IMMUNOLOGY

Loss of Immunological Reactivity of an Alpha-2 Globulin after Prolonged Freezing of Serum

MOST investigators who preserve sera for future analysis take it for granted that the serum proteins are stable at temperatures around −20° C. Some evidence supports this assumption: the titres of certain antibodies or of rheumatoid factor, for example, remain unaltered after even several years in a deep freeze. This communication shows that most human sera, from both healthy and sick people, contain an α-2 globulin which loses its antigenic (precipitin) characteristics after prolonged storage at −20° C.

An antihuman serum was made by injecting a rabbit intravenously with 1 ml. of pooled human serum three times weekly for eighteen months. This pool was made by combining equal amounts of serum from 200 patients in a State hospital. Immunoelectrophoresis using the antihuman serum and several human sera showed antibodies to various proteins including about 10 α-globulins. The antihuman serum was absorbed with another serum pool made by combining equal amounts of serum from 20 blood donors. 1 ml. of the antiserum was mixed with 25 μl. of the blood donors' pooled serum, left at room temperature for 30 min and then centrifuged at 3,500 r.p.m. for 10 min. Using a small-scale double-diffusion-in-agar technique[1], 0·5 μl. amounts of each reactant were placed in wells the centres of which were 8 mm apart and the edges 4 mm apart. The preparations were left in a moist chamber at room temperature for 48 h.

When a recently drawn human serum and the absorbed antiserum were used as reactants, two precipitin lines could usually be seen. The first (line 1) was a dense line, occasionally closely split, present about 1 mm from the edge of the well containing the human serum. The second (line 2) was a hazy, faint line present about 1 mm from the edge of the well containing the absorbed antiserum. There was no reaction between the absorbed antiserum and saline, nor between human serum and rabbit serum taken before immunization had been started.

When the human serum had been kept for a long time in a deep freeze at −20° C, line 2 tended to disappear, an observation made in more than 1,100 sera taken from both healthy and ill persons. Table 1 shows the findings in 763 different sera for which the dates of drawing were precisely known. Line 2 was seen in all freshly drawn sera; in about 30 per cent of sera stored for 9 months; and in only 5 per cent of sera stored for 30 months. Among 36 freshly drawn sera, line 2 was present in all; on retesting 14 weeks later, 5 of them had entirely lost line 2. The disappearance of line 2 depended more on the length of time the serum had remained frozen than on either mere freezing or the number of times the serum had been frozen and thawed, because there was no diminution in the intensity of line 2 in 20 freshly drawn sera which had been 20 times rapidly brought to the temperature of dry ice and then thawed. Nor did heating the serum at 56° C for 30 min cause line 2 to change intensity.

Both line 1 and line 2 were shown by immunoelectrophoresis on cellulose acetate to be α-2 globulins. Line 1 was immunologically identical with that produced by the S_f 0-400 lipoproteins (isolated in an analytical ultracentrifuge, and provided by the Institute of Medical Physics). Line 2 has not been identified. Use of commercially available serum fractions and antisera showed it to be neither cæruloplasmin nor α-2 macroglobulin. The absorbed antiserum did not react with fibrinogen, and addition of neither heparin nor thrombin to fresh sera abolished line 2—so that line 2 is probably neither a cryofibrinogen nor the heparin-precipitable protein described by Thomas, Smith and Von Korff[2]. Siderophyllin, Cohn's fractions II, III and IV 5 + 6, and an isolated cryoglobulin did not react with the absorbed antiserum.

Table 1. PRESENCE OR ABSENCE OF LINE 2 IN 763 DIFFERENT SERA FOR WHICH THE DATES OF DRAWING WERE KNOWN

Age of serum	Total No.	Line 2 present	Line 2 absent
3 days	79	79 (100%)	0
2 weeks	80	80 (100%)	0
1–3 months	91	89 (98%)	2 (2%)
3–6 months	88	71 (81%)	17 (19%)
6–9 months	86	59 (69%)	27 (31%)
9–12 months	68	18 (26%)	50 (74%)
12–15 months	40	13 (32%)	27 (68%)
30–36 months	231	12 (5%)	219 (95%)

These investigations show that most human sera contain an α-2 globulin which loses its immunological reactivity after a long period in the deep freeze. Although stored sera are used all over the world for numerous investigations, systematic investigations have not been made of the effects of lengthy storage of the serum on the various proteins; nor has a comparison been made of the various methods of preservation to determine the least deleterious. The results of such inquiries would be valuable.

This work was supported by grant *MH*04581–02 from the National Institutes of Health, Bethesda, Maryland.

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Development of the Fœtus in Female Rats injected with Homologous Heart Tissue

RECENT observation of the occurrence in human beings of auto-antibodies specific to heart tissue has given support to the hypothesis of an auto-immune ætiology in rheumatic fever, following myocardial infarction and postcardiotomy state[1,2]. In the rabbit, heart-specific antibodies have been produced readily against heterologous heart tissue and much less readily against homologous heart tissue[3–5]. While there is good evidence from clinical and experimental sources of the existence of antiheart antibodies whether from autologous, homologous or heterologous stimuli, there is considerable confusion as to the part they play in cytotoxic action on the heart. If antiheart antibodies are cytotoxic then they could readily influence developing embryonic heart tissue to produce either arrested growth with persistent anomalies or death. The possibility that such an immune (auto-immune) mechanism may play a part in the development of congenital heart disease seemed worthy of investigation.

Mature Sprague–Dawley female rats were immunized with homologous rat heart tissue one week before mating, approximately at the time of mating, and one week following mating. The number of new-born, their weight, and the gross and histological changes in the heart of the new-born were evaluated. Antigen was prepared from adult rat hearts of Sprague–Dawley rats removed shortly after death. The hearts were trimmed of connective tissue, minced with scissors and rinsed with cold saline to remove visible traces of blood. Extracts were made by homogenation with 4 times the tissue volume of saline carried out for 3 days at 4° C. The antigen was mixed with equal parts of complete Freund's adjuvant and emulsified. Female rats were divided into 3 experimental groups and 3 corresponding controls. Group I consisted of 5 female rats, 4 injected 7 days and 1 injected 12 days before mating (1 ml. of the antigen emulsion, 0·5 ml. subcutaneously and 0·5 ml. intraperitoneally). Group II consisted of 4 females immunized the morning following mating, and Group III consisted of 3 females immunized 7 days following mating. Control animals were mated at the same time as the experimental counterpart but were