

INHIBITION OF RESPONSES TO WEAK ANTIGENS IN CONTACT SENSITIZATION IN THE GUINEA PIG

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INHIBITORY phenomena among antigens reacting with circulating antibodies are well recognized, but there is little reference to such phenomena in contact sensitization. A report by Epstein and Kligman¹ indicates that development of contact sensitization to a weak antigen may be inhibited by the prior application of a stronger antigen, but their work has not been confirmed.

It seems of interest, therefore, to report two animal experiments in which there appeared to be two distinct types of inhibition of a weak antigen by a stronger antigen in the course of contact sensitization.

EXPERIMENT NO. 1

Animals were sensitized to a strong antigen and subsequently tested with mixtures containing essentially constant proportions of a weak antigen and varying proportions of the strong antigen.

Materials and Methods.—

Animals: Twelve albino guinea pigs of the Hartley strain were used.* Each animal weighed approximately 500 grams at the start of experimentation and each was maintained as previously described.² The animals were of both sexes, but this factor will be ignored in this report since gender seemed to have no influence on the results of the experiments.

Antigens: DNCB, 1-chloro-2, 4-dinitrobenzene,† was used as the strong antigen.

A single batch of Conti olive oil was used as the weak antigen. This “antigenic olive oil” had been found to produce an approximately 80 per cent rate of epidermal contact sensitization in guinea pigs, but was considered weakly antigenic because of the limited duration of sensitization it produced.²

Preliminary sensitizing dose of strong antigen: As the first step in the experiment, one drop of 0.5 M DNCB in acetone (NF), approximately 5 mg. of DNCB, was applied by dropper to the skin of each animal in an area of the back which had just been clipped.

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Subsequent testing procedures: After the preliminary application of the strong antigen DNCB, each animal was repeatedly tested with a battery of tests consisting of six successive tenfold dilutions of DNCB in a diluent composed of equal parts of the weak antigen (Conti olive oil) and acetone (USP). The diluent alone was used as the seventh test of the battery. (See Table I.)

Each individual test in the battery consisted of a single drop of solution delivered through a 26 gauge needle from a tuberculin syringe held in a vertical position 1 cm. above the skin surface. The average drop was approximately 0.005 ml., so that one drop of the 10^{-2} M solution contained about 0.01 mg. of DNCB.

The tests were applied to the freshly clipped skin of the posterolateral aspect of the animal's thorax and abdomen and read at 24 hours. The individual tests of each battery were applied in varying sequence so that the responses could be read in "blind" fashion. The inflammatory responses observed at 24 hours consisted of varying degrees of erythema without wheal formation or vesiculation. The responses were consistent and reproducible and no variation of response was found which might be attributed to the area of the skin used.

Results (Table I).—

When first subjected to the battery of tests, 2 weeks after their initial exposure to DNCB, 9 of the 12 animals showed positive reactions in the first, or in the first and second, dilutions in the battery. These reactions were taken to indicate sensitization to DNCB, since animals similarly sensitized to DNCB will usually react to DNCB in these dilutions.

In the second application of the battery of tests 3 weeks later, 10 animals showed similar reactivity to DNCB, but one animal (No. 4) showed reactivity in the third dilution as well. One animal (No. 10) had weakly positive reactions in all dilutions. This seemed to indicate sensitization to olive oil because the last dilution, which contained no DNCB, showed the same degree of response as the other dilutions.

In the tests applied at 8 weeks, all of the animals appeared to be reactive to DNCB, as indicated by the strong reactions in the first dilutions. Two animals (Nos. 7 and 10) appeared to be weakly reactive to olive oil as well, as evidenced by their positive responses to the diluent.

In the fourth group of tests, done at 14 weeks, all but one of the animals appeared to be sensitive to the antigenic olive oil. This apparent sensitivity, which subsequently appeared in the one negative animal at the next test, persisted in the majority of the animals for 3 or 4 months. Although not shown in the data given, the DNCB sensitivity tended to persist for several months after the sensitivity to oil had disappeared.

During the period between the fourteenth and twenty-ninth weeks, a period in which simultaneous sensitivity to both antigens appeared to prevail in most of the animals, each animal was subjected to the battery of tests on four occasions—a total of 48 test batteries. As indicated by the asterisks in Table I, in 27 of these 48 tests the intermediate dilutions, usually 10^{-4} and 10^{-5} , showed distinctly less positive responses than did the other dilutions in the battery. On

TABLE I. REACTIONS TO MIXTURES OF CONTI OLIVE OIL AND DNCB IN GUINEA PIGS PREVIOUSLY EXPOSED TO DNCB ALONE

	WEEKS AFTER PRELIMINARY APPLICATION OF DNCB						
	2	5	8	14	22	25	29
1	2000000	3000000	3000000	2000000	3210022*	3211111	3311111
2	2100000	3100000	2100000	2211222*	3211223*	2111122*	3211111
3	2220000	2100000	2000000	2211122*	3322233*	3222333*	3210002*
4	1100000	2210000	2110000	2111122*	3322333*	3222233*	2000000
5	2000000	3100000	2000000	2112233*	3211222*	3322233*	2100012*
6	3000000	2000000	2200000	3221122*	3322333*	3322233*	2101222*
7	3200000	3000000	2211111	3222222	0000000	1111111	0000000
8	2200000	2200000	2200000	2222211	1110000	1001111*	2221112*
9	2000000	2000000	2000000	3222221	3322333*	3333333	2222222
10	0000000	1111111	2111111	2211122*	2233333	2221123*	0000000
11	0000000	0000000	2000000	0022223	1110022*	1111111	0000000
12	0000000	2000000	2101000	2211222*	2222222	2221133*	2020002

Legend: Each group of seven digits represents a battery of seven individual tests. Reading from left to right the individual tests represented by each digit were: (1) 10^{-2} M DNCB in diluent, (2) 10^{-3} M, (3) 10^{-4} M, (4) 10^{-5} M, (5) 10^{-6} M, (6) 10^{-7} M, and (7) diluent alone (equal parts of Conti olive oil and acetone USP).

The individual numbers represent the following degrees of response to the individual tests in the battery: 0—No visible reaction, 1—Faint pink, 2—Distinct pink, and 3—Brisk red.

*Denotes batteries showing decreased response in the intermediate range of dilution.

five occasions the response in the intermediate range was totally negative, contrasting sharply with the positive responses obtained with the 10^{-2} dilution and with the control. More often, however, there was simply a diminution of the positivity in the mid-range: a drop from a maximum to a minimum and back to a maximum when the tests were read in order from the 10^{-2} M dilution to the control.

It was noted that this apparent suppression of response to the olive oil obtained only when there was a simultaneously strong reaction to both dinitrobenzene and olive oil. Control studies during this period, using peanut oil as a diluent in place of the antigenic olive oil in the battery of tests, showed the reactivity of dinitrobenzene to be limited in the first two dilutions (10^{-2} M and 10^{-3} M).

Comment.—

In previously reported work with antigenic olive oil, animals repeatedly exposed to the oil alone developed sensitivity to the oil in an average of 4 or 5 weeks.² In the above experiment the sensitization had not appeared in most of the animals until 14 weeks after their initial exposure to the oil.

Since preliminary experiments suggested that the technique of simultaneous testing with the combination of DNCB and antigenic olive oil was not a factor in the apparently delayed sensitization to the oil, the following experiment was performed to see if the prior sensitization to DNCB might not be responsible for the delay.

EXPERIMENT NO. 2

Materials and Methods.—

Four groups of 12 guinea pigs each, a total of 48 animals, were used. The animals met the specifications given in Experiment I and were maintained in similar fashion.

All of the animals were given a preliminary exposure to DNCB, as described in Experiment I, and then each group was repeatedly tested with the same battery of tests used in the first experiment. The animals of Group I had their initial test with the battery (their first exposure to antigenic olive oil) one week after their preliminary exposure to DNCB. In Groups 2, 3, and 4, the first testing with the battery was 2, 3, and 4 weeks, respectively, after the preliminary DNCB exposure. Each group was subsequently tested with the battery at intervals of 3 weeks and the appearance of positive reactions to the control tests in the battery (the diluent alone) was used as the criterion for the development of sensitivity to the antigenic olive oil.

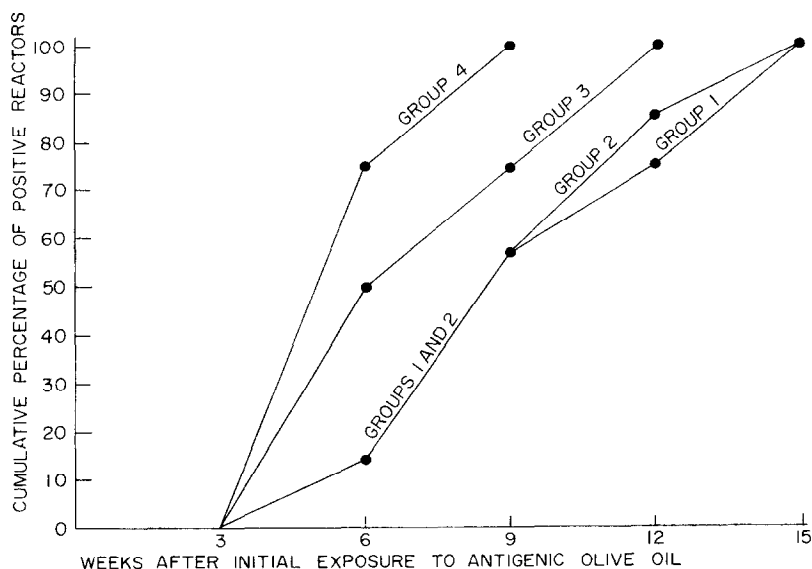


Fig. 1.

Results.—

The results are shown in Fig. 1, in which the abscissa represents the period of time in the weeks elapsing from the initial exposure to antigenic olive oil in the battery of tests. The ordinate represents the cumulative percentage of animals reactive to the oil. In computing the percentages, animals which failed to react to the olive oil were excluded. The percentage was calculated solely on the basis of the final total of animals that had shown a positive reaction to the oil at some time. The total number of reactive animals used for calculating the percentage in each group was 7, 7, 8, and 4 for Groups 1, 2, 3, and 4, respectively—an over-all sensitization rate of 54 per cent of the original 48 animals.

It is noted that no guinea pig reacted to either the first or second applications of the oil. At the third application of the battery, however, 6 weeks after the first exposure to the antigenic oil, there are distinct differences among the groups: the percentage of positive reactors varies inversely with the time interval between the preliminary exposure to DNCB and the initial exposure to

antigenic olive oil. Groups 1 and 2, initially exposed to antigenic olive oil one or two weeks after DNCB, respectively, show 14 per cent reactors (1/7). Group 3, with an initial exposure to oil 3 weeks after DNCB, shows 50 per cent reactors (4/8), and Group 4 shows 75 per cent (3/4).

Following the experiment to its conclusion, it is seen that all of the guinea pigs in the fourth group had reacted by the ninth week (the fourth application of the oil). Group 3 required 12 weeks to obtain this percentage of reactivity, and Groups 1 and 2 required 15 weeks.

DISCUSSION AND SUMMARY

The experiments reported above suggest two ways in which a strong antigen may inhibit the reaction to a weaker antigen in contact sensitization of the guinea pig.

In the first experiment, certain dilutions of the stronger antigen (DNCB) seemed to suppress reactivity to the weaker antigen (antigenic olive oil) when both were applied simultaneously. It seems probable that the suppression depends on an immunologic phenomenon, rather than on a chemical interaction between the two antigens, since it appeared only when the animals were demonstrably allergic to both antigens and since the dilution zones involved in the suppression were variable.

The second experiment suggests that development of an allergic response to a weak contact antigen may be temporarily blocked if the weak antigen is applied while the animal is developing sensitivity to a stronger antigen. The small number of reactive cavies in Group 4 weakens the experiments, but experience with other animals in similar types of experiment indicates that the result is valid. The findings are parallel to, and tend to confirm, the results obtained by Epstein and Kligman in their work with DNCB and para-nitrosodimethyl aniline in the human being.¹

REFERENCES

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