

The Journal of Allergy

VOL. 28

MAY, 1957

No. 3

Original Articles

THE ROLE OF THE CARRIER IN THE FORMATION OF COMPLETE ANTIGENS

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WITH THE TECHNICAL ASSISTANCE OF D. JACONIA

NUMEROUS chemical substances produce skin sensitizations of the delayed type with great regularity upon epidermal or intradermal application to guinea pigs; among the particularly strong sensitizers are primulin, paraphenylenediamine, phenylhydrazine, picryl chloride, 2,4-dinitrochlorobenzene, and others. However, sensitization by the epidermal route leads not only to the delayed type of hypersensitiveness, but also, as Landsteiner and Chase¹ and Chase² have demonstrated, to allergies of the immediate or anaphylactic type, which is demonstrable by challenge with the corresponding hapten-protein complex.

Parenteral administration of these same substances, as for instance by intraperitoneal or intravenous injection, under careful avoidance of contact with the epidermis, produces regularly sensitizations of the immediate type, but only occasionally sensitizations of the delayed type. However, if the parenteral preparation is accompanied by a simultaneous administration of certain unspecific adjuvants, particularly killed mycobacteria suspended in paraffin oil (Freund's adjuvant) or certain mycobacterial waxes, the intensity and the frequency of epidermal sensitizations increase considerably. Using mycobacteria, for instance, as the adjuvant, sensitizations of the delayed type in increased number and intensity were obtained when guinea pigs were sensitized to picryl chloride,³ egg albumin,⁴ and tuberculin.⁵

Landsteiner and Chase^{1, 2} have proposed various explanations for this delayed-reactivity-promoting action of tubercle bacilli or their lipopolysaccharides, such as an improvement of the antigenic effect, an enhancement of sensitization, or a modification of the response to antigens. Raffel^{4, 5} believes that the adjuvant brings about an actual modification of the allergic response.

From the Research Department, CIBA Pharmaceutical Products, Inc.
Received for publication March 18, 1957.

We have never, in all our previous studies of experimental sensitizations, found an adjuvant capable of effecting any qualitative change of an allergic response, particularly a substitution of the delayed type of sensitization for the immediate type. One can readily observe skin sensitizations of the delayed type after the parenteral injection of sensitizing agents in the absence of adjuvant, although in smaller numbers and lesser strength than in the presence of appropriate adjuvants. Tuberculin alone is likewise capable, under favorable conditions, of producing typical tuberculin hypersensitivity of the delayed type.

In a previous publication, the following explanation of the sensitization-promoting effect of certain adjuvants was proposed⁶:

All chemicals of low molecular weight which are frequent causes of skin allergies, such as picryl chloride, paraphenylenediamine, and many other simple chemicals, are haptens which become complete antigens and true sensitizers only when they are attached to carriers of high molecular weight. Most of these haptens, or their metabolites, are chemically reactive and firmly combine with proteins—in fact, with any kind of protein they may encounter at the point of introduction into the body. When a hapten is injected into the skin, it there encounters two predominant types of protein, namely, collagen and keratin (and their precursors), both of which are fibroid and rigid proteins with which the hapten will combine, often under formation of cross-links. If, however, this same hapten is injected intraperitoneally, it will come in contact with very little collagen and no keratin at all. It will, however, encounter almost exclusively the globular proteins, albumins and globulins, and will combine with them to form complete antigens.

The presence of tubercle bacilli and waxes in the peritoneal cavity and in close proximity to the haptens provides the hapten with collagen and thereby enables it to form complete antigens containing fibroid proteins. Mycobacteria and their waxes produce at the site of deposition tubercles composed of epithelioid and giant cells. In these tubercles, collagen is formed and accumulated at a very early stage. In the presence of mycobacteria, collagen is thus accumulated at sites which are normally very low in collagen content (the normal collagen content of the organs is about $\frac{1}{20}$ that of skin). If, therefore, picryl chloride is injected intraperitoneally together with a tubercle-producing adjuvant, it will encounter in its quest for a carrier with which it can combine to form a complete antigen not only the proteins which are normally abundant in the peritoneal cavity, namely, albumins and globulins, but also collagen which it normally would find in abundance only in the skin.

It is therefore likely that the chemical composition of complete antigens formed after penetration of a hapten into the skin, the peritoneum, or any

organ in the presence or absence of mycobacteria is not the same since the proteinic carriers, to which they become attached, are different. They unquestionably have different solubilities, rigidities, and shapes. Consequently, one may expect that the antibodies formed by the presence of these diverse complete antigens are likewise different.

According to accepted theories, the specific antibodies are fabricated on the templates of the protein-synthesizing enzymes modified by the presence of specific antigens, and are complementary in shape and form to the specific antigens. Thus, it may be expected that an antibody formed on a template modified by an antigen containing globular proteins as the carrier will be different from an antibody molded on templates modified by the presence of complete antigens containing fibroid proteins as the carrier. One may anticipate that the introduction of haptens into the peritoneal cavity normally induces the development of humoral antibodies of the anaphylactic or "immediate type" of allergy, while in the presence of collagen in the tubercle sessile antibodies of the "delayed reaction type" are fabricated in addition to the humoral antibodies. This theory would explain why the injection of a hapten directly into the peritoneal cavity is usually followed by the development of a hypersensitivity of the immediate type, while the injection of the same hapten into the peritoneal cavity together with a mycobacteria-containing adjuvant will favor a considerable increase in number and severity of the reactivities of the delayed type.

We have tested this hypothesis by substituting, as an adjuvant, mycobacteria or their waxes by a purified collagen preparation (procollagen). As previously reported,⁶ guinea pigs injected parenterally with picryl chloride in the presence of procollagen as the adjuvant reacted after an appropriate sensitization period upon epidermal challenge with delayed reactivities in increased number and strength; the delayed-reaction-promoting effect of the procollagen adjuvant was of the same magnitude as that obtained with Freund's adjuvant containing mycobacteria. In the present article, we report on additional experiments in which guinea pigs were sensitized to picryl chloride or Old Tuberculin by parenteral application with and without the addition of different "adjuvants," such as mycobacteria, procollagen, crystalline albumin, and paraffin oil.

EXPERIMENTAL

1. *Animals*.—In all experiments guinea pigs weighing approximately 250 grams were used.

2. *Antigens*.—Solutions of recrystallized picryl chloride were prepared in saline with the aid of absolute alcohol according to the method of Landsteiner and Chase.¹⁻³ The doses used for sensitization were 0.2 mg. picryl chloride dissolved in 0.5 ml. of diluent. Procollagen, prepared as described by Orekhovich and associates,⁷ was dissolved with 1:10,000 formic acid to form

5 per cent semisolutions and then emulsified with the oily components of Freund's adjuvant. The composition of the procollagen adjuvant used was as follows:

Procollagen solution, 5.0%	40 ml.
Tween 80	0.5 ml.
Dihydrostreptomycin	20 mg.
Penicillin	21 mg.
Bayol F	100 ml.
Arlacel C	4 ml.

The final approximate concentration of procollagen in this adjuvant* preparation was 1.37 per cent. (It is probable that the procollagen may have been partially transformed into gelatin during the preparation of the adjuvant.) An "albumin adjuvant" containing approximately 1.4 per cent crystallized albumin was prepared in an identical way as the above-mentioned "procollagen adjuvant."

Complete Freund's adjuvant was obtained from the Difeo Laboratories.

3. *Method of Injection.*—In order to avoid contact with the skin, injections were made as first reported by Landsteiner and Chase¹ by first slitting the abdominal skin of the guinea pigs and introducing the needle slantwise through the musculature, with the use of all precautions noted by these authors.

4. *Challenge.*—Four weeks after the first sensitizing injection, the animals were challenged by (a) a single, percutaneous application of one drop of 1.5 per cent picryl chloride dissolved in olive oil, spread over approximately a 10 sq. cm. area on the right shaved flank, or (b) intradermal injection of 0.1 ml. of a saline solution containing 20 mcg. of picryl chloride. In the case of sensitization to Old Tuberculin, the challenging dose was administered by the intradermal injection of 0.1 ml. of 1 per cent and 10 per cent Old Tuberculin, diluted in saline.

RESULTS

We have performed eight experiments thus far, in which the conditions of the sensitizing procedures were varied; of these experiments, three are described hereafter.

1. In the *first experiment*, forty-five guinea pigs, divided into three groups of fifteen animals each (Groups II, III, and IV of Table II), were sensitized to picryl chloride by intraperitoneal injection, with and without the addition of the "procollagen adjuvant" or Freund's adjuvant, and challenged, four weeks after the first sensitizing injection, by the percutaneous and intracutaneous application of the allergen. Group I, consisting of fifteen nonsensitized controls, was kept untreated until the time of challenge. The animals in Group II were sensitized to picryl chloride alone, those of Group III were sensitized to picryl chloride combined with procollagen, and those of Group IV were

*I am grateful to my associate, Dr. H. Heymann, for the preparation of the procollagen and the adjuvant.

TABLE I. FIRST EXPERIMENT. SCHEDULE OF SENSITIZING INJECTIONS

DAYS	PREPARATIONS ADMINISTERED INTRAPERITONEALLY PER GUINEA PIG		
	GROUP II	GROUP III	GROUP IV
1	—	0.5 ml. procollagen preparation combined with 0.2 mg. picryl chloride	0.5 ml. Freund's adjuvant
2	—	—	—
3	0.2 mg. picryl chloride in 0.5 ml. saline	—	0.2 mg. picryl chloride in 0.5 ml. sodium chloride
4	—	—	—
5	—	0.5 ml. procollagen preparation combined with 0.2 mg. picryl chloride	0.5 ml. Freund's adjuvant
6	—	—	—
7	—	—	—
8	0.2 mg. picryl chloride in 0.5 ml. saline	—	0.2 mg. picryl chloride in 0.5 ml. saline

sensitized to picryl chloride combined with Freund's adjuvant. Thirty-seven animals were alive at the end of the four-week incubation period. The schedule of sensitizing injections is given in Table I, the results of the challenge in Tables II and III and Fig. 1.

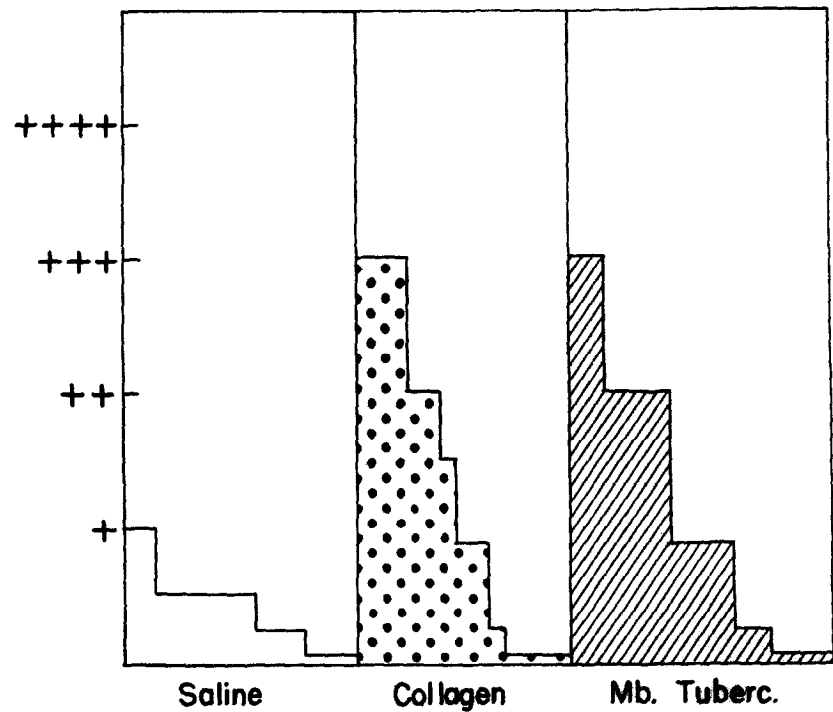


Fig. 1.—First experiment. Challenge reactions after sensitization to picryl chloride with saline, collagen adjuvant, and Mycobacterium tuberculosis (Freund's) adjuvant.

TABLE II. FIRST EXPERIMENT. RESULTS OF CHALLENGE APPLICATION

SENSITIZING TREATMENT	GUINEA PIG NO.	CHALLENGED BY SKIN SMEAR (24 HOUR READING)	CHALLENGED INTRA- DERMALLY (24 HOUR READING)
Group I. Normal, unsensitized controls	1	tr	0
	2	tr	0
	3	tr	0
	4	tr	0
	5	tr	0
	6	tr	0
	7	tr	0
	8	tr	0
	9	tr	0
	10	0	0
	11	0	0
	12	0	0
	13	0	0
	14	0	0
	15	0	0
Index		0	0
Group II. Picryl chloride in saline	16	++	+
	17	+	(+)
	18	(+)	(+)
	19	(+)	(+)
	20	(+)	0
	21	(+)	0
	22	(+)	0
	23	(+)	0
	24	tr	0
	25	tr	0
	26	tr	0
	27	0	0
	28	0	0
	29	0	0
Index		0.11	0.05
Group III. Picryl chloride with "procollagen adjuvant"	30	+++	+++
	31	++	++
	32	++	++
	33	++	++
	34	++	+ ½
	35	+ ½	+ ½
	36	+	+
	37	+	+
	38	(+)	+
	39	(+)	(+)
	40	tr	tr
	41	0	0
	42	0	0
	43	0	0
	44	0	0
Index		0.28	0.3
Group IV. Picryl chloride with Freund's adjuvant	45	+++	++
	46	++	+
	47	++	+
	48	+	0
	49	+	0
	50	tr	0
	51	0	0
	52	0	0
Index		0.32	0.16

In this and the following tables the intensities of reactions were recorded as 0, tr (trace), (+), +, + ½, ++, ++ ½, +++, +++ ½, and +++++. The index values for each group were calculated as follows: 0 and trace were considered as negative, the others were assigned numerical values of 1 to 8, respectively. The index for each group was obtained by dividing the total numerical value calculated for each group by the theoretical maximum value obtainable (number of animals per group × 8).

TABLE III. SUMMARY OF FIRST EXPERIMENT. RELATIVE INDEX VALUES

GROUP	TREATMENT	SMEAR CHALLENGE (24 HOUR READING)	INTRADERMAL CHALLENGE (24 HOUR READING)
I	Controls (unsensitized)	0.00	0.00
II	Picryl chloride only	0.11	0.05
III	Picryl chloride and procollagen	0.28	0.30
IV	Picryl chloride and Freund's adjuvant	0.32	0.16

Results.—The comparison of the challenge reactions observed twenty-four hours after tests with skin smear and by intradermal injection indicate that epidermal challenge usually produced a slightly higher number of generally weak positive reactions. In this experiment, preparation with picryl chloride in saline without addition of any adjuvant resulted in only two distinctly positive reactions (Group II). The addition of the procollagen adjuvant resulted in eight strongly positive reactivities (Group III). The addition of Freund's adjuvant was followed by a comparable increase of positive reactions (Group IV). As seen from Table III, the relative index values of the two groups (III and IV) receiving either adjuvant were three times as high as the index of Group II, which had received only picryl chloride.

2. In the *second experiment* the influence of a "procollagen adjuvant" and of an "albumin adjuvant" on the number of delayed sensitizations was investigated. Seventy-five guinea pigs, divided into four groups, were sensitized to picryl chloride alone or with addition of various adjuvants containing procollagen, bovine serum albumin, and Freund's adjuvant, respectively. The injection schedule is inscribed in Table IV, the individual results in Table V, and the index values in Table VI (Figs. 2 and 3).

TABLE IV. SECOND EXPERIMENT. SCHEDULE OF SENSITIZING INJECTIONS

DAY	PREPARATIONS ADMINISTERED INTRAPERITONEALLY PER GUINEA PIG			
	GROUP II	GROUP III	GROUP IV	GROUP V
1	—	0.5 ml. procollagen preparation combined with 0.2 mg. picryl chloride	0.5 ml. bovine serum albumin combined with 0.2 mg. picryl chloride	0.5 ml. Freund's adjuvant
2	—	—	—	—
3	0.2 mg. picryl chloride in 0.5 ml. saline	—	—	0.2 mg. picryl chloride in 0.5 ml. saline
4	—	—	—	—
5	—	0.5 ml. procollagen preparation combined with 0.2 mg. picryl chloride	0.5 ml. bovine serum albumin combined with 0.2 mg. picryl chloride	0.5 ml. Freund's adjuvant
6	—	—	—	—
7	—	—	—	—
8	0.2 mg. picryl chloride in 0.5 ml. saline	—	—	0.2 mg. picryl chloride in 0.5 ml. saline

Group I: untreated controls.

TABLE V. SECOND EXPERIMENT. RESULTS OF CHALLENGE APPLICATIONS

SENSITIZING TREATMENT	GUINEA PIG NO.	CHALLENGED BY SKIN SMEAR (24 HOUR READING)	CHALLENGED INTRA- DERMALLY (24 HOUR READING)
Group I. Normal, not sensi- tized controls	1	+	+
	2	(+)	(+)
	3	(+)	tr
	4	(+)	tr
	5	(+)	tr
	6	(+)	tr
	7	tr	tr
	8	tr	0
	9	tr	0
	10	tr	0
	11	tr	0
	12	0	0
	13	0	0
	14	0	0
Index		0.06	0.03
Group II. Picryl chloride in saline	15	++	++
	16	+	+
	17	+	+
	18	(+)	+
	19	(+)	(+)
	20	(+)	tr
	21	(+)	tr
	22	tr	0
	23	tr	0
	24	tr	0
	25	tr	0
	26	tr	0
	27	0	0
	28	0	0
Index		0.1	0.1
Group III. Picryl chloride with "procollagen adju- vant"	29	+++	+++
	30	+++	++
	31	++ $\frac{1}{2}$	++
	32	++	+ $\frac{1}{2}$
	33	++	+
	34	+ $\frac{1}{2}$	+
	35	+ $\frac{1}{2}$	+
	36	+	+
	37	+	+
	38	(+)	+
	39	tr	(+)
	40	tr	(+)
	41	tr	tr
	42	tr	tr
Index		0.3	0.3
Group IV. Picryl chloride and bovine serum albumin	43	+	+
	44	tr	(+)
	45	tr	(+)
	46	tr	tr
	47	tr	tr
	48	tr	0
	49	0	0
	50	0	0
	51	0	0
	52	0	0
	53	0	0
	54	0	0
Index		0.03	0.04

TABLE V—CONT'D

SENSITIZING TREATMENT	GUINEA PIG NO.	CHALLENGED BY SKIN SMEAR (24 HOUR READING)	CHALLENGED INTRA- DERMALLY (24 HOUR READING)
Group V. Picryl chloride and Freund's adjuvant	55	+++	+++
	56	+++	+++
	57	+++	++
	58	+++	++
	59	+++	++
	60	++	++
	61	++	++
	62	++	+ ½
	63	++	+
	64	++	+
	65	+ ½	+
	66	+ ½	+
	67	+	+
	68	+	(+)
	69	+	(+)
	70	+	(+)
	71	+	(+)
	72	(+)	(+)
	73	(+)	(+)
	74	(+)	(+)
	75	(+)	tr
	76	tr	tr
	77	tr	0
	78	tr	0
	79	0	0
	80	0	0
Index		0.34	0.25

Group I: untreated controls.

TABLE VI. SUMMARY OF SECOND EXPERIMENT. RELATIVE INDEX VALUES

GROUP	TREATMENT	SMEAR CHALLENGE (24 HOUR READING)	INTRADERMAL CHALLENGE (24 HOUR READING)
I	Controls	0.06	0.03
II	Picryl chloride only	0.1	0.1
III	Picryl chloride and procollagen	0.3	0.3
IV	Picryl chloride and serum albumin	0.03	0.04
V	Picryl chloride and Freund's adjuvant	0.34	0.25

Result.—While sensitizations with picryl chloride alone produced only a small number of positive reactions, with a relative index value of 0.1, the addition of procollagen gave rise to a considerable increase in number as well as intensity of positive reactions, with a threefold increase in the index value (0.3). The reactions obtained with Freund's adjuvant were very slightly stronger, having an index of 0.34 for the epidermal challenge, but slightly weaker with an index of 0.25 upon intradermal challenge. On the other hand, the addition of albumin resulted in a considerable decrease in reactivities with index values of only 0.03 to 0.04.

3. In the *third experiment* recorded, Old Tuberculin (O.T.) was used as sensitizing agent and the influence of procollagen adjuvant, albumin adjuvant,

and Freund's adjuvant upon the production of a sensitization of the delayed type was investigated. As in the previous experiment, the O.T., as well as the adjuvants, was injected intraperitoneally. In this experiment, seventy-five

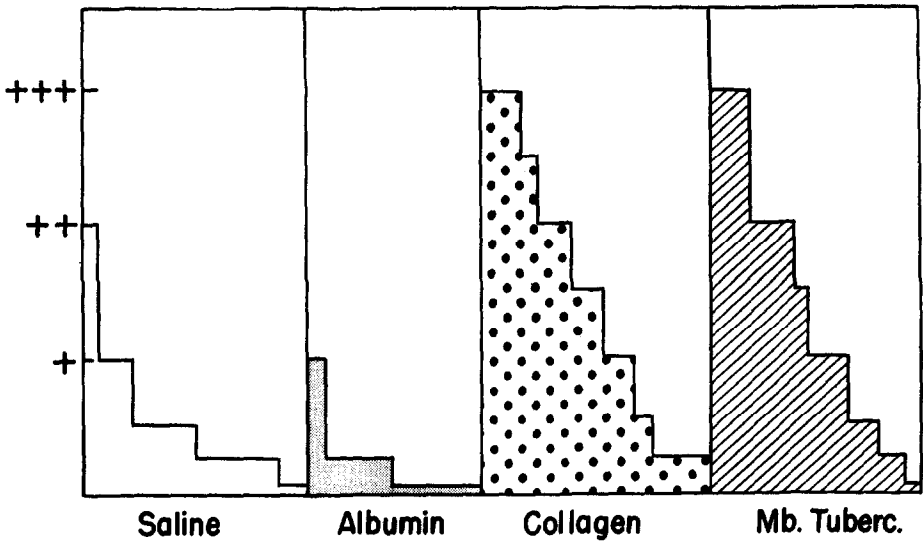


Fig. 2.—Second experiment. Challenge reactions after sensitization to picryl chloride with saline, albumin, collagen, and *Mycobacterium tuberculosis* (Freund's) adjuvant.

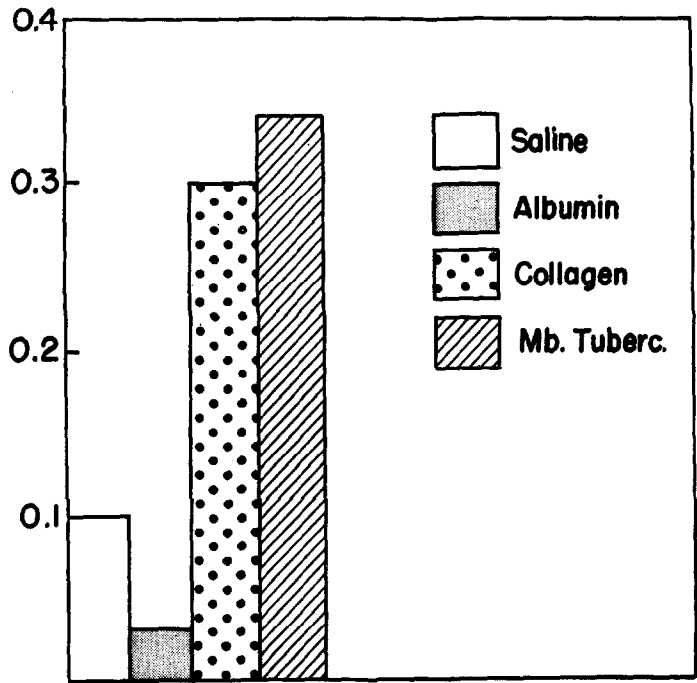


Fig. 3.—Second experiment. Challenge reaction, indices.

TABLE VII. THIRD EXPERIMENT. SCHEDULE OF SENSITIZING INJECTIONS

DAYS	PREPARATIONS ADMINISTERED INTRAPERITONEALLY PER GUINEA PIG			
	GROUP II	GROUP III	GROUP IV	GROUP V
1	Nothing	0.5 ml.* procollagen combined with 0.1 ml. Old Tuberculin	0.5 ml. serum albumin combined with 0.1 ml. Old Tuberculin	0.5 ml. Freund's adjuvant
2	Nothing	Nothing	Nothing	Nothing
3	0.1 ml. Old Tuberculin in 0.5 ml. saline	Nothing	Nothing	0.1 ml. Old Tuberculin in 0.5 ml. saline
4	Nothing	Nothing	Nothing	Nothing
5	Nothing	0.5 ml.* procollagen combined with 0.1 ml. Old Tuberculin	0.5 ml. serum albumin combined with 0.1 ml. Old Tuberculin	0.5 ml. Freund's adjuvant
6	Nothing	Nothing	Nothing	Nothing
7	Nothing	Nothing	Nothing	Nothing
8	0.1 ml. Old Tuberculin in 0.5 ml. saline	Nothing	Nothing	0.1 ml. Old Tuberculin in 0.5 ml. saline

*The Old Tuberculin mixtures of procollagen and serum were used immediately upon preparation.

guinea pigs were sensitized to O.T. in groups of fifteen animals each. Seventy-one of the animals were alive at the moment of the challenge. The injection schedule of this experiment is inscribed in Table VII, the individual results in Table VIII, and the index values in Table IX and Fig. 4.

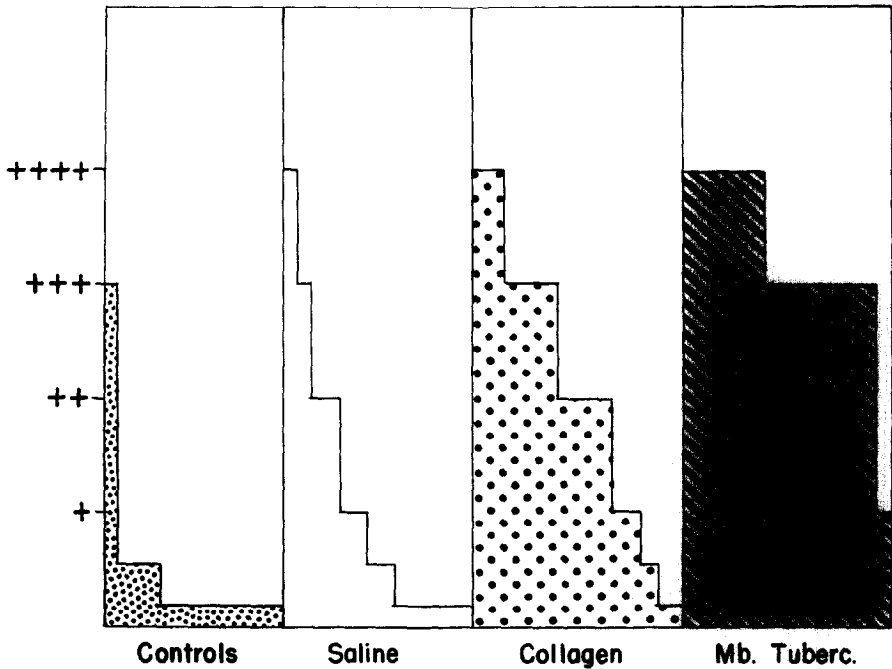


Fig. 4.—Third experiment. Challenge reactions after sensitization to Old Tuberculin with saline, collagen, and Mycobacterium tuberculosis (Freund's) adjuvant.

TABLE VIII. THIRD EXPERIMENT. RESULTS OF CHALLENGE APPLICATIONS

SENSITIZING TREATMENT	GUINEA PIG NO.	INTRADERMAL CHALLENGE 10%—24 HOURS
Group I. Normal controls (not sensitized)	1	+++
	2	(+)
	3	(+)
	4	(+)
	5	0
	6	0
	7	0
	8	0
	9	0
	10	0
	11	0
	12	0
	13	0
Index		0.09
Group II. Old Tuberculin in saline	14	++++
	15	+++
	16	++
	17	++
	18	+
	19	+
	20	(+)
	21	(+)
	22	0
	23	0
	24	0
	25	0
	26	0
	27	0
Index		0.25
Group III. Old Tuberculin and procollagen	28	++++
	29	++++
	30	+++
	31	+++
	32	+++
	33	+++
	34	++
	35	++
	36	++
	37	++
	38	+
	39	+
	40	(+)
	41	0
	42	0
Index		0.40
Group IV. Old Tuberculin and bovine serum albumin	43	+++
	44	+++
	45	+++
	46	++
	47	+
	48	+
	49	0
	50	0
	51	0
	52	0
	53	0
	54	0
Index		0.27

TABLE VIII.—CONT'D

SENSITIZING TREATMENT	GUINEA PIG NO.	INTRADERMAL CHALLENGE 10%—24 HOURS
Group V. Old Tuberculin and Freund's adjuvant	55	++++
	56	++++
	57	++++
	58	++++
	59	++++
	60	++++
	61	+++
	62	+++
	63	+++
	64	+++
	65	+++
	66	+++
	67	+++
	68	+++
	69	+
Index		0.81

TABLE IX. SUMMARY OF THIRD EXPERIMENT. RELATIVE INDEX VALUES

GROUP	TREATMENT	10%—24 HOURS
I	Controls	0.08
II	Old Tuberculin in saline	0.25
III	Procollagen and Old Tuberculin	0.40
IV	Bovine serum albumin and Old Tuberculin	0.27
V	Freund's adjuvant and Old Tuberculin	0.81

Results.—The results of this experiment indicate that a number of animals were tuberculin positive prior to the first O.T. injection of control Group I and the first challenge of the animals of Group II, since, under the conditions of this experiment, no sensitizations to O.T. could be expected unless an effective adjuvant was added. There was one strong positive reaction in the control group and four strong reactions in the animals of Group II treated with two injections of tuberculin alone and then held for four weeks without further treatment. Four positive reactions were also noted in Group IV, in which all animals received serum albumin in addition to O.T. The index values in these two groups were 0.25 and 0.27, respectively.

Despite this interference, the influence of the collagen adjuvant upon the successful sensitization was very noticeable. The percentage of strongly positive reactions in Group III (animals receiving procollagen) was almost twice that of the Group II guinea pigs receiving O.T. alone, with an index value for Group III of 0.40 as against 0.25 for Group II. It could be expected that the injection of Freund's adjuvant resulted in a strong increase of positive challenge reactions with an index of 0.8.

DISCUSSION

The results of the three experiments reported here indicate, in confirmation of our previous experiments, that in sensitizations of guinea pigs to picryl chloride procollagen exerts the same delayed-reaction-promoting effect as do tubercle bacilli or lipopolysaccharides isolated from the wax fraction of mycobacteria. Indeed, the challenge reactions of the delayed type were considerably stronger and more numerous when the sensitizations were carried out

with the addition of procollagen than in its absence. The activity of mycobacteria, however, in promoting delayed reactions upon sensitization with O.T. was markedly superior to that of procollagen.

While there can be little doubt that the hypersensitivities produced with picryl chloride represent allergies of the contact type, this is not a priori certain in the case of sensitizations obtained to O.T. The question arises whether the reactivities observed in the collagen-treated group are actually true delayed reactions of the tuberculin type and not immediate reactions of the Arthus type.

It was often observed that the positive skin reactions resulting from experimental sensitizations with O.T. alone faded more rapidly than those accompanying a tuberculous infection or following sensitization with dead mycobacteria. It has been questioned on this basis whether sensitizations after preparation with O.T. alone were indeed specific sensitizations to tuberculin and the skin reactions actual "tuberculin reactions." We have observed a similar, more rapid fading of delayed skin reactions in our experiments including those in which sensitizations to O.T. were obtained by the simultaneous use of the collagen adjuvant. On the other hand, the strongly positive challenge reactions persisted even after seventy-two hours in approximately 50 per cent of those animals which had been sensitized to O.T. with the aid of Freund's adjuvant; after seventy-two hours virtually all reactions induced by the use of procollagen and albumin, as well as those produced with O.T. alone, had faded. Nevertheless, it seems probable that these reactivities do not represent an Arthus type of immediate reaction, but types of true delayed reaction. It is known that in most instances the Arthus-type reaction reaches the maximum intensity after several hours and then slowly decreases to almost one-half this maximum after twenty-four hours, whereas the delayed type of reaction continues to increase in intensity up to twenty to twenty-four hours, when it reaches its maximum. In our case, maximum intensity was also reached after twenty-four hours. It seems, therefore, that the differences in the persistence of the skin reactions of sensitizations obtained in the presence of tubercle bacilli and of those obtained in association with collagen may be due to quantitative differences of the same (namely, tuberculin) type of reactivity, rather than to a different type. There is the possibility, however, that these reactions are delayed reactions in a hypersensitiveness not to the specific tuberculin protein, but to a nonspecific protein present in the Old Tuberculin. New experiments with purified protein fractions are under way.

We believe that these reported results represent additional support for our hypothesis that (1) the delayed reactivity is mediated by a complete antigen whose protein carrier is formed by a fibroid protein, such as collagen, while the immediate reactivity is mediated by a complete antigen whose protein carrier is a globular protein, such as albumin or globulin, and (2) that the delayed-reactivity-promoting effect of mycobacteria and associated waxes is due to the formation of tubercles and, within these tubercles, of collagen on sites normally poor in fibroid proteins.

SUMMARY

1. Procollagen, like mycobacteria, increases the delayed reactions following sensitization of guinea pigs with picryl chloride or with Old Tuberculin by the parenteral route. Albumin does not exert this effect.

2. The effect of tubercle bacilli and lipopolysaccharides isolated from the wax fraction of tubercle bacilli upon the production of sensitizations of the delayed type after intraperitoneal injection is explained by the formation of specific tubercles and consequent formation of fibroid protein, particularly collagen, at sites in the vicinity of the injected hapten, thus enabling the hapten to combine with collagen to form complete antigens containing a carrier of fibroid protein.

3. The theory is discussed that the sessile antibody of the delayed type of sensitization is formed by complete antigen with fibroid proteins as carrier, whereas the humoral antibodies of the immediate or anaphylactic type are produced by complete antigen with globular proteins as carriers.

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