BINDING OF DINITROCHLOROBENZENE WITH DERMAL PROTEINS AND USE OF CONJUGATES FOR SEROLOGIC DIAGNOSIS OF CONTACT ALLERGY

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Antibodies against dinitrochlorobenzene, its conjugate with homologous dermal proteins, and against carrier protein were found in the blood serum of guinea pigs with contact allergy to dinitrochlorobenzene.

Induction of hypersensitivity of delayed type by simple chemical compounds is associated, most workers believe [3, 9], with the ability of the hapten to combine in vivo with skin proteins. Some workers consider that the chief site for fixation of hapten with proteins is the epidermis [4, 5]. Most writers have obtained complete antigens in vitro with the use of heterologous or homologous serum proteins, as a rule, as carrier. However, sensitization with such conjugates causes antibody formation in high titers against the foreign protein also. Only isolated reports of the use of homologous skin proteins, again mainly epidermal proteins, can be found in the literature.

The object of the present investigation was to obtain conjugates of 2,4-dinitrochlorobenzene (DNCB) with the globular fraction of homologous dermis and to use them for the serologic study of contact allergy to this hapten. The obtaining of such a conjugate in vivo would show indirectly whether these proteins of the dermis participate in the formation of complete antigen following cutaneous application of hapten.

EXPERIMENTAL METHOD

Protein conjugates with DNCB were obtained in vivo and in vitro. To isolate proteins bound with DNCB in vivo, the skin of guinea pigs from which the hair had been shaved was painted with 10 ml of 10% DNCB solution in acetone. The animals were sacrificed 18 h later, and after removal of the epidermis by careful scraping, their skin was used to obtain globular dermal proteins. The proteins were extracted with physiological saline at pH 8.6 [2]. For preparation of the conjugate in vitro, the same fraction of proteins isolated from the skin of intact guinea pigs was used as carrier. The protein content was determined by the method of Lowry et al. [10]. Conjugation of DNCB with proteins was carried out as follows: 0.1 ml of 2% DNCB solution in acetone was added to 2 mg protein in 1 ml phosphate buffer, pH 8.0, and incubated for 3 h at room temperature. The resulting conjugate was freed from unbound DNCB by gel filtration on a column with Sephadex G-25, coarse [1], and kept in ampules at 4°.

RESULTS

In the experiments of series I, to determine whether DNCB was in fact bound with dermal proteins, 30 guinea pigs (weighing initially 250-270 g) were sensitized with the resulting conjugates. The animals were divided into three groups. The guinea pigs of group 1 were injected intraperitoneally with 4 mg of the

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TABLE 1. Antibody Titer in Blood of Guinea Pigs Sensitized with Conjugate against DNCB, Globular Fraction of Homologous Dermis, and Their Conjugates, M ± m*

Conjugate for sensitization	Test-antigen					
	DNCB		globular proteins		conjugates of DNCB + globular proteins	
	13th day	21st day	13th day	21st day	13th day	21st day
Prepared in vitro	2.1 ± 0.2	2.8 ± 0.1	1.7 ± 0.1	1.9 ± 0.4	2.5 ± 0.1	3.3 ± 0.2
Prepared in vivo	2.1 ± 0.2	3.0 ± 0.3	1.6 ± 0.2	1.9 ± 0.4	2.7 ± 0.2	3.4 ± 0.1

*Log of titers of antibodies.

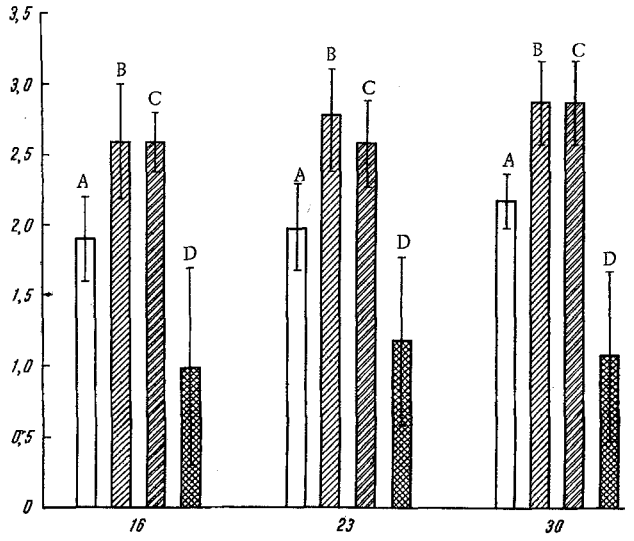

Fig. 1. Blood antibody titer in guinea pigs with contact allergy to DNCB: A) antibodies against DNCB; B) antibodies against conjugate obtained in vivo; C) antibodies against conjugate obtained in vitro; D) antibodies against carrier protein. Abscissa, day of testing; ordinate, antibody titer (in $\log_{10}$ units).

conjugate of DNCB with globular proteins obtained in vitro, with simultaneous intramuscular injection of 0.2 ml Freund's adjuvant. On the fifth day the animals were revaccinated by intracardiac injection of 1.5 mg of conjugate. The revaccination had to be given in fractional doses, because signs of anaphylactic shock were observed. The guinea pigs of group 2 were sensitized by the same scheme, but using conjugate obtained in vivo. The animals of group 3 acted as the control.

The level of allergy was tested percutaneously with 0.2% DNCB solution in acetone on the fifth day after revaccination, the reaction being read after 18 h. Antibody formation against DNCB, dermal proteins, and their conjugates was determined by the passive hemagglutination test (PHT).

The results of the skin tests showed significant development of hypersensitivity of delayed type against DNCB in guinea pigs sensitized with conjugates compared with intact animals.

The results of the PHT are given in Table 1. Results of the study of sera of intact animals are not shown in this table, because humoral antibodies against the tested antigens were not found in any of them. As Table 1 shows, antibodies against DNCB were discovered in all experimental animals. This means that its conjugates with globular dermal proteins could be obtained both in vivo and in vitro. Antibodies against the carrier proteins themselves also were found. Conjugates obtained in vivo and in vitro evidently were only slightly different antigenically, for in animals sensitized with them no significant differences in antibody formation could be found.

In the experiments of series II, the conjugates as well as DNCB and globular proteins of the intact dermis were used to detect antibodies in the presence of contact allergy to this hapten. Contact allergy was produced in 36 guinea pigs by five daily cutaneous applications of 3 drops of a 2% solution of DNCB in acetone. On the 16th, 23rd, and 30th days from the beginning of sensitization blood was taken for determination of antibodies against DNCB, cutaneous proteins, and their conjugates in the PHT.

Antibodies against DNCB were detected with the use of tanninized erythrocytes sensitized both to hapten alone and to its conjugates (Fig. 1). At all times of sensitization, antibody formation against the conjugates was significantly higher than against pure DNCB. Antibodies against globular proteins of the intact dermis, i.e., to the carrier protein, were also found, but the titer of antibodies against them was always significantly lower than the titer of antibodies against the conjugate.

The results thus indicate that DNCB, when applied to the skin, forms conjugates not only with proteins of the epidermis, as the investigations of Eisen and Tabachnik [6] showed, but also with proteins of the dermis. The present results agree with those obtained by Frey and Geleick [7], who succeeded in producing contact allergy to DNCB by subcutaneous injection of the hapten, i.e., avoiding the epidermis, and also with the findings of Macher and Sennlaub [11], who obtained contact allergy in animals by injection of DNCB into lymph glands.

When the present investigation was completed, the writers learned of the work of Watanabe and Ofuji [12], who also detected antibodies against conjugates of DNCB with homologous skin proteins.

The results of these investigations suggest the formation of humoral antibodies during contact allergy to DNCB, but in low titers, in agreement with the observations of Johnson [8]. Formation of antibodies is observed not only against the hapten, but even more intensively against the hapten–protein complex, and also against the carrier protein.

The use of conjugates thus greatly improves the chance of serologic detection of contact allergy to chemical compounds, a matter of special importance during the study of weak chemical allergens.

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