

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 grouped using only saline techniques but no blood was crossmatched or transfused. The patient was re-admitted 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 α_1 globulin 0.31 g.%, α_2 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 cross-matching (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

of known genotype, O R₁R₂, O R₁R₁, O R₂R₂, O R₀, of known Kell, Duffy, Kidd, Lewis, P, M N S, Lutheran, and Sutter groups (Knickerbocker Panocell 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 1.0 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 immunoelectrophoresis, 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 immunological 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 agglutination 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 neoplastic 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.

We are grateful to Dr. B. Bevan, of the Welsh Regional Blood Transfusion Service, and Dr. G. Franglen for their examination of this serum, and to Mr. C. Havard for permission to publish.

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