

Effect of Highly Radiolabelled 2,4-Dinitrochlorobenzene (DNCB) on Experimental DNCB Contact Dermatitis in Guinea Pigs

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With the aid of epicutaneous application of 2,4-dinitrochlorobenzene (DNCB) solution in acetone, we induced a cutaneous allergic reaction of the delayed type. Our question was whether the development of the DNCB cutaneous sensitivity could be suppressed by highly radiolabelled DNCB. On the basis of the clonal selection theory and our own results with other *in vivo*-experimental animal models, one could suppose that the highly radiolabelled DNCB as haptens binds to the Ig-membrane receptors of the genetically determined T-lymphocyte clone, and that the conjugated radioactivity (¹²⁵I) causes a selective radioactive damage to this competent T-lymphocyte subpopulation. By means of intracardially applied radiolabelled DNCB, we are able to induce either complete or very significant suppression of the cutaneous DNCB immune response. In the second experiment, the highly radiolabelled DNCB was not able to inhibit sensitization to a simultaneously applied 4-ethoxy-methylene-2-phenyl-oxazolone (oxazolone). This result clearly demonstrates the antigen specificity of this form of immune suppression.

Key words: antigen-specific suppression of DNCB-allergy; deletion of T-cell clone by radio-labelled DNCB; antigen-specific suppression of delayed immune reaction.

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According to the theory of clonal selection, the specificity of immunological responses is genetically determined and controlled. In the frame of an unending spectrum of antigens, each antigenic determinant corresponds to a genetically determined clone of B- and/or T-lymphocytes. In other words, each antigen can stimulate only one distinct subpopulation of B- and/or T-lymphocyte clone.

The "one signal model" says that primary interaction between antigen and Ig-membrane receptors of lymphocytes leads directly through reorganization of membrane receptors, through patching, capping and endocytosis to proliferation and differentiation of immuno-

competent cells. In the sense of the "two signal model", the antigen can trigger proliferation of B-lymphocytes, but is unable to induce differentiation of B-lymphocytes into antibody-forming and -secreting plasma cells, except thymus independent antigens. The differentiation of B-cells into antibody-producing cells requires a second signal delivered by T-helper lymphocytes. It is very likely that the same fate is also valid for the highly radioactively labelled antigens: the antigenic determinant binds to the Ig-receptor of B- and/or T-cells of the competent lymphocyte clone and brings about, by means of bound radioactivity, a selective damage and death of this cell subpopulation

without suppressing other not related immune responses. The antigen-specific inhibition of the immune responses by means of highly hot labelled antigens has been termed "suicide phenomenon" and has been tested in different *in vitro*- (1, 8, 9, 13) and *in vivo*- (3a, 3b, 4, 5, 6, 14) models.

In the new present experiment, we asked whether it would be possible to suppress antigen – specifically the experimental allergic 2,4-dinitrochlorobenzene (DNCB) contact dermatitis, with the aid of previously intracardially injected highly hot labelled DNCB.

The basis of the cutaneous DNCB-allergy is the clonal expansion of the genetically competent T-lymphocyte subpopulation. The combination of chemical substances of low molecular weight with the skin proteins occurs through the NH_2 -group of lysin, SH-group of cysteine, phenol-group of tyrosine or through the imidazole-group of histidine. There exist possibilities for coupling the hornless epidermis with keratin, procollagen and with the serum proteins in the blood. This is valid for paraphenylendiamine, 2,4-dinitrochlorobenzene, picrylchloride and other chemical compounds.

MATERIAL AND METHODS

Group 1. In 12 control white guinea pigs (Strain: Bor: supplied by Winkelmann, Borchem, FRG), 0.4–0.5 ml of 2,4-dinitrochlorobenzene (Merck, FRG) 30% solution in acetone was applied epicutaneously on the 1st day to the shaved skin of the right flank on an area of 30×50 mm.

The provocation test followed on the 12th day after sensitization with 0.05 ml of 0.1% DNCB solution in acetone on the shaved skin of the left flank on an area of 30×30 mm. The DNCB solution was put on the skin with the aid of a pipette. Visual control followed 12, 24 and 48 h after provocation. 48 h after testing, the animals were sacrificed for histological examination.

Group 2. The guinea pigs of this group were injected intracardially on day –3 with non-radioactive cold DNCB in alcoholic solution.

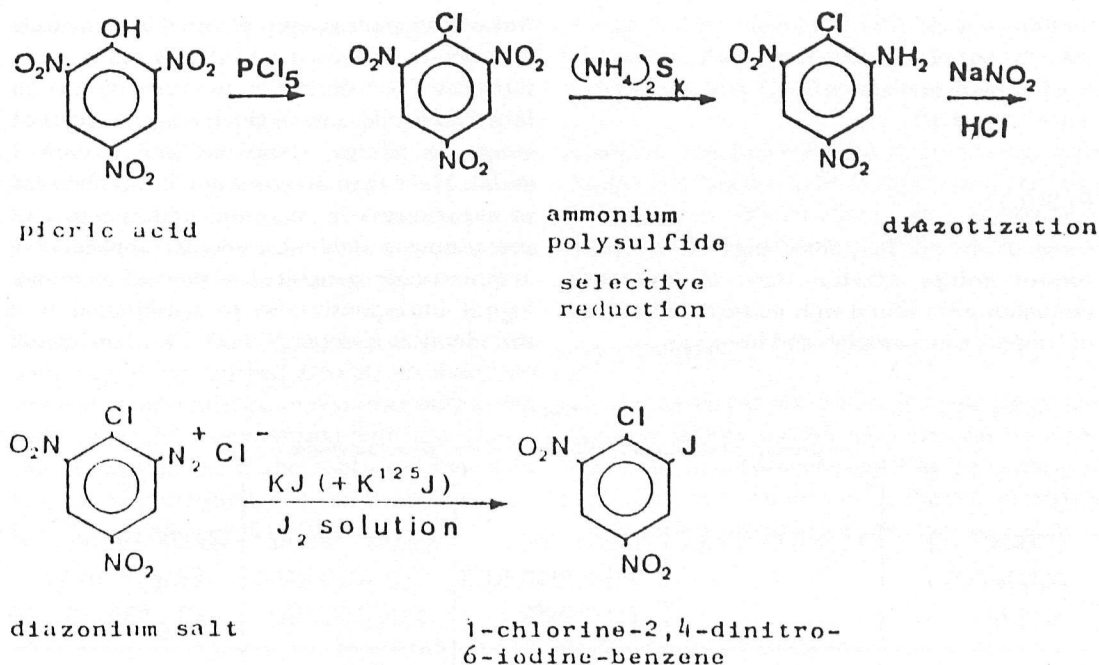
The total amount of DNCB substance injected in 1 ml of 0.01% alcoholic solution per animal was 0.1 mg. Sensitization with DNCB followed on day +1, and the provocation procedure was performed on day +12.

Group 3. On day –3, 12 guinea pigs received intracardially highly hot labelled growth hormone (HGH): 80–90 μCi ^{125}I /mg HGH (Institut National des Radioéléments, Belgium). The purpose of this combination of radioactivity and growth hormone was to prevent the binding of ^{125}I to the thyroid glands. Sensitization on day +1 and provocation test on day +12 were performed as in Groups 1 and 2.

Group 4. Twelve guinea pigs were injected intracardially on day –3 with highly hot labelled DNCB in 1 ml physiological saline solution of 80–90 μCi DNCB ^{125}I /dose/animal. The form of administration was as follows: 0.01 mg hot DNCB + 0.09 mg cold DNCB, i.e. a total of 0.1 mg. We should mention here that the Group 2 animals received 0.1 mg cold DNCB intracardially. Sensitization on day +1 and provocation procedure on day +12 were the same as in the preceding groups.

The labelling of DNCB with ^{125}I was carried out in the following manner: As dinitrochlorobenzene is insoluble in water, conventional labelling with radioactive iodine was not practicable. We were therefore obliged to use another method: The initial product was picric acid (1, 3, 5 trinitrophenol). Picric acid was transformed into trinitrochlorobenzene with the aid of phosphopentachloride. With ammoniumpolysulfide, trinitrochlorobenzene was then partially and selectively reduced to 1,3-dinitro-5-aminochlorobenzene. After diazotization, this last product was then transformed in the presence of J_2 -solution ($\text{KJ} + \text{KJ}^{125}$) into 1,3-dinitro-5-jod-chlorobenzene.

In chemical formulas:



Group 5. Nine guinea pigs were sensitized simultaneously with two different antigens:

- a) with 0.4–0.5 ml DNCB 30% solution as in the previous groups on the shaved skin of the right flank, and
- b) with 0.25 ml 20% solution in acetone of 4-ethoxy-methylene-2-phenyl-oxazolone (oxazolone: Hoffmann - La Roche, Basle) on the shaved skin of the left flank on an area of 30×50 mm.

The provocation tests were performed on the 12th day after sensitization:

- a) with DNCB in the same manner as in the previous groups on the shaved skin of the left flank on an area of 30×30 mm, 3.0 cm from the sensitization area with oxazolone, and
- b) with 0.05 ml 0.20% solution in acetone of oxazolone on the shaved skin of the right flank on an area of 30×30 mm, 3.0 cm from the sensitization area with DNCB.

Group 6. Ten guinea pigs were injected intracardially on day -3 with highly hot labelled DNCB in 1 ml physiological saline solution of 80–90 μCi DNCB ^{125}I /dose/animal. The form of administration was identical to that in Group 4. The sensitization of animals followed on day +1 in the same manner as in Group 5, i.e. with two different antigens: DNCB and oxazolone. On day +12 provocation with both substances was performed.

In Groups 1–4, the degrees of contact sensitivity were first graded macroscopically and then microscopically: the density of the inflammatory cells, and the intensity of infiltration of mononuclear cells were decisive in rating the degree of cutaneous sensitivity.

In Groups 5 and 6, the intensity of the inflammatory reaction was ascertained by lymphocyte, histocyte and basophil counts, by means of graticule II of Zeiss Company at $\times 400$. The intensities of reactions were graded as follows:

0(+) = 0–1
 + = 2–3

+(+) = 4-5
++ = 6-7
++(+) = 8-9
+++ = ≥ 10

RESULTS

Group 1. In all 12 guinea pigs of the pure control groups, classical signs of cutaneous dermatitis were found with massive infiltrations of lymphocytes, basophils and histocytes.

Group 2. In guinea pigs pretreated intracardially with non-radiolabelled cold DNCB we found in three cases very distinct, in two cases distinct, in four cases mild and in three cases no signs of cutaneous allergy, compared with Groups 1 and 3. More than 50 years ago, it was observed in experiments in man and guinea pigs that pretreatment with intravenously applied neoarsphenamine generated a state of immunological unresponsiveness to sensitization with the identical allergen (7. 10). The same result

Table 1
Intensity of cutaneous DNCB allergy in four guinea pig groups.

INTENSITY OF CUTANEOUS ALLERGY	1. CONTROL GROUP	2. GROUP PRE - TREATMENT WITH COLD DNCB	3. GROUP PRETREA - TED WITH RADIO - LABELLED HGH	4. GROUP PRETREA - TED WITH HIGHLY HOT LABELLED DNCB
0		● ● ●		● ● ● ● ● ● ● ●
+		● ● ● ●		● ●
++		● ●		
+++		● ● ●	● ●	
++++	● ● ● ● ● ● ● ●		● ● ● ● ● ● ● ●	

The Wilcoxon test (without correcting for the absence of continuity of the basic distribution) was used in an investigation of the significance. A comparison of duplicates was carried out (bilateral test). Level: 95 %.

- Comparison 1-2 significant differences
1-3 no significant differences
1-4 highly significant differences
2-3 significant differences
3-4 highly significant differences
2-4 significant differences

All groups with the exception of 1 to 3 differ significantly at the 95 % level.

could be achieved by feeding of contact allergens (2, 11).

Group 3. In guinea pigs, pretreated with highly radioactive labelled HGH, there was no sign of suppression of cutaneous allergy. Infiltrations of eosinophils, lymphocytes and histocytes were in 10 cases as massive as in the first pure control group, in two cases, one degree milder.

Group 4. In 10 of 12 guinea pigs pretreated with highly hot labelled DNCB, we found no signs of contact allergy, in two cases only a very mild reaction in comparison with the cutaneous inflammation in the control Group 1 or Group 3, pretreated with radiolabelled non-specific protein, i.e. of HGH.

Group 5. The intensity of DNCB and oxazolone cutaneous allergy was more dispersed than the intensity of DNCB inflammation in Group 1.

Group 6. The pretreatment of guinea pigs with highly hot labelled DNCB suppressed very significantly the DNCB allergy, the development of cutaneous oxazolone allergy remained undisturbed.

DISCUSSION

In the experimental dinitrochlorobenzene cutaneous allergy model, we were able to show again the possibility of suppressing the delayed type immune response with the aid of highly radioactively labelled antigen. We suppose that

Table 2
Intensity of cutaneous DNCB- and oxazolone-allergy in two mixed guinea pig groups

INTENSITY OF CUTANEOUS ALLERGY	5. CONTROL GROUP		6. GROUP PRETREATMENT WITH DNCB 125 I	
	DNCB - SENSITIZATION DNCB - PROVOCATION	OXAZOLONE - SENSITIZATION OXAZOLONE - PROVOCATION	DNCB - SENSITIZATION DNCB - PROVOCATION	OXAZOLONE - SENSITIZATION OXAZOLONE - PROVOCATION
0 (+)	●		●●●●●●●●	
+		●	●	●●●●●
++ (+)	●●●	●●●●●	●●	●●
+++	●●	●●●		●●●
++++ (+)	●			
+++++	●●			

Concerning DNCB-allergy there exists a highly significant difference between the statistical data distributions in Groups 5 and 6. The Wilcoxon test refuted the hypothesis that both distributions were equal at a level of significance $\alpha \geq 0.86\%$. This was even more strongly confirmed by the χ^2 test results at $\alpha \geq 0.5\%$. In the case of oxazolone allergy at a level of significance $\alpha \geq 5\%$, none of the tests refutes the hypothesis of identical distributions in Groups 5 and 6 (12).

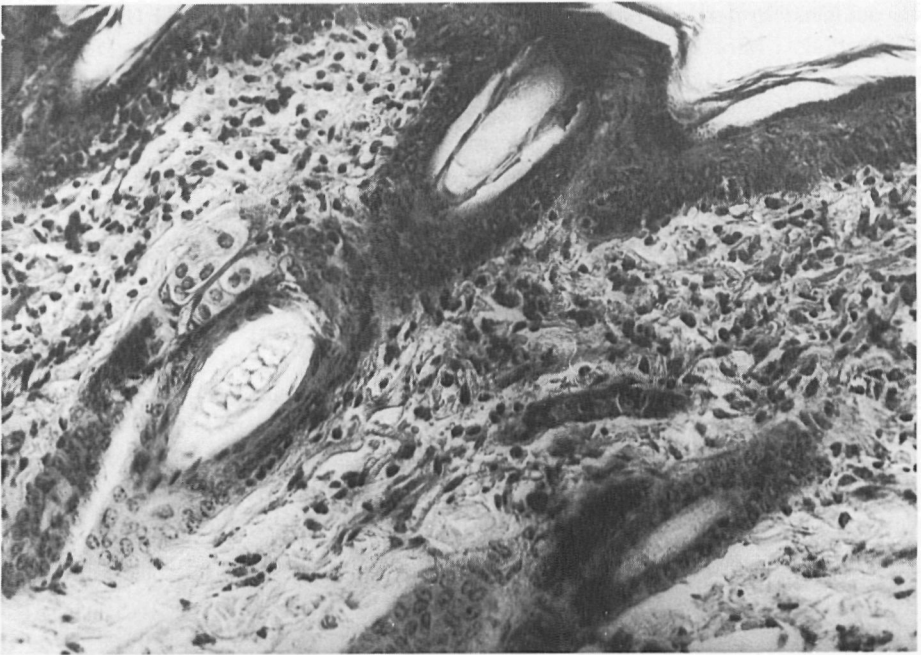


Fig. 1. DNCB contact allergy with an intensive inflammatory infiltration of the cutis H.E. $\times 250$.

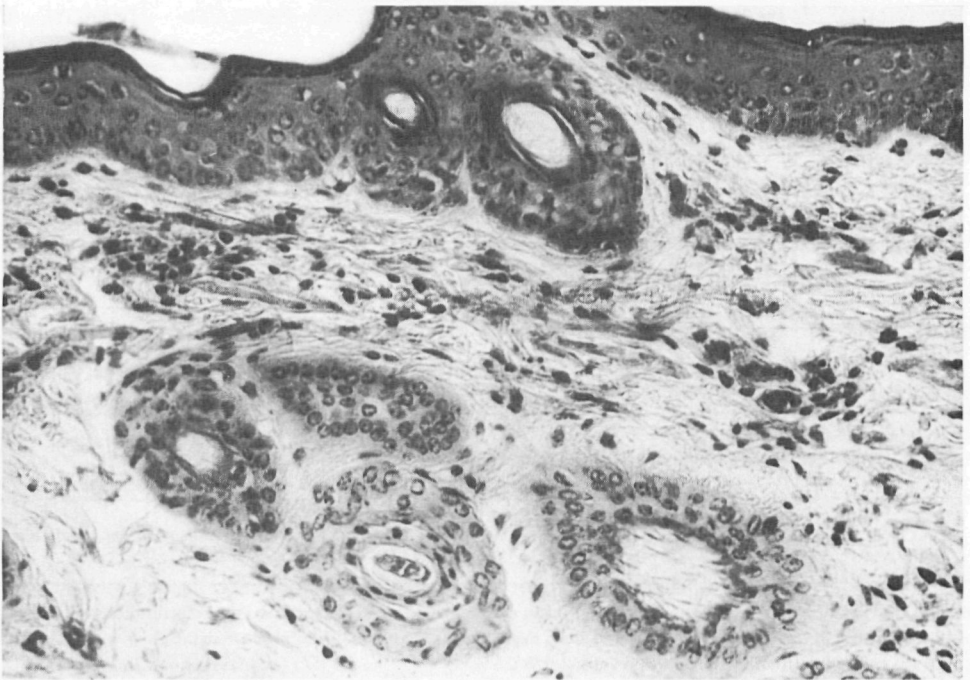


Fig. 2. Suppressed DNCB contact allergy with a slight inflammatory infiltration of the skin H.E. $\times 250$.

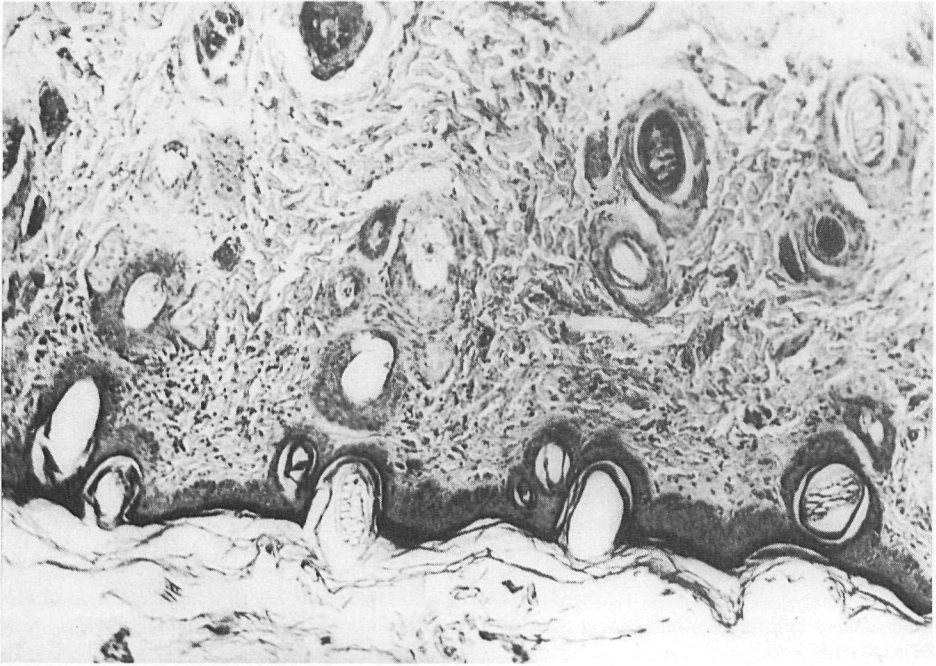


Fig. 3. Complete suppression of DNCB contact allergy with a largely regular histology of the epidermis and cutis H.E. $\times 100$.

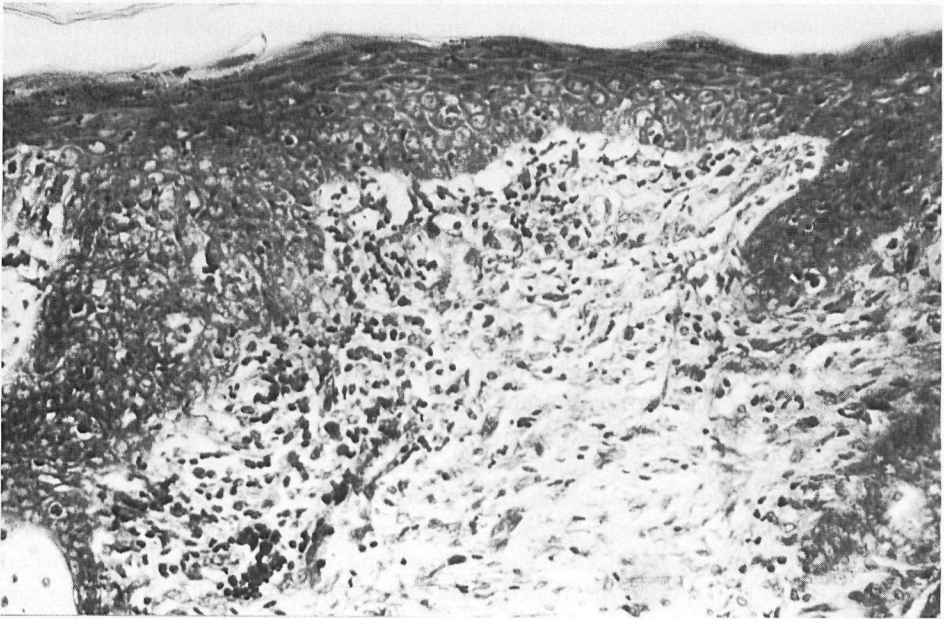


Fig. 4. Intense delayed hypersensitivity reaction on the DNCB test site in the 5th control group. H.E. $\times 40$.

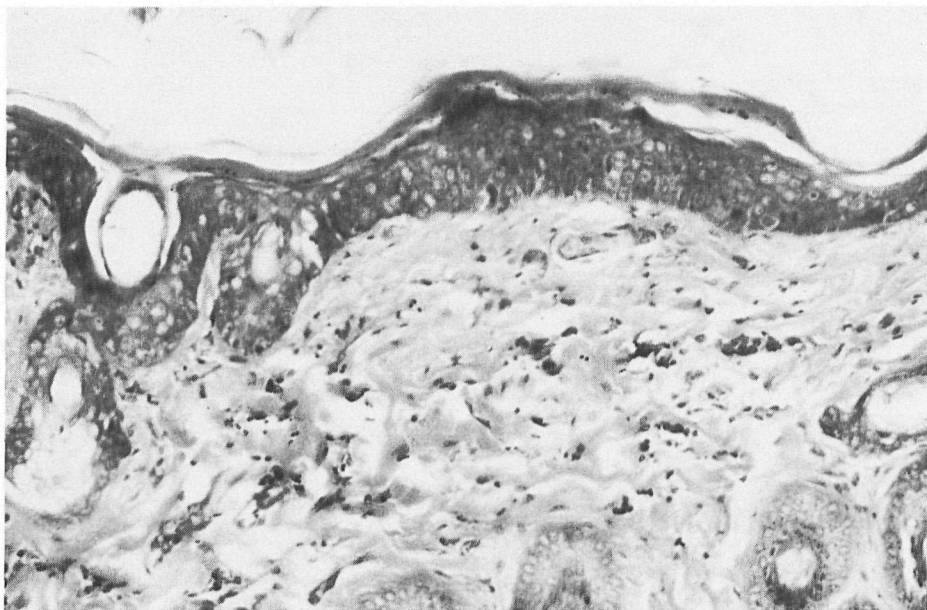


Fig. 5. Virtually absent inflammatory infiltrate on the DNCB test site in the 6th experimental group pretreated with highly radioactive DNCB. H.E. $\times 40$.

the highly hot labelled DNCB as hapten binds in the sense of the clonal selection theory to the specific receptors of the competent T-lymphocyte clone and that the bound radioactivity causes a selective damage to this T-cell subpopulation.

The pretreatment of guinea pigs with highly radiolabelled DNCB was not able to modify the intensity of the cutaneous oxazolone allergy. Consequently, the effect of highly radioactively labelled DNCB is an antigen-specific phenomenon.

The intravenous application of cold DNCB suppresses the DNCB immune response in line with older experimental results. The degree of this suppression corresponds to the term of partial immunological tolerance and is probably either due to deletion of the competent T-lymphocyte clone or to activation of specific T-suppressor cells. The intensity of suppression of DNCB cutaneous allergy in Group 4, pretreated with highly radiolabelled DNCB, was significantly higher than in Group 2, pretreated with cold DNCB.

In other words: the intravenous application of cold DNCB evoked in all probability a partial immunological unresponsiveness, the pretreatment with highly hot labelled DNCB induced practically complete immunological tolerance.

Up to the present we have used ^{125}I for labelling the antigens and autoantigens in an activity of 80–90 μCi per animal and per injection. This antigen-bound radioactivity was enough to bring about isolated antigen-specific damage to the corresponding B- and/or T-lymphocyte clone, without suppressing other not related immune responses. In the near future, we intend to use radioactive materials with a shorter life-time.

We know that the therapeutical use of highly hot labelled antigens, allergens and autoantigens in immunopathological and allergic processes is still out of the question. It should be emphasized that antigen-specific immune suppression can only be applied in immunopathological and autoimmune processes, where the antigen or autoantigen give rise to the synthesis of such antibodies or sensitized T-lymphocytes

which represent the pathogenic basis, the prime movers of the diseases.

The intention of this paper and of others on this subject is first the presentation of our experimental results concerning the possibility of antigen-specific immune suppression of a T-cell type of immune response, and second the presentation of the ideology of the *in vivo* induction of the state of immunological tolerance by means of highly hot labelled antigens.

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