# ANTIGENIC PROPERTIES OF HEART VALVE TISSUES PRESERVED BY DIFFERENT METHODS

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It was shown by the anaphylaxis method in guinea pigs that treatment of hog heart valves with γ-rays does not reduce their allergic properties, while the ability of the valves preserved in formalin solution to produce anaphylactic shock is considerably reduced. Treatment of the valves with solutions of metaperiodate and glutaraldehyde (the "conditioning" method) led to a sharp decrease in the sensitizing and anaphylactogenic properties of the tissues. Similar results were obtained by the study of increased sensitivity of delayed type by the method of allergic skin tests. The results are evidence of differences in the value of the xenogenetic methods used in preserving the tissues as factors reducing their antigenicity.

The widespread clinical use of xenogeneic grafts as replacements for irreversibly changed heart valves is limited by the frequent development of late degenerative changes in the graft tissues, leading to disturbance of its function. In recent years an increasingly large number of reports have been published in which the genesis of these lesions has been associated largely with the development of an immunological reaction of the recipient to the transplanted tissue [6, 7, 16, 17], although many investigators have been unable to observe signs of rejection of the transplanted valves or to discover circulating antibodies [5, 8, 11, 19]. The use of various conservation methods (keeping the tissues in formalin solution, irradiation with γ-rays followed by freezing) has only slightly improved the late results of transplantation [4, 6, 12, 18]. More hopeful clinical results have been obtained by transplanting valves treated by the "conditioning" method suggested by Carpenter et al. [7] and based on the more complete removal of antigenic components of the valves [6, 14].

Investigation of the various methods used in practice to preserve valves could be valuable not only in the choice of the most adequate method of preserving the grafts, but also in connection with the study of the effect of various factors on the antigenic structure of the tissue.

The object of the investigation described below was to study the allergic properties of the tissues of hog heart valves preserved by different methods used in clinical practice and to compare them with the antigenicity of intact valves.

## EXPERIMENTAL METHOD

The allergic properties of native hog aortic valves and of valves treated by various methods were studied. These methods included: γ-ray irradiation (3 Mrad) followed by freezing [15]; 4% formaldehyde solution buffered to pH 5.6 for 3 weeks and 6 months, respectively [10]; the conditioning method ( a solution of sodium metaperiodate and glutaraldehyde solution [7]) with conservation for 3 days and 3 months, respectively. Saline extracts of the tissues were investigated as fractions possessing the properties of

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TABLE 1. Anaphylactogenicity of Extracts of Hog Valves Preserved by Different Methods

| Sensitization | Severity of shock after reacting to injection of | | | |
|---|---|---|---|---|
| | AGN | AGF | AGG | AGC |
| AGN | 3,8/26 | — | — | 0,18/22 |
| AGF | 4,0/10 | 2,7/16 | — | — |
| AGG | 3,7/4 | — | 4,0/9 | — |
| AGC: | | | | |
| 3 days | 0,16/25 | — | — | 0/29 |
| 3 months | 0/6 | — | — | — |

Note. Here and in Table 2: AGN – extract from native valves; AGF – extract from valves treated with formalin; AGG – extract from irradiated valves; AGC – extract from valves treated by conditioning. Numerator gives mean anaphylactic index (severity of shock assessed by a 5-point system); denominator gives number of animals in group.

TABLE 2. Allergic Skin Tests for Sensitization by Extracts of Hog Valves Preserved by Different Methods*

| Sensitization | Intensity of reaction after reacting injection of | | | |
|---|---|---|---|---|
| | AGN | AGF | AGG | AGC |
| AGN | 2,7/6 | 1,3/3 | — | 0,6/3 |
| AGF | 2,3/6 | 1,4/7 | — | — |
| AGG | — | — | 2,5/3 | — |
| AGC | 0,5/3 | — | — | 0,3/3 |

* See legend and note to Table 1.

transplantation antigens [2, 3, 13]. The homogenized tissue was kept in physiological saline (1 : 5) at 4°C for 24 h, the supernatant was isolated by centrifugation, and its protein was determined by Lowry's method.

Experiments were carried out on 147 guinea pigs weighing 250-300 g, sensitized by injection of 0.025 ml of extract together with an equal volume of Freund's complete adjuvant into the plantar pad of all four limbs (total dose 10-15 μg protein per animal). After 24 days, anaphylactic shock was induced in the animals by an injection of 0.5-1 ml antigen (100-200 μg protein) into the heart, and the severity of the shock in the various groups was assessed by means of an anaphylactic index [20]. Increased sensitivity of delayed type was detected by allergic skin tests, the intensity of which was assessed by Eisen's method [9]. The $\chi^2$ criterion was used for the statistical analysis of the results [1].

## EXPERIMENTAL RESULTS

The results showing the reproduction of anaphylactic shock in the guinea pigs are given in Table 1. They indicate marked sensitizing properties of the tissues of native hog valves. Signs of anaphylactic shock appeared 1-2 min after injection of the reacting dose into guinea pigs, and in 46% of cases death of the animals ensued.

The same high sensitizing activity was also found in the tissues of valves treated by γ rays or formalin. The duration of conservation of the valves in formaldehyde solution had no effect on the degree of their antigenicity.

Injection of 0.5-1 ml of extracts of antigens from native valves (GN) as reacting dose in animals sensitized with antigens from valves treated with formalin (AGF) or γ rays (AGG) induced severe anaphylactic reactions in the animals with death in 60-70% of cases. Injection of the corresponding "specific" antigenic extract into immunized guinea pigs showed that the reactivity of the AGF was much less than that of AGN and AGG (anaphylactic index 2, 7, 3.8, and 4, respectively). Of the 16 animals sensitized with AGF, only four developed a severe anaphylactic reaction to a second injection, and in no case did the animal die.

Different results were obtained in groups of animals sensitized with extracts of valves treated by the conditioning method. Of 25 animals sensitized with extracts from valves preserved for 3 days, only three developed slight signs of anaphylactic shock in response to a second injection of AGN. In guinea pigs sensitized with AGC preserved for 3 months, no signs of an anaphylactic reaction developed in any animal. When AGC was used as the reacting injection, the almost complete absence of anaphylactogenic activity was demonstrated (tne anaphylactic index for animals sensitized with AGN was 0.18, and for animals sensitized with AGC it was 0).

The study of hypersensitivity of delayed type showed a similar pattern (Table 2). The appearance of distinct hypersensitivity of delayed type after sensitization with extracts from native valves and valves treated with γ rays or formalin will be noted. The allergic properties of components extracted from conditioned valves were slight in degree. Meanwhile, after intradermal injection of 0.1 ml AGF into sensitized animals, the skin reactions were significantly less marked than after injection of AGN. Consequently, in these experiments the AGF showed lower allergenic activity than AGV or AGG. The allergenic activity of AGC was minimal (0.3-0.6).

These results indicate that the methods used to conserve grafts in practice differ in importance as factors reducing their antigenicity. The method of preference must be regarded as conditioning: treatment of the valves with solutions of metaperiodate and glutaraldehyde leading to a sharp decrease in the antigenicity of the tissues.

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