

COMPARATIVE STUDY OF ANTIGENIC PROPERTIES OF NATIVE AND FORMALINIZED SKIN

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The method of anaphylaxis with desensitization and Ouchterlony's gel-diffusion test were used to study the antigenic composition of saline extracts of native and formalinized skin, while the composition of the protein fractions of these tissues was investigated by electrophoresis in acrylamide gel. No difference in the antigenic properties of extracts of native and formalinized tissues and no significant difference in the qualitative composition of their protein fractions were detected.

According to recent reports [1, 3, 5, 8], the incompatibility reaction is absent during transplantation of skin homografts preserved in weak (0.5-1%) solutions of formalin. This has been taken to indicate changes in the antigenic properties of the tissues after treatment with formalin. However, no changes have been found in the group or organ-specific antigens in formalinized tissues [2, 4].

The object of the investigation described below was to study the effect of formalin on the antigenic properties of skin.

EXPERIMENTAL METHOD AND RESULTS

The antigenic properties of native and formalinized tissues were studied by the anaphylaxis with desensitization test and by Ouchterlony's gel-diffusion test. The qualitative protein composition of native and formalinized skin was determined by electrophoresis in acrylamide gel.

The anaphylaxis with desensitization test was carried out on 46 guinea pigs: 27 animals (group 1) were sensitized by subcutaneous injection of a saline extract of rabbit's skin untreated with formalin, and 19 guinea pigs (group 2) received an injection of saline extract of rabbit skin kept in 0.5% formalin solution for 1 month. The animals of group 1 were desensitized with the extract of formalinized skin, while the animals of group 2 were desensitized with extract of native skin. An extract of skin identical with that taken for sensitization was used for the reacting dose.

The results of the anaphylaxis with desensitization test are given in Table 1.

They show that desensitization of the animals of both groups was complete and that the reacting injection was not accompanied by anaphylactic reactions.

Consequently the anaphylaxis with desensitization tests revealed no difference in the antigenic composition of the saline extracts of native and formalinized skin.

In Ouchterlony's gel-diffusion test, the antiserum formed the same number of precipitation bands with extracts of both native and formalinized skin. The precipitation bands ran smoothly into one another, demonstrating the identity of the antigens of the native and formalinized skin.

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TABLE 1. Results of Study of Native and Formalinized Skin by the Anaphylaxis with Desensitization Test

Group of animals	Expt. No.	Number of animals	Sensitization			Desensitization			Test of completeness of desensitization			Reacting injection		
			antigen	protein content (in mg)	reaction	antigen	protein content (in mg)	reaction	antigen	protein content (in mg)	reaction	antigen	protein content (in mg)	reaction
1st	1	4	Extract of native skin	3.2	-	Extract of for-malinized skin	1.26	+	Extract of for-malinized skin	2.5	-	Extract of native skin	3.8	-
	2	5		3.9	-		1.3	+		2.6	-		4.9	-
	3	5		4.1	-		2.1	+		2.8	-		4.5	-
	4	4		4.4	-		1.9	+		3.9	-		8.4	-
	5	4		3.6	-		1.2	+		1.6	-		3.8	-
2nd	1	3	Extract of for-malinized skin	3.3	-	Extract of native skin	1.4	+	Extract of native skin	3.4	-	Extract of for-malinized skin	3.7	-
	2	4		3.6	-		1.4	+		3.4	-		3.7	-
	3	4		3.5	-		1.5	+		3.5	-		3.5	-
	4	4		3.9	-		1.8	+		3.6	-		4.8	-

Note. The control sensitized animals (not shown in the table) in every case died a few minutes after injection of the reacting dose of protein from anaphylactic shock.

By electrophoresis in acrylamide gel, three principal protein fractions were detected in the extracts of the native and formalinized skin. The protein fractions from formalinized skin were more weakly stained and had less sharp outlines than the fractions of native skin. No other significant differences could be found in the composition of the protein fractions of native and formalinized skin.

These experiments thus revealed no differences between the native and formalinized tissues. The conclusions stating that the antigenic properties of a tissue are changed by treatment with formalin [3] can evidently be explained by technical inaccuracies. In their experiments on anaphylaxis, the workers cited used extracts which were equal in volume and, therefore, unequal in protein content, for an extract of formalinized skin contains much less protein per unit volume than native extracts [6].

The weakening of the incompatibility reaction during grafting of tissues treated with formalin is evidently associated with the marked decrease in their content of water-soluble proteins, on account of the formation of methylene-bis-compounds, which are not hydrolyzed [7]. The amount of antigens entering the recipient's body from the formalinized graft is therefore considerably reduced, and this contributes to the decrease in activity of the rejection reaction.

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