EFFECT OF METHOD OF CONSERVATION ON IMMUNOGENICITY AND ANTIGENIC COMPOSITION OF XENOGENEIC HEART-VALVE TISSUES

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The effect of treatment of the aortic valves of pigs with γ rays, 4% formaldehyde solution, with sodium metaperiodate and glutaraldehyde (by Carpentier's method) on the immunogenicity and antigenic composition of the tissue was studied. No new antigenic specificities were found in saline extracts of the preserved valves. The immunogenicity of extracts of the valves treated with γ rays and formaldehyde solution was fully preserved. Formaldehyde slightly reduced the antigenic activity of the valve tissue. When Carpentier's method was used, the immunogenic and antigenic properties of the xenogeneic valves were considerably suppressed.

Differences in the value of methods used in surgical practice to treat xenogeneic valves (usually valves from pigs' hearts), as factors influencing the allergenic properties of the valves, were established in previous investigations in which anaphylaxis and hypersensitivity of delayed type in guinea pigs were used as models.

These observations, together with results indicating that patients may develop immunological reactions to the transplantation of xenogeneic valves [3-6, 8, 10], have necessitated a more detailed study of the antigenic composition of the tissues of pig heart valves and of their immunogenicity after preservation by different methods.

EXPERIMENTAL METHOD

Saline extracts of the valves were used: native (AGN), treated with γ rays (AGG) [7], with 4% formaldehyde solution (AGF) [9], or by conditioning (AGC) [4].

The homogenized tissue was covered with physiological saline (1 : 5) and stirred for 2 h with a magnetic mixer at 20°C, after which it was allowed to stand for 15-18 h in a refrigerator (4°C).

Antisera against water-soluble antigens of the valves and pig serum were obtained by immunizing rabbits by the method of Gusev and Yazova [1] (the total immunizing dose of each antigen as protein was 160-200 μg). The methods of investigation were the double diffusion test in agar and immunoelectrophoresis in agar on plates measuring 9 × 12 cm in veronal-medinal buffer (pH 8.4-8.6) with a voltage of 120 V and current of 40 mA. Extracts equalized for protein content, determined by Lowry's method, were used in the reactions.

EXPERIMENTAL RESULTS

The immunogenicity of the saline extracts of the valves treated by the various methods and studied by rabbit immunization methods varied significantly both in the titer of the sera obtained (Table 1) and in the assortment of antibodies in them.

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TABLE 1. Titers of Precipitating Antibodies in Rabbit Sera

Antigen for immunization	Titers of antibodies against antigens of native valve	Titers of antibodies against homologous antigens
AGN	1:128—1:256	1:128—1:256
AGF	1:128—1:256	1:4 —1:8
AGG	1:128—1:256	1:128—1:256
AGC	1:2 —1:16	1:2 —1:4

TABLE 2. Protein Concentration in Saline Extracts of Valves

Method of preservation	Protein concentration (in mg/ml)
Native	5,14 (5,17—5,32)
Irradiation with γ rays	4,28 (3,46—5,09)
4% formaldehyde solution	0,57 (0,50—0,67)
Protein concentration (in mg/ml)	0,54 (0,45—0,62)

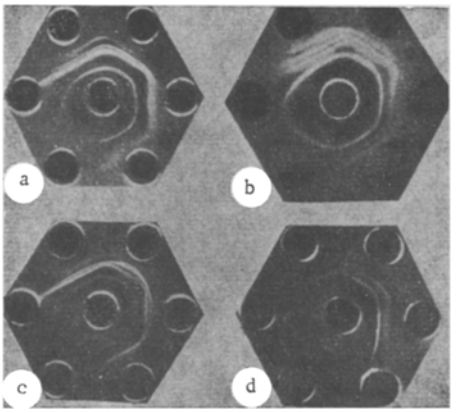

Fig. 1. Antibodies in antisera against extracts of heart valves preserved by different methods. Central wells contain antisera against extracts of valves: a) native, b) treated with γ rays c) treated with formaldehyde solution, d) conditioned. Peripheral wells contain extract of tissue of native valves in dilutions of 1 : 4-1 : 128.

It will be clear from Table 1 that the titers of sera obtained by immunization of rabbits with the AGN, AGG, and AGF extracts and in the precipitation test with AGN differed essentially from the titers of the sera of the animals immunized with AGC extracts. If these sera were tested with homologous antigens, it was found that AGF gave precipitation bands only in low titers (1 : 4-1 : 8), whereas AGG with the homologous serum worked in those same titers. The AGC obtained as described above was inert, for only when it was concentrated tenfold was a reaction observed in titers of 1 : 2-1 : 4.

Immunization of the animals with extracts of valves preserved by the different methods led to the formation of different spectra of antibodies, as shown by the precipitation test and by immunoelectrophoresis in agar. For instance, antisera against extracts from native valves (ASN) or valves treated with formaldehyde solution (ASF) and γ rays (ASG) regularly formed four precipitation bands in Ouchterlony's test with AGN antigens (Fig. 1a, b, c). Serum against extract of the conditioned valves (ASC) gave only one or two bands with AGN (Fig. 1d). These differences in the antigenic activity of the valve tissues were clearer still in the immunoelectrophoresis test. Antisera against extracts from the native, irradiated, and formalinized valves in all cases formed four main precipitation arcs when reacting with AGN. When the sera of animals immunized with extract from conditioned valves were used, under these conditions only two precipitation bands were detected (Fig. 2).

The first step in the study of the antigenic composition of the valve extracts was to detect whether or not they contained antigens in common with serum proteins. For this purpose immunoelectrophoresis of AGN and of pig serum (PS) was carried out with pig antiserum (ASP) and ASN. The results showed that extract of the native valves formed four chief precipitation arcs with the homologous antiserum in the zones of albumins and α_2-, β_1-, and γ-globulins. Meanwhile, in the reaction with ASP precipitation bands were discovered only in the zones of albumins and α_2-globulins, but not of the γ-globulins. Comparison between these results and those of the reactions of pig blood serum with ASP and ASN shows that besides antigens common to the valves and serum, the valve extracts also contained components specific for the valve tissues only.

The second stage of the investigation was to study changes produced in the antigenic composition of the valves by the various methods of treatment. Extracts of the valves treated with γ rays, like AGN, formed four precipitation bands in the reaction with ASN and the homologous antiserum (ASG) (Fig. 3a, b)

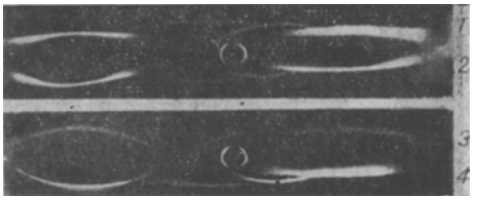

Fig. 2. Immunophoretic analysis of antibodies against extracts of tissues from preserved heart valves. Central wells contain extract of native valves, gutters contain antisera against antigens of valve tissues: 1) native, 2) treated with formaldehyde solution, 3) conditioned, 4) treated with γ rays.

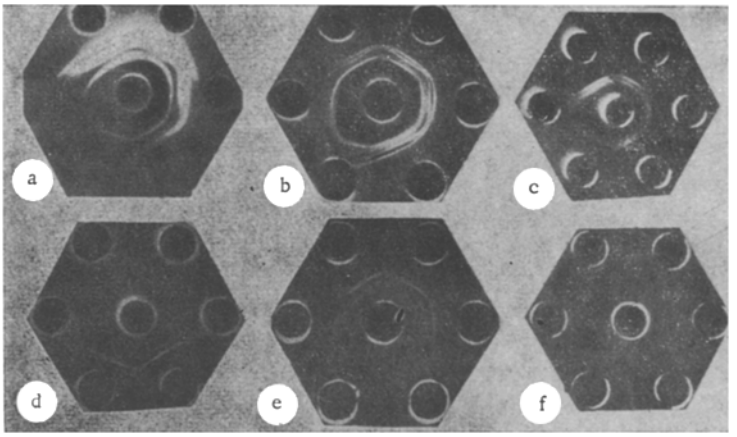

Fig. 3. Antigenic composition of tissues of heart valves treated by various methods. Central wells contain antigens of valves: a, b) treated with γ rays, c, d) treated with formaldehyde solution, e, f) conditioned; peripheral wells contain antisera: a, c, e) against extract of native valves, b, d, f) homologous.

while extract from AGF gave only 1-2 lines (Fig. 3c, d) and extract from the conditioned valves (AGC) was inert in the tests with homologous serum (Fig. 3e, f). Similar results were obtained in the immunoelectrophoresis test. Antibodies characterizing a group of antigens specific for pig heart valves were found in all the antisera. Meanwhile no new antigens were found in the extracts of the treated valves. This is evidence that treatment of the valves does not lead to the formation of new antigenic specificities.

It can be concluded from these results that treatment of heart valves by the conditioning method considerably diminishes the immunogenicity and the antigenic properties of the tissues. The advantage of this method becomes particularly clear when the protein content in the saline extracts from homogenates of valves treated by different methods is compared (Table 2).

Treatment of the valves with 4% formaldehyde solution, like the conditioning method, led to a sharp decrease in the extraction of water-soluble proteins from the tissue. However, this was accompanied by only a very small decrease in the antigenic properties of the valves and by the preservation of their immunogenicity. Meanwhile the nitrogen content (determined by the Kjeldahl method) showed only slight variation whatever method was used to preserve the valves (Table 3).

This suggests that the changes detected in the antigenic properties of the tissue are connected primarily with blocking of the antigenic determinants by the chemical preservatives (formaldehyde, a mixture of glutaraldehyde with sodium metaperiodate) rather than with the removal of the proteins themselves from the tissues. These processes are probably responsible also for the "inertia" of the saline extracts of the valves treated with formaldehyde solution and by the conditioning method in the gel diffusion tests.

TABLE 3. Nitrogen Content in Tissue of Valves Preserved by Different Methods

Method of preservation	Nitrogen content (in mg/g)
Native	17,82 (15,42—18,52)
Irradiation with γ rays	15,84 (12,17—18,43)
4% formaldehyde solution	13,21 (11,81—16,15)
Conditioning	18,13 (15,12—20,01)

The results show that from the immunological standpoint treatment of xenogeneic heart valves by conditioning is the most promising of the modern methods of preservation for clinical use.

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