STUDIES ON HYPERSENSITIVITY

IV. The Relationship between Contact and Delayed Sensitivity: A Study on the Specificity of Cellular Immune Reactions*

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In a previous study of the relationship between contact sensitivity to picryl chloride and delayed sensitivity to the picryl group of protein conjugates (1), the following observations were made:

1. Delayed sensitivity to the picryl group itself could be demonstrated in animals sensitized with a picryl conjugate and tested with a different picryl conjugate. Large testing doses were, however, required owing to the limited size of the picryl group. Delayed sensitivity to picryl protein conjugates generally appeared to entail a considerable degree of carrier specificity, that is specificity involving the environment of the picryl group on the carrier protein.

2. Guinea pigs immunized with picryl protein conjugates were shown to become contact-sensitive to picryl chloride, especially if homologous protein conjugates, such as picrylated guinea pig skin or picryl guinea pig gamma globulin, were used. The contact reactions were less intense than the delayed reactions to the picryl conjugates used for immunization.

3. Guinea pigs sensitized with picryl chloride showed delayed sensitivity reactions to conjugates of picryl with homologous proteins, as well as contact reactions to picryl chloride; but the delayed reactions were far less severe than the contact reactions.

These results were interpreted to mean that the relationship between contact sensitivity to picryl chloride and delayed sensitivity to picryl conjugates is that of two cross-reacting immunological systems with different carrier specificity; it is indeed impossible to duplicate carrier specificities equivalent to that resulting from the reaction of picryl chloride with the numerous autologous proteins with which it is able to conjugate *in vivo*, by immunization with a single picryl protein conjugate, even though a homologous protein

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carrier is used. This interpretation, generalized to other immunological systems, would define contact sensitivity as a form of delayed cellular sensitivity to autologous conjugates indistinguishable in its immunological mechanism from other classical forms of delayed sensitivity to proteins.

In the experiments reported previously and with the antigens studied, large immunizing doses of picryl protein conjugates had to be used to render the animals contact sensitive to picryl chloride. In view of the relatively small amounts of picryl chloride necessary to sensitize for contact reactivity, the question was raised by Eisen (2) whether trace amounts of unreacted picryl chloride which could conceivably be present in the protein conjugate preparation, in spite of all efforts for purification, was not responsible for the contact sensitivity to picryl chloride observed in animals immunized with large doses of these picryl proteins. Moreover, Eisen, working with another system, dinitrofluoro- and dinitrochlorobenzene (DNFB,[1] DNCB) and dinitrophenyl (DNP) proteins, failed to observe contact sensitivity after immunization of guinea pigs with DNP conjugates, whether heterologous or homologous. On the strength of these findings and of the observed affinity of chemical sensitizers for lymphocytes *in vitro* (2), this investigator felt that contact sensitivity represented a different form of immune reactivity to be distinguished from other immune responses.

In the present study, the problem is investigated further using several chemical sensitizers, picryl chloride, dinitrochlorobenzene, parachlorbenzoyl chloride (pCBCl), orthochlorbenzoyl chloride (oCBCl), and their conjugates with homologous and foreign proteins. Our earlier observations with the picryl system are confirmed and extended with the picryl, pCBCl, and oCBCl systems. It is shown that guinea pigs immunized with small amounts of these haptens conjugated with guinea pig serum albumin become regularly contact-sensitive to the corresponding chemical sensitizer. To induce a state of contact reactivity, the minimal necessary amounts by weight of hapten, when attached to pro-

[1] The following abbreviations, listed in order of appearance, are used throughout the paper.

DNFB, dinitrofluorobenzene
DNCB, dinitrochlorobenzene
DNP, dinitrophenyl
pCBCl, parachlorbenzoyl chloride
oCBCl, orthochlorbenzoyl chloride
PicCl, picryl chloride
PicBGG, picryl bovine gamma globulin
PicOva, picryl ovalbumin
PicGpAlb, picryl guinea pig albumin
DNPOva dinitrophenyl ovalbumin
DNPBSA, dinitrophenyl bovine serum albumin
DNPGpAlb, dinitrophenyl guinea pig albumin
pCBOva, parachlorbenzoyl ovalbumin
pCB, parachlorbenzoyl
pCBBSAlb, parachlorbenzoyl bovine serum albumin
pCBGpAlb, parachlorbenzoyl guinea pig albumin
oCBGpAlb, orthochlorbenzoyl guinea pig albumin
CitGpAlb, citraconyl guinea pig albumin
Ova, ovalbumin
BγG, bovine gamma globulin
BSA, bovine serum albumin
PicBγG, picryl bovine gamma globulin
PCA, passive cutaneous anaphylaxis

tein, are of the same order of magnitude as those of the corresponding simple chemical itself, showing that chance of contamination of conjugates with small amounts of unreacted sensitizer no longer need be considered as a possible explanation for the contact reactivity observed.

The DPN system alone is found to behave differently; in confirmation of Eisen's findings (2), contact reactivity cannot be induced by immunization with DNP protein conjugates. The reasons for the atypical behavior of this system will be discussed. The results to be presented show definitely that contact sensitivity is a form of delayed sensitivity to autologous conjugates with a considerable degree of carrier specificity and can be considered as an example of partial autosensitization.

Making use of these various systems of haptens conjugated with heterologous and homologous proteins, a study has also been made of the immunological specificity of the delayed reaction in order to compare it more particularly with the specificity of the Arthus reaction mediated by circulating antibodies.

Materials and Methods

Antigens.

Picryl antigens: Picryl chloride (PicCl); picryl bovine gamma globulin (PicBGG) 6.6 per cent picryl; picryl ovalbumin (PicOva) 6 per cent picryl; picryl guinea pig albumin (PicGpAlb) 9.5 per cent picryl.

Dinitrophenyl antigens: Dinitrofluorobenzene (DNFB); Dinitrochlorobenzene (DNCB); dinitrophenyl ovalbumin (DNPOva) 4 per cent dinitrophenyl (DNP) residue; dinitrophenyl bovine serum albumin (DNPBSA) 4.1 per cent DNP; dinitrophenyl guinea pig albumin (DNPGpAlb)-preparation III 6.16 per cent DNP (about 20 groups/molecule), preparation IV 2.08 per cent DNP (about 7 groups/molecule), preparation V 0.95 per cent DNP (about 3 groups/molecule).

Parachlorbenzoyl antigens: Parachlorbenzoyl chloride (pCBCl); *p*-chlorbenzoyl ovalbumin (pCBOva) 8.7 per cent pCB; *p*-chlorbenzoyl bovine serum albumin (pCBBSAlb) 9.8 per cent pCB; *p*-chlorbenzoyl guinea pig albumin (pCBGpAlb) 7.4 per cent pCB.

Orthochlorbenzoyl antigens: orthochlorbenzoyl chloride (oCBCl); orthochlorbenzoyl guinea pig albumin (oCBGpAlb) hapten content not estimated.

Citraconyl guinea pig albumin (CitGpAlb): guinea pig albumin treated with 20 per cent by weight of citraconic anhydride, hapten content not estimated.

Ovalbumin, 3 times recrystallized (Ova).

Bovine gamma globulin (BγG) and *Bovine serum albumin* (BSA), both from Armour Laboratories, Chicago.

Guinea pig albumin, prepared from pooled guinea pig serum by starch electrophoresis.

The various conjugated antigens were prepared according to the technique described before for the preparation of picryl conjugates (1, 3).

The hapten content of each conjugate was estimated by measuring the optic density at specific wave length in the region of peak absorption for the hapten concerned, and comparing it with the optic density of a known solution of hapten conjugated with glycine. The following wave lengths were used: 365 mμ for DNP antigens, 345 mμ for picryl antigens, and 250 mμ for pCB antigens.

Animals.—Male and female albino guinea pigs weighing 300 to 450 gm. were used.

Immunization.—The water soluble antigens were diluted with physiological saline, mixed with an equal volume of Difco complete adjuvants containing 2 mg. M butyricum per ml. and emulsified. The chemical sensitizers, not soluble in water were first dissolved in small volumes of alcohol; these were then diluted with physiological saline and emulsified with an equal volume of Difco adjuvants.

The immunizing injections were made in the foot-pads of the hind legs in a total volume of 0.2 ml.

Skin Tests.—The animals were carefully shaved on their flanks and the antigens to be tested were injected intradermally in a volume of 0.1 ml. of physiological saline. Animals, non-immunized or having received adjuvant alone, were also injected as controls. No more than 4 tests were made per animal. The specificity of the Arthus and delayed reactions were explored by testing the animals with:

1. The conjugated protein used for immunization.
2. An equal amount of the same hapten on other protein carriers.
3. The same carrier alone or with a different hapten.

Contact tests were generally made 24 hours before the intradermal test injections to avoid possible specific desensitization. The chemical sensitizers were dissolved in a non-irritant mixture made of four parts of acetone and one part of olive oil. A small drop was applied and spread on the skin of the flank. The following concentrations were used which were verified to be non-irritant: Picryl 0.5 per cent DNFB 0.15 per cent, pCBCl 2 per cent, oCBCl 5 per cent.

The animals were observed at 2, 4, 6, and 24 hours, and the nature of the reactions, immediate, delayed, or contact were differentiated and graded. The immediate Arthus-type reactions had a different appearance from the delayed reactions. They showed edema and in severe reactions necrosis, often with slight or severe hemorrhage. They were graded as follows: ± slight edema, + definite edema, ++ severe edema and slight hemorrhage, +++ severe edema with necrosis and hemorrhage, ++++ marked hemorrhagic necrosis. The delayed reactions were generally more extensive and showed mainly redness and induration. They were recorded according to size: ±, 0 to 5 mm.; +, 5 to 10 mm.; ++, 10 to 15 mm.; +++, 15 to 20 mm.; ++++, larger than 20 mm. The contact reactions were graded as follows: ±, definite but spotty erythematous inflammation; +, definite confluent erythematous inflammation; ++, as above, but slightly raised with palpable edge; +++, as above, more marked induration; ++++, maximal reaction. In several experiments, the antibody response against the conjugated proteins was studied by investigating whether the animals were anaphylactically sensitized. In other cases, the animals were bled prior to skin testing and the antibody content of the sera was assayed by passive cutaneous anaphylaxis (PCA) (4).

RESULTS

In Table I, the intensity of contact reactivity to picryl chloride and of delayed sensitivity to PicGpAlb and to PicBγG was compared in animals immunized with PicCl, PicGpAlb or PicOva. The minimal amount of the chemical sensitizer or of the protein conjugates in adjuvants required to render the animals contact-sensitive was investigated. For this, doses were used which were about equivalent with respect to the amount of picryl residue: PiCcl 0.032 to 8 μg., picryl conjugates 0.4 to 100 μg.

The number of animals with positive reactions and the average intensity of the reactions only are tabulated, since it is impracticable to describe individually the large number of animals used in these experiments and in similar experiments with other systems (Tables III, IV, VII).

The data show that animals immunized with PicgpAlb develop contact reactivity to PicCl nearly as readily as animals immunized with equivalent amounts of PicCl. Animals immunized with PicOva develop contact reactivity only to a very limited extent, in spite of large immunizing doses.

Delayed sensitivity to PicgpAlb, in the absence of antibodies detectable by

TABLE I

A Comparison of Contact Reactivity and Intradermal Delayed Sensitivity of Animals Sensitized with Picryl Chloride, Picryl Guinea pig Albumin, and Picryl Ovalbumin

Immunizing antigen	Test antigen PicCl 0.5 per cent		PicGpAlb 20 μg.		PicBGG 20 μg.		PCA reaction	
μg.								
PicCl	8 D	13 D	8 D	13 D	8 D	13 D	8 D	13 D
0.032	−	−	−	−	−	−	−	−
0.16	+1/6	±4/5	−	−	−	−	−	−
0.8	+5/6	+3/5	−	−	−	−	−	−
8.0	++4/5	++4/4	++*4/5	+1/4	±	+1/4	−	−
PicGpAlb								
0.4	+1/5	−	+2/5	++2/5	−	−	−	−
2.0	+3/6	±	+++	++4/5	+	−	−	−
10.0	+4/6	++	++++	++++	+	−	−	−
100.0	+4/5	++4/5	+++	C	±	A	±	++
PicOva								
0.4	−	−	−	−	−	−	−	−
2.0	−	−	−	A	−	A	−	+
10.0	−	±1/5	−	A	−	A	−	++
100.0	±1/5	+4/5	+2/5	A	±2/5	A	−	++

C, combined (delayed and Arthus) reaction; A, Arthus reaction; D, day.

The fraction indicates the number of animals showing reactions. No fraction implies that all animals in the group reacted. The grade refers to the intensity of the positive reactions.

± indicates some doubtful reactions.

Different groups of animals were used, at each immunizing dose level, for the tests on the 8th and 13th day after immunization.

* 50 μg. test dose of picGpAlb.

anaphylaxis, is very prominent in the groups sensitized with this antigen, but very weak in animals sensitized with PicCl and nearly absent in animals sensitized with equivalent amounts of PicOva.

Two other observations were made: (*a*) PicOva is a much better antigen than PicGpAlb in inducing antibody formation, as evidenced by the presence of Arthus and anaphylactic reactivity, with low immunizing doses, (*b*) the specificity of the delayed sensitivity in animals immunized with picryl conjugated with homologous, normally non-antigenic guinea pig albumin, involves clearly more than the picryl group itself, as only weak reactions are observed

to PicBγG, in presence of very severe reactions to the immunizing antigen, PicGpAlb. A considerable participation of the normally immunologically nonreactive guinea pig albumin in the specificity of the delayed reaction has thus been demonstrated. The homologous guinea pig albumin does more than act as an inert carrier for the hapten; it has been rendered antigenic by the presence of the hapten and participates in the specificity. This is, however, only complementary antigenicity because, although the specificity involves more than the picryl group, the hapten is always required, no reaction was observed with guinea pig albumin conjugated to another hapten, the citraconyl group.

This model allows for a better understanding of the mechanism of sensitivity in the case of immunization with the chemical sensitizer PicCl, where contact sensitivity can be considered as a form of autosensitization with a number of autologous proteins modified and rendered antigenic by combination with the picryl group. Presumably, many different conjugates of picryl with autologous proteins are the antigens responsible for the contact sensitivity of these animals. In contrast, the contact reactivity to PicCl observed in guinea pigs sensitized with PicGpAlb depends upon the formation during the contact test of the identical antigen PicGpAlb, the only picryl conjugate to which these animals are specifically sensitized. Therefore, while both groups of animals immunized with PicCl or with PicGpAlb show identical contact reactions to PicCl, the two reactions should have different immunological specificity and should be liable to be differentiated by specific desensitization. This was attempted in the experiments described in Table II.

Three experiments were carried out:

A. Two groups of guinea pigs were immunized with 0.8 μg. of PicCl or 10 μg. of PicGpAlb; then, on the 11th day after immunization, desensitization of half of the animals with PicGpAlb was carried out as described in Table II. Immediately after desensitization, the contact tests were applied. The group of animals immunized with PicGpAlb and desensitized with the same antigen were totally unreactive to contact with PicCl. In contrast, the contact sensitivity of animals immunized with PicCl was virtually unaffected by desensitization with PicGpAlb.

B. Another group of guinea pigs immunized with 10 μg. of PicGpAlb was contact tested with PicCl to evaluate the level of sensitivity before desensitization. They were tested again after desensitization; in every animal, the contact reactivity disappeared after the treatment.

C. A control experiment was carried out to ensure that the results of desensitization were immunologically specific and were not the result of the depletion of some intermediate effector of delayed sensitivity. Two groups of animals were immunized with 10 μg. BGG as well as with 10 μg. PicGpAlb or with 10 μg. PicCl; on the 7th day post immunization, half of the animals were desensitized to BGG as described in Table II. (This was done on the 7th rather than the 11th day because BGG is a strong antigen and considerable antibody production may occur after the 7th day). After this unrelated desensitization, PicCl contact tests were applied to all groups. No significant difference was observed between the contact sensitivity of control animals and of animals desensitized to BGG. Therefore, non-specific factors incident to desensitization cannot be considered responsible for the results observed after desensitization with PicGpAlb in Experiments A and B.

These experiments confirm our previous hypothesis and establish that the immunological specificity of the contact reaction is different after immunization with PicCl or with PicGpAlb; only in the latter case do we know the exact specificity.

In the course of the desensitization procedure, an interesting incidental ob-

TABLE II

Effect of Desensitization with Picryl Guinea pig Albumin on Contact Reactivity to Picryl Chloride, of Animals Sensitized with Either Picryl Chloride or Picryl Guinea pig Albumin

Immunizing antigen									
PicCl 0.8 μg.		PicGpAlb 10 μg.		PicGpAlb 10 μg.		PicCl 10 μg. + BGG 10 μg.		PicGpAlb 10 μg. + BGG 10 μg.	
I. Control	II. Desensitized	III. Control	IV. Desensitized	V. Before desensitized	After desensitized	VI. Control	VII. Desensitized to BGG	VIII. Control	IX. Desensitized to BGG
++++±	+++±	+++±	−(Died)	++	−	+++	++	+	(Died)
+++	++	++	−	++	−	++	+	+	++
+++±	++	++	−	+	−	+	+	±	±
+	+±	++	−	±	−	±	−	±	±
±	±	+±	−	±	−	±	−	±	±
−	±	+	−	−	−	−		−	−
−	−	±	−						
−	−	−	−						
−	−	−	−						

Groups II and IV: Animals desensitized on day 11 after immunization and tested together with I and III on day 12. Desensitization schedule; PicGpAlb 0.2 mg. intradermally, a few hours later 2 mg. intraperitoneally, next day 2 mg. intravenously. One animal in IV died, and 3/8 showed skin rashes 5 hours after the intravenous injection.

Group V: Animals tested on day 10 after immunization; desensitized on day 11 and tested as for Groups II and IV.

Groups VII and IX: Animals desensitized on day 7 after immunization and tested together with IV and VIII on day 8. Desensitization schedule: BGG 50 μg. intradermally, a few hours later 2 mg. intraperitoneally, next day 2 mg. intravenously. One animal in IX died, and 5/11 animals showed skin rashes 5 hours after the intravenous injection.

servation was made. After the initial intradermal and intraperitoneal injections, anaphylactic reactivity was generally nearly suppressed; 2 animals only out of 21 died of anaphylaxis as a result of the intravenous injections. However, a number of these animals (about 50 per cent) developed around the 5th to 6th hour after the intravenous desensitization a maculopapular skin rash not unlike human measles. The rash was in some cases still detectable 24 hours later.

In interpreting the mechanism of these rashes, it should be kept in mind not only that these animals were very highly delayed sensitive previous to

desensitization, but also that rapid antibody production is generally stimulated by the desensitizing injections. While it is tempting to consider the rashes as

TABLE III

A Comparison of Contact Reactivity and Delayed Intradermal Sensitivity in Animals Immunized with p-Chlorbenzoyl Chloride (pCBCl), pCB-Guinea pig Albumin (pCBGpAlb), pCBBSA, and pCB-ovalbumin (pCBOva)

Immunizing antigen	Animals tested with						
	Contact pCBCl 2 per cent	pCB-GpAlb 25 μg.	BSA 25 μg.	pCB-BSA 25 μg.	Ova 5 μg.	pCB-Ova 25 μg.	Anaphylaxis or PCA 1 to 2 mg.
μg.							
pCBCl							
4	±2/5	−		−			
20	++	+		−			pCBBSAlb:
100	+±	+3/5		−			negative
500	+±	++*A				+ A	
pCBGpAlb							
4	±1/5	++				−	
20	±3/5	+++				−	
100	+	+++±				±1/5	
500	+4/5	+± C		+ C			
pCBBSAlb							
4	±2/5	++	++±	++±±		−	
20	+1/9	+	+±	+++		−	pCBBSAlb: 2/9
100	−	−−4/5 C	+	+++ C			" 5/5
pCBOva							
4	−	−			++	+±	pCBOva: 0/5
20	−	+				++++	" 1/5
100	±2/5	±				+++±	" 5/5

All animals were contact tested on the 7th day and intradermally tested on the 8th day after immunization.

A, Arthus reaction; C, combined reaction. The fraction gives number of animals in the group showing positive reactions, the grade the severity of the positive reactions: no fraction implies that all animals in the group reacted.

* Test dose 50 μg.

the manifestation of localization of sensitive cells, it is not possible to rule out the participation of antibody in their mechanism. Nevertheless, the experimental procedure described above may provide a good technique to investigate further the pathogenesis of skin rashes that are observed in the course of infectious diseases.

The results obtained with the parachlorbenzoyl and the orthochlorbenzoyl

systems are presented in Tables III and IV. They are similar to those reported with the picryl system and confirm the observations made with that system. Immunization with pCBGpAlb or with oCBGpAlb induces contact sensitivity to the corresponding chemical sensitizer. Equivalent amounts of pCBBSAlb or pCBOva are not able to provoke the same degree of contact sensitivity.

Animals immunized with pCBGpAlb show also excellent delayed sensitivity to the immunizing antigen, but not to pCB Ova. These results illustrate that, in this system also, the specificity of the delayed reaction involves far more than the hapten alone. Animals sensitized with pCBCl show excellent contact reactivity to pCBCl, but much weaker delayed sensitivity to pCBGpAlb.

TABLE IV

Contact Reactivity to Orthochlorbenzoyl Chloride in Guinea pigs Immunized with oCBGpAlb

Immunizing antigen oCBGpAlb	Contact oCBCl 5 per cent	oCB-GpAlb 25 μg	pCB-GpAlb 25 μg.	Anaphylaxis oCBGpAlb 1 to 2 mg.
μg.				
25	+	++++	+++	0/5
	±	+++	++	
	±	+++	++	
	±	++	+	
	−	+++	++	
100	++	+++	+++	3/4
	+	+++	++	
	±	+++	++	
	±	+++	++	

The severity of the delayed reactions are recorded for individual pigs; no immediate reactions were observed. The animals were contact tested and intradermally injected on the 8th day after immunization.

Guinea pigs immunized with pCBBSAlb were skin-tested with equivalent amounts of the immunizing antigen, BSA, pCBGpAlb, and also pCBOva. As expected, the most severe reactions were observed with pCBBSAlb; however, good reactions were observed with pCBGpAlb, while the animals were negative to pCBOva. This is explainable by the cross-reactivity which one may expect to exist between the two protein carriers BSA and guinea pig albumin. These data extend to two other immunological systems the observations made with picryl conjugates. If our interpretation is correct, the specificity of the contact reaction in animals immunized with pCBCl and in animals immunized with pCBGpAlb is different. As in the case of the picryl system, this difference in specificity was demonstrated by a desensitization experiment (Table V). Animals immunized with pCBGpAlb could be desensitized to contact with pCBCl by treatment with the immunizing antigen pCBGpAlb,

showing that this is the antigen responsible for the contact reactivity in these animals. In contrast, the contact sensitivity of guinea pigs immunized with pCBCl was hardly affected by the same course of desensitization.

In Table VI, a study of the specificity of sensitivity in animals immunized with pCBCl or pCBGpAlb was carried out with respect to both the delayed and Arthus reactions. The animals sensitized with 20 μg. pCBCl and tested on the 12th day did not have a sufficient amount of antibody against the pCB

TABLE V

Effect of Desensitization with p-Chlorbenzoyl Guinea pig Albumin on Contact Reactivity to p-Chlorbenzoyl Chloride of Guinea pigs Sensitized with pCBCl or pCBGpAlb

Immunizing antigen			
pCBCl 20 μg.		pCBGpAlb 100 μg.	
Controls	Desensitized	Controls	Desensitized
++++	++++	++	−
+++	+++	++	−
+++	++±	++	−
++±	++±	+	−
++	++	+	−
++	+±	+	−
	−	+	−
		±	−
		±	−
		±	−
		−	−
		−	−

The animals were desensitized on the 11th day after immunization; desensitization schedule: pCBGpAlb 0.2 mg. intradermally, a few hours later 2 mg. intraperitoneally, next day 2 mg. intravenously; no deaths from anaphylaxis were observed. 2 per cent pCBCl was then applied for the contact test.

group for Arthus reactions to occur. They showed much stronger contact reactions to pCBCl than delayed reactions to pCBGpAlb. The only significant delayed reactions were observed to pCBGpAlb, none to pCBOva or to pCB-BSAlb.

The animals sensitized with 100 μg. pCbGpAlb were also tested on the 12th day when, with this antigen, enough antihapten antibody was present to elicit mild Arthus reactions, but not enough to interfere with the reading of the delayed reactions 24 hours later. Nearly identical Arthus reactions were observed with the three antigens bearing the pCB group, pCBGpAlb, pCBBSAlb, pCBOva. The delayed reactions 24 hours later were far more specific; they were most severe with the immunizing antigen pCBGpAlb, less intense with

pCBBSAlb which cross-reacts with it to a large extent, and neglibible to pCB-Ova. The Arthus reaction depending upon circulating antibody can be elicited equally well by the same hapten on different protein carriers, as the strong binding capacity of the antibody for the hapten is sufficient to cause the re-

TABLE VI

Specificity of the Sensitization in Animals Immunized with p-Chlorbenzoyl Chloride and Parachlorbenzoyl Guinea pig Albumin

Immunizing antigen	pCBCl 2 per cent	PCB GpAlb 25 μg.		pCBBSAlb 25 μg.		pCBOva 25 μg.		
	Contact	Arthus	Delayed	Arthus	Delayed	Arthus	Delayed	
			mm.		*mm.*		*mm.*	
pCBCl								
20 μg.	++++	−	15 × 15	−	−	−	−	
	+++	−	8 × 8	−	4 × 4	−	−	
	++	−	11 × 11	−	4 × 4	−	−	
	++±	−	5 × 5	−	−	−	−	
	++	−	15 × 15	−	−	−	−	
	+++	−	15 × 15	−	−	−	−	
								Anaphylaxis
pCBGpA								1 mg. pCBOva
100 μg.	±	±	20 × 20	±	15 × 15	±	−	Dead
	+	++	20 × 20	+	15 × 15	+	−	"
	++	++	30 × 25	++		++	−	"
	±	+	25 × 25	+	−	+	−	"
	++	−	25 × 25	−	15 × 20	−	4 × 4	"
	+	+++	30 × 30	+++	25 × 25	+++	6 × 6	"
	±	+	20 × 20	+	15 × 15	+	−	"
	+	+	25 × 25	±	20 × 20	±	−	
	+	++	30 × 20	±	15 × 20	+	−	
	−	++	30 × 30	++	−	++	−	
	−	+++	30 × 30	+++	15 × 15	+++	−	"
	++	+	25 × 25	+	20 × 20	+	4 × 4	

All the delayed reactions elicited to pCBBSAlb were of less intensity than those to pCBGpAlb.

Animals were tested for contact 12 days after immunization and injected intradermally next day.

action. The delayed reaction, depending upon cellular sensitivity, exhibits greater carrier specificity. These observations are in agreement with our previous findings.

The results obtained with the dinitrobenzene system are presented in Table VII. With this system, we failed to provoke contact reactivity to DNFB by immunization with protein conjugates, even when guinea pig albumin was used as the carrier, regardless of the dose used and the number of hapten groups

TABLE VII

A Comparison of Contact Reactivity and Intradermal Delayed Sensitivity, in Animals Immunized with Dinitrochlorbenzene (DNCB), Dinitrophenyl Guinea pig Albumin (DNPGpAlb), and Dinitrophenyl-ovalbumin (DNPOva)

Immunizing antigen	DNFB contact 0.15 per cent	DNP-GpAlb 50 μg.	Cit-GpAlb 50 μg.	DNP-BSA 37 μg.	Ova 5 μg.	DNP-Ova 5 μg.	PCA DNPova 1.0 mg.
μg.							
DNCB							
10	±3/5	±1/5					Neg.
100	++5/5	+4/5					Neg.
DNPGpAlb: III. 20 groups/mol.		25 μg.					
20	−	++++±	−	++4/5			DNPGpAlb +1/3
200	−	++++C	−	++			++3/3
DNPGpAlb: IV. 7 groups/mol.							
20	−	++++±	−	+±4/5			Neg.
200	−	++++	−	++			Neg.
DNPGpAlb: V. 3 groups/mol							
20	−	++±	−	±2/6			Neg.
200	−	+++	−	++			Neg.
DNPOva: 8 groups/mol							DNPOva
2	−	−			+++	++±	neg.*
20	−	±A3/5			++++±	+++ ±A2/5	+*
200	−	+A5/5			+++	+++ +A5/5	+*

A, Arthus reaction; C, combined reactions.

Fraction indicates number of animals in group showing reactions, grade refers to severity of positive reactions. No fraction indicates that all animals in the group showed reactions.

All animals were contact-tested for contact on the 8th day and injected intradermally on the 9th day after immunization.

* All these animals immunized with DNPOva. were also challenged intravenously with 1 mg. ovalbumin on day 11; none showed anaphylaxis.

per molecule of antigen, thus confirming the observations of Eisen with this system (2). It appears, therefore that the DNB system behaves differently from other chemical sensitizers. It is possible that these differences are related to the fact that both DNFB and DNCB are much more irritant to the skin than PicCl, pCBCl, or oCBCl. The non-irritant concentration of DNFB which can be used to test for contact sensitivity, 0.15 per cent is much smaller than that of the other sensitizers. Moreover, the fact that DNCB and DNFB cause inflammatory reactions at comparatively lower concentrations than the other sensitizers indicates that unknown reactions and transformations of the antigen may occur in the tissues, which render it more difficult to duplicate the exact specificity in an effort to induce contact sensitivity with an homologous protein conjugate prepared *in vitro*.

In the same Table VII, we present the results of an experiment concerning the effect of the number of DNP group per molecule of DNPGpAlb used for immunization on the severity and specificity of the delayed and Arthus reactions. As little as an average of 3 groups per molecule is sufficient to induce delayed sensitivity with a considerable degree of carrier specificity. As the number of groups is increased, the reactions are more severe and antibody production is induced. A comparison between the sensitizing capacity of DNP-Ova and DNPGpAlb with about the same number of groups per molecule shows that DNP on a foreign protein, such as ovalbumin, causes more Arthus reactivity and therefore antibody production than DNP on homologous guinea pig albumin.

DISCUSSION

The identification of contact sensitivity as a special case of delayed hypersensitivity has always been a matter of some difficulty, in spite of the obvious analogies between the two states. The classical work of Landsteiner and Chase (5–7) with the picryl system went some way to establish the link between the two, when they showed, firstly that the skin was not uniquely associated with the stimulation of contact sensitivity, since intraperitoneal injection could evoke it under appropriate circumstances (5), and secondly, that the free chemical itself was not essential, since a conjugate of picrylated red cell stromata was a very effective stimulant for contact sensitivity (6). Nevertheless, a satisfactory correlation between delayed sensitivity to heterologous protein conjugates and contact sensitivity to the simple chemical, which might have been expected in view of the free cross-reactivity of anti picryl antibodies with numerous picrylated proteins, was never demonstrable (2).

In the work reported here, it is shown that for systems other than DNP and with a proper choice of conjugate, a very much more satisfactory correlation can be shown between contact sensitivity and delayed (intradermal) reactivity to conjugate in animals sensitized, either with free chemical or with

conjugate. The failure of this correlation to be complete can readily be explained by the striking carrier specificity of the delayed reaction, that is by the marked effect of the carrier protein on the total specificity, even when that protein, being homologous, is itself non-antigenic in the species. Thus, contact reactivity is likely to be conditioned by the carrier specificities of an unknown number of autologous proteins, one of which may be analogous with the stromatic protein of Landsteiner and Chase (6) and another be identical or cross-reactive with serum albumin.

A further factor which may explain why conjugates with serum albumin are so successful in establishing the delayed hypersensitive state, including the state of contact reactivity, is the intrinsically poor antigenicity of the hapten homologous albumin conjugate itself, as a stimulant of antibody production against the hapten. Serum albumins, such as BSA are poor antigens in the guinea pig and homologous conjugates are less powerful than heterologous conjugates in provoking anti-hapten antibody (Table I). The use of a kind of protein which is a weak antigen and in its homologous form gives a minimal stimulus to the antibody production, and the fact that this appears to enhance delayed sensitization certainly suggests a sequential relationship between the two kinds of immune response. A study of contact sensitivity and antibody production over a period of time in animals immunized with a single dose of homologous albumin conjugate may throw light upon the progress from one state to the other.

The relationship between the specificity of the delayed reaction, in which the carrier protein plays an essential part, and the specificity of antibodies when they are produced against the same conjugate, which is dominated by the haptenic group, is most easily described as a change in specificity between the two stages. This implies no more than that the specificity of the observed events is narrower in the one case than in the other. Clearly binding forces of a different order of magnitude may be needed for a cellular interaction, by means of which mononuclears from the blood stream must be guided towards a site of antigen concentration in the tissues, as compared with the forces needed for the interaction of two soluble proteins, whether free in the tissues or *in vitro*; the observed differences may be the result of a difference in these forces, rather than of any difference in the actual interacting sites.

SUMMARY

In earlier observations with the picryl system, it was concluded that contact sensitivity was a form of delayed (cellular) hypersensitivity to conjugates of sensitizer with autologous proteins indistinguishable in its immunological mechanism from other classical forms of delayed hypersensitivity to proteins. This conclusion has been confirmed and extended with the picryl and chlorbenzoyl chloride systems.

1. It is shown that to induce a state of contact sensitivity, the minimal necessary amounts of hapten are of the same order of magnitude, whether this hapten is conjugated with protein or the free reactive chemical itself. From this, it is evident that contamination of conjugates with small amounts of unreacted sensitizer plays no part in the induction of contact reactivity by the conjugate. With the dinitrophenyl system, no contact sensitivity could be induced by the conjugates used; possible reasons for this discrepancy are discussed.

2. Animals sensitized to contact by homologous conjugate can be completely desensitized by injections of such a conjugate in large amount; a similar injection schedule has no effect on the contact sensitivity of animals sensitized with the free reactive sensitizer.

3. The capacity of heterologous (ovalbumin) conjugates to evoke antihapten antibodies is shown to be greater than that of homologous (guinea pig seralbumin) conjugates: the reverse is true of their capacity to induce delayed reactivity.

4. Evidence is brought forward to suggest that in animals sensitized with homologous albumin conjugates, the specificity of the delayed reaction involves more than the hapten alone, even though the carrier protein is non-antigenic on its own. The contrast with the apparent lesser specificity of the antibodies later produced is discussed.

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