of the liver.

Crowley and Hyland (1962) postulate that the
substance must be of small molecular size in that it
can be transferred across the placenta. However, in
our case it was clearly of larger size than could pass
across the AutoAnalyzer dialysing membrane. After
fractionation of the proteins it was found that activity
was present in the globulin fraction, with maximal
activity in β and γ fractions (Crowley and Hyland,
1962). The serum studied by us showed a moderate
increase in the globulin fraction, although an
abnormal fraction could not be detected by other
tests, including immuno-electrophoresis.

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